BaxΔ2 Promotes Apoptosis through Caspase-8 Activation in Microsatellite-Unstable Colon Cancer

Honghong Zhang1, Yuting Lin1, Adriana Mañas1, Yu Zhao1, Mitchell F. Denning2, Li Ma2, and Jialing Xiang1

Abstract

Loss of apoptotic Bax due to microsatellite mutation contributes to tumor development and chemoresistance. Recently, a Bax microsatellite mutation was uncovered in combination with a specific alternative splicing event that could generate a unique Bax isoform (BaxΔ2) in otherwise Bax-negative cells. Like the prototype Baxα, BaxΔ2 is a potent proapoptotic molecule. However, the proapoptotic mechanism and therapeutic implication of BaxΔ2 remain elusive. Here, the isolation and analysis of isogenic subclones are described that represent different Bax microsatellite statuses from colorectal cancer. Colon cancer cells harboring Bax microsatellite G7/G7 alleles are capable of producing low levels of endogenous BaxΔ2 transcripts and proteins. Interestingly, BaxΔ2-positive cells are selectively sensitive to a subgroup of chemotherapeutics compared with BaxΔ2-negative cells. Unlike other Bax isoforms, BaxΔ2 recruits caspase-8 into the proximity for activation, and the latter, in turn, activates caspase-3 and apoptosis independent of the mitochondrial pathway. These data suggest that the expression of BaxΔ2 may provide alternative apoptotic and chemotherapeutic advantages for Bax-negative tumors.

Implications: "Bax-negative" colorectal tumors expressing a Bax isoform are sensitive to selective chemotherapeutics. Mol Cancer Res; 12(9): 1225–32. ©2014 AACR.

Introduction

A microsatellite is a short stretch of repeating DNA sequence that is prone to deletion or insertion due to the slippage of DNA polymerase (1–3). Dysfunction of the DNA mismatch repair (MMR) system often results in microsatellite instability (MSI; refs. 4, 5). The classic example of MSI is Lynch syndrome (6), in which patients have a high risk of developing many types of cancer, especially hereditary nonpolyposis colorectal cancer (HNPCC; refs. 7, 8). Over 90% of patients with Lynch syndrome have a high level of microsatellite instability (MSI-H; refs. 9–11). If a microsatellite sequence is in the DNA coding region, frameshift mutations can disrupt the translational reading frame and cause truncation or premature termination. Many genes with microsatellite frameshift mutations are cancer related, such as Bax, TGF-β, IGFRII, and TCF-4, which are closely associated with MSI-H colon cancer (12–16). Loss of these key components significantly contributes to tumor development and chemoresistance (17–19).

Bax is a proapoptotic Bcl-2 family member (20–22). Typically, Bax promotes apoptosis through the activation of the mitochondrial death pathway (23, 24). Under a nonstimulated condition, Bax localizes in the cytosol as monomers. Upon stimulation by a death signal, Bax translocates to the mitochondrial membrane where it disrupts the mitochondrial membrane, causes the release of cytochrome C, and sequentially activates caspase-9 and caspase-3 for cell death (24, 25). Bax has several isoforms, mostly generated by alternative splicing between exons 1 and exon 3 or between exon 5 and exon 6 from the prototype Baxα pre-mRNA (21, 26–30). These isoforms either universally exist in normal and cancer cells or are only detectable in certain tissues (29). The proapoptotic activity of Bax isoforms can be well preserved as long as the Bax functional BH domains remain intact (31, 32). Some of the Bax isoforms, such as Baxβ, change the stability of proteins, whereas others change their potencies of cell death (30, 33). Nevertheless, most Bax isoforms promote apoptosis using the same mechanism as the prototype Baxα, that is, through the activation of the mitochondrial death pathway (23, 30, 33). Bax is one of the genes that are frequently mutated in MSI tumors (16, 35). The inactivation of Bax by a frameshift mutation is found in 50% of HNPCC (16). The deletion of a single guanine nucleotide (G) in the Bax exon 3 microsatellite tract (from G8 to G7) results in a reading frameshift and "Bax-negative" phenotype due to a premature termination codon. The loss of Bax often promotes tumor growth and increases resistance to chemotherapeutics (17–19). Recently, we found that the mutation-mediated loss of Bax could be restored by unique alternative splicing that...
produces a novel Bax isoform, BaxΔ2, which exists only in cells harboring the Bax microsatellite G7 mutation (36, 37). BaxΔ2 transcripts can be detected in both MSI cancer cell lines and primary tumors (36). BaxΔ2 is a more potent apoptotic inducer than Bax (36). In this study, we show that BaxΔ2 proteins determine the chemosensitivity of colon cancer cells. Unlike Bax, the proapoptotic activity of BaxΔ2 is mediated by the activation of the caspase-8 pathway rather than the mitochondrial death pathway. Our results uncover a distinct mechanism by which BaxΔ2 induces apoptosis and suggest that BaxΔ2 is a potential target for cancer therapy.

Materials and Methods

Materials
Antibody against Bax (N20, against the Bax N-terminus) was purchased from Santa Cruz Biotechnology. The BaxΔ2 monoclonal antibody was generated as described (36). Antibodies against cleaved human-specific caspase-8 and mouse-specific caspase-8, and human-specific Bid were from Cell Signaling Technology. The antibody against full-length caspase-8 (LS-C99287) was from Lifespan BioSciences. The antibody against cleaved human-specific caspase-3 and mouse-specific caspase-9, and human-specific Bid were from Cell Signaling Technology. The antibody against full-length caspase-9 (LZRS-caspase-9, D/N; ref. 38) and Bid siRNA (5’GGGCAAAAGC UUACAAAUAUU3’), as well as controls, were transfected using Lipofectamine 2000 according to the manufacturer’s instruction (Life Technology).

Transfection and RNAi
Cells were grown in 6-well plates to 70% to 80% confluences before transfection. Caspase-9 dominant negative construct (LZRS-caspase-9, D/N; ref. 38) and Bid siRNA (5’GGGCAAAAGC UUACAAAUAUU3’), as well as controls, were transfected using Lipofectamine 2000 according to the manufacturer’s instruction (Life Technology).

Immunoprecipitation and immunostaining
Cell pellets were lysed in NP-40 buffer (145 mmol/L NaCl, 5 mmol/L MgCl, 1 mmol/L EGTA, 0.25% NP-40, 20 mmol/L HEPES, pH 7.4) with a cocktail of protease inhibitors at 4°C for 30 minutes. Cell lysates were incubated with the appropriate antibody-conjugated beads overnight at 4°C. The beads were washed with NP-40 buffer for three times and the immunocomplexes were separated by SDS-PAGE and subjected to immunoblotting analysis. For immunostaining, cells were grown on cover slips coated with 1% gelatin. Cells were fixed and permeabilized with ice-cold methanol and incubated with primary antibodies diluted in PBS plus 0.3% Triton-X and 3% BSA. Following PBS washing, cells were incubated with Alexa fluorescence-conjugated secondary antibodies. Cell nuclei were stained with DAPI (4’, 6-diamidino-2-phenylindole). The fluorescence images were collected by Leica S5P Laser Scanning Confocal microscope using a ×40 objective.

Mitochondrial membrane potential assay
Cells (5 × 10⁶ per well) in 6-well plates were treated with different stimuli for a period of time as indicated in the figure legends. For the positive control, cells were treated with FCCP (Carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone, 100 μmol/L) for 30 minutes before staining. Mitochondrial membrane potentials (MMP) were analyzed by using a mitochondrial-specific cationic dye (JC-1) according to the manufacturer’s instruction and the images were collected by Nikon TE-2000 fluorescence microscope. The quantitation of MMP was performed using ImageJ program with at least five random fields for each sample.

Colony formation and cell invasion assays
A standard colony formation assay was performed as described (39). Briefly, total 1.5 × 10⁴ cells were seeded in each well in a 6-well plate. After culturing for 3 to 4 weeks, the colonies were visualized by staining with crystal violet and triplicate samples were counted under the dissecting...
The expression of Bax

Results

The expression of Bax2 proteins can be detected in colon cancer cells harboring the Bax microsatellite G7 mutation allele

Bax2 is a functional Bax isoform produced by a unique combination of a microsatellite mononucleotide deletion (G8 to G7) in Bax exon 3 and alternative splicing of Bax exon 2 (36). HCT116 colon cancer cells contain mixed populations with different Bax microsatellite statuses as follows: the majority of these cells (94%) have mixed Bax alleles (G8/G7); 4% of these cells have pure Bax G7/G7; and 2% of these cells have pure Bax G8/G8 (17). It has been shown that further deletion of the Bax G8 allele in the Bax G8/G7 cells results in a Bax null phenotype and leads to partial chemoresistance (17). We speculated that the population of HCT116 cells harboring the Bax G7 mutation is capable of producing Bax2, thus remaining sensitive to chemotherapeutic treatment. To test this scenario, we isolated single-cell populations using a standard 96-well plating method (Fig. 1A; ref. 17). More than 54 isogenic subclones were isolated and genotyped. The following 20 subclones were further analyzed by both genomic sequence and splicing analyses: 14 clones contained Bax G8/G7; six clones contained G7/G7; and none of the clones contained G8/G8 (Table 1). Interestingly, all six Bax G7/G7 clones contained both detectable transcript products from constitutive splicing and alternative splicing, as determined by RT-PCR (Table 1). In contrast, all Bax G8/G7 clones contained only constitutive splicing transcripts, and none of these clones contained a detectable transcript from alternative splicing (Table 1). These results suggest that not all alleles containing Bax microsatellite mutations are capable of alternative splicing.

To study the ability of the isogenic cells to generate Bax2 transcripts, we further analyzed HCT116 sublines, namely clone #10 (Bax G7/G7) and clone #28 (Bax G8/G7). RT-PCR analysis with primers in Bax exons 1 and 3 revealed that clone #10 (Bax G7/G7) contained both constitutive splicing (upper band) and alternative splicing (lower band), although the constitutive splicing product was predominant (Fig. 1B). In contrast, clone #28 (Bax G8/G7) only contained constitutive splicing products, albeit the Bax G7-mutated allele. Furthermore, only less than 20% of the total pre-mRNA from the Bax G7/G7 population went through the exon 2 alternative splicing (Fig. 1B). To further confirm that the alternative splicing product (lower band) was a Bax2 transcript, the lower band was excised and subjected to sequence analysis. In addition, the BaxΔ2-specific 5’ primer covering the junction of exons 1 and 3 was used in the PCR analysis. As expected, the BaxΔ2 transcript was only detected in clone #10 and the BaxΔ2-positive control, but it was not detected in clone #28 and the BaxΔ6-negative control (Fig. 1B). Thus, BaxΔ2 transcripts can be easily detected in cells containing the Bax G7/G7 allele.

We then determined the expression of endogenous BaxΔ2 proteins in the HCT116 clones #10 and #28 subline cells using a specific anti-BaxΔ2 antibody (36). Immunoblotting analysis revealed that the endogenous BaxΔ2 protein levels were extremely low in clone #10 and undetectable in clone #28 (Fig. 1C). However, BaxΔ2 proteins were easily detected in clone #10 when the cells were treated with MG-132, a proteasomal inhibitor (Fig. 1C). Under the
same conditions, clone #28 had no detectable BaxD proteins. This result was consistent with previous observations (Fig. 1B) that Bax G7/G7 cannot generate detectable BaxD2 transcript and protein. Of note, both clone #10 and clone #28 had no detectable parental Baxa proteins, as analyzed by immunoblotting using several anti-Baxa antibodies (Fig. 1C and data not shown). Taken together, these results indicate that cancer cells harboring Bax G7/G7 mutations are capable of generating BaxD2 proteins, although the BaxD2 proteins seem to be unstable and prone to proteasomal degradation.

To analyze the physiologic characteristics of these two HCT116 isogenic sublines, the growth rate, invasion ability, and colony formation of both clones #10 and #28 cells were analyzed. We found that clones #10 and #28 were quite similar to each other in terms of growth rate (Fig. 1D) and invasive ability (Fig. 1E). However, the BaxΔ2-positive clone #10 had significantly less capability in colony formation compared with the BaxΔ2-negative clone #28 (Fig. 1F). These results indicate that BaxΔ2-positive cells may be less tumorigenic in tumor development.

**BaxΔ2-positive subline cells are sensitive to a subgroup of chemotherapeutics**

We have previously shown that cancer cells with Bax microsatellite mutations, such as prostate cancer 104-R cells (G7/G7) and colon cancer LoVo cells (G7/G9), are more sensitive to adriamycin treatment than cancer cells with wild-type Bax (36). However, the heterogeneity of different cell lines often adds complexity to data interpretation. To address this issue, we compared HCT116 isogenic subline clone #10 (BaxΔ2-positive) and clone #28 (BaxΔ2-negative) cells for their sensitivities with chemotherapeutic agents. For the initial screening, we selected a panel of different classes of commonly used chemotherapeutic drugs. Cells were treated with a series of doses of each drug, and the results of cell death are shown in Table 2. BaxΔ2-positive clone #10 was more sensitive to adriamycin than BaxΔ2-negative clone #28, which was consistent with the previous report that BaxΔ2-positive cancer cells are more sensitive to adriamycin than BaxΔ2-negative cancer cells (36). In addition, BaxΔ2-positive clone #10 was also highly sensitive to 5-FU, a pyrimidine analogue compared with the BaxΔ2-negative clone #28 (Table 2). Interestingly, the sensitivity seems selective, even within the same class of chemotherapeutic drugs because there was no significant difference when both clones were treated with daunorubicin, which is also an anthracycline antibiotic with a similar structure as,

### Table 1. Analysis of the Bax MSI and splicing statuses in HCT116 isogenic subcell lines

<table>
<thead>
<tr>
<th>Clone #</th>
<th>Bax MSI status</th>
<th>Splicing</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>G8/G7</td>
<td>Constitutive</td>
</tr>
<tr>
<td>10</td>
<td>G7/G7</td>
<td>Constitutive, alternative</td>
</tr>
<tr>
<td>11</td>
<td>G8/G7</td>
<td>Constitutive</td>
</tr>
<tr>
<td>20</td>
<td>G7/G7</td>
<td>Constitutive, alternative</td>
</tr>
<tr>
<td>22</td>
<td>G7/G7</td>
<td>Constitutive, alternative</td>
</tr>
<tr>
<td>24</td>
<td>G8/G7</td>
<td>Constitutive</td>
</tr>
<tr>
<td>28</td>
<td>G8/G7</td>
<td>Constitutive</td>
</tr>
<tr>
<td>32</td>
<td>G8/G7</td>
<td>Constitutive</td>
</tr>
<tr>
<td>37</td>
<td>G8/G7</td>
<td>Constitutive</td>
</tr>
<tr>
<td>38</td>
<td>G8/G7</td>
<td>Constitutive</td>
</tr>
<tr>
<td>39</td>
<td>G8/G7</td>
<td>Constitutive</td>
</tr>
<tr>
<td>40</td>
<td>G8/G7</td>
<td>Constitutive</td>
</tr>
<tr>
<td>41</td>
<td>G8/G7</td>
<td>Constitutive</td>
</tr>
<tr>
<td>42</td>
<td>G8/G7</td>
<td>Constitutive</td>
</tr>
<tr>
<td>44</td>
<td>G7/G7</td>
<td>Constitutive, alternative</td>
</tr>
<tr>
<td>48</td>
<td>G8/G7</td>
<td>Constitutive</td>
</tr>
<tr>
<td>49</td>
<td>G8/G7</td>
<td>Constitutive</td>
</tr>
<tr>
<td>50</td>
<td>G7/G7</td>
<td>Constitutive, alternative</td>
</tr>
<tr>
<td>52</td>
<td>G8/G7</td>
<td>Constitutive, alternative</td>
</tr>
<tr>
<td>54</td>
<td>G8/G7</td>
<td>Constitutive</td>
</tr>
</tbody>
</table>

### Table 2. Initial screening for chemosensitivities in the HCT116 isogenic subcell lines

<table>
<thead>
<tr>
<th>Class</th>
<th>Name</th>
<th>Chemosensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkylating</td>
<td>Cytoxan</td>
<td>BaxΔ2&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Cysplatin (CDDP)</td>
<td>ns</td>
</tr>
<tr>
<td>Antimetabolites</td>
<td>5-FU</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>Hydroxyurea</td>
<td>++</td>
</tr>
<tr>
<td>Antibiotics</td>
<td>Doxorubicin</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>Daunorubicin</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>Epirubicin</td>
<td>++</td>
</tr>
<tr>
<td>Topoisomerase inhibitor</td>
<td>Etoposide</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>Irinotecan (CPT-11)</td>
<td>++</td>
</tr>
<tr>
<td>Akaloids</td>
<td>Taxol</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>Vinblastine</td>
<td>ns</td>
</tr>
</tbody>
</table>

**Note:** Cell viability was determined at 48 hours posttreatment. +, less than 20%; ++, 30% to 50%; ++++, >60%. Abbreviation: ns, no significant difference between the BaxΔ2<sup>-</sup> and BaxΔ2<sup>+</sup> groups.
BaxΔ2 promotes apoptosis through activation of the caspase-8 pathway

Bax typically targets the mitochondria upon activation and results in the activation of the caspase-9 and caspase-3 cascade for cell death (40). To determine the underlying mechanism by which BaxΔ2 promoted cell death, HCT116 clones #10 and #28 were treated with adriamycin or 5-FU in the presence of different caspase inhibitors. We found that the chemodrug-induced apoptosis was significantly augmented in clone #10 and could be effectively inhibited by either a caspase-8 or caspase-3 inhibitor, but not caspase-1 or caspase-9 inhibitor (Fig. 2A). The caspase-3 activity was confirmed by the caspase-3 fluorometric assay (Fig. 2B). The activation of caspase-3 was not surprising because it is an executioner caspase downstream of many caspase-mediated cell death events, including the Bax mitochondrial death pathway (24, 25). However, the activation of caspase-8 was unexpected, as the Bax family usually utilizes the mitochondrial death pathway (24, 25). Consistent with this notion, immunoblotting analysis using an anti-active caspase-8 antibody revealed that caspase-8 was activated, as indicated by the appearance of the cleaved caspase-8 fragments (43 kDa and 18 kDa), in clone #10 when treated with adriamycin or 5-FU but not in clone #28 (Fig. 2C). Thus, BaxΔ2 promoted apoptosis through the activation of caspase-8 and its downstream executioner caspase-3.

Activation of the Bid mitochondrial pathway is not essential for the onset of BaxΔ2-induced apoptosis

Caspase-8 is one of the initiator caspases in the extrinsic death receptor pathway (41). Once activated, caspase-8 directly activates the executioner caspase-3, or cleaves the BH-3-only protein Bid into tBid, which in turn targets mitochondria and triggers the release of cytochrome c for apoptosis (42, 43). We next determined whether the Bid-dependent mitochondrial death pathway was required for BaxΔ2 to promote apoptosis. We found that Bid was partially degraded in the BaxΔ2-positive clone #10 cells treated with 5-FU or adriamycin but not in the BaxΔ2-negative clone #28 cells (Fig. 3A). However, inhibition of Bid activity by its specific siRNA or inhibitor did not significantly affect the chemodrug-induced apoptosis (Fig. 3B). Similar results were obtained using a caspase-9 inhibitor or ectopic expression of the caspase-9 dominant negative mutant (ref. 44; Fig. 3C). Furthermore, caspase-8 was activated as early as 8 hours and reached to its maximum activity by 16 hours, but the mitochondria membrane potential remained reasonable intact 24 hours posttreatment, as evidenced by simultaneously monitoring the caspase activity and MMP (Fig. 3D and E). Taken together, these data indicate that the Bid-dependent mitochondrial death pathway may not be essential for the onset of chemodrug-induced apoptosis in the BaxΔ2-positive cells. Direct activation of caspase-3 by caspase-8 is likely required for the onset of the apoptosis.

BaxΔ2 activates caspase-8 by recruiting it into proximity

Caspase-8 is usually activated by death receptor-mediated self-processing, that is, the proximity-induced dimerization, followed by aggregation and self-cleavage/activation (45). We speculated whether BaxΔ2 oligomers or aggregates might serve as a platform to recruit caspase-8 into the proximity for activation. To test this hypothesis, we first examined whether BaxΔ2 and caspase-8 were localized together. Bax null MEFs were transfected with BaxΔ2 and the activation of caspase-8 was confirmed by immunoblotting analysis with an anti-cleaved caspase-8 antibody (Fig. 4A). Immunostaining showed that in the absence of BaxΔ2, the staining of caspase-8 was weak and appeared as diffused fine granules (Fig. 4B, top). Upon the expression of BaxΔ2, caspase-8 became aggregated and colocalized with BaxΔ2 (Fig. 4B, bottom). To determine whether BaxΔ2 and caspase-8 physically interacted with each other, we transfected BaxΔ2 into BaxΔ2-negative HCT116 clone #28 cells. Coimmunoprecipitation in combination with immunoblotting analysis revealed that the amount of the caspase-8 cleaved fragment (p43) was significantly higher in the immunocomplex with the anti-BaxΔ2 antibody than that of caspase-8.
with the IgG control (Fig. 4C). These data suggest that Bax
d2 oligomers may serve as a platform for caspase-8 aggregation and activation.

Discussion

Bax is a proapoptotic tumor suppressor and is expressed in almost all types of human cells (21, 46). Exon 3 of Bax contains a microsatellite sequence that is prone to mutation due to replication slippage if the MMR system is impaired (47, 48). A single guanine nucleotide deletion from G8 to G7 is the most common mutation in colorectal cancer with MSI, thus resulting in an apparent Bax null phenotype (47, 48). Interestingly, alternative splicing of Bax exon 2 can rescue the frameshift mutation, generating a unique and functional Bax\textsuperscript{D2} isoform (36). In this report, we demonstrated that cancer cells harboring Bax G7/G7 alleles were capable of producing Bax\textsuperscript{D2} transcripts and proteins, although the levels of Bax\textsuperscript{D2} transcripts and proteins were extremely low and unstable (Fig. 1B and C). Bax\textsuperscript{D2}-positive cells were selectively sensitive to a subgroup of chemotherapeutics, such as 5-FU and adriamycin (Table 2 and Fig. 2). Surprisingly, Bax\textsuperscript{D2}-promoted apoptosis relied on the activation of caspase-8 and downstream caspase-3 (Fig. 2). The Bid-mitochondrial pathway appeared not essential for onset of the apoptosis (Fig. 3). The mechanism underlying caspase-8 activation by Bax\textsuperscript{D2} was most likely through physical interactions between Bax\textsuperscript{D2} and caspase-8 resulting in the formation of aggregates thereby triggering the apoptotic process (Fig. 4).

There are two criteria in the generation of Bax\textsuperscript{D2}. First, the Bax gene must have the deletion of a single guanine nucleotide (G8 to G7) in its exon 3 microsatellite tract.
BaxΔ2 Activates Caspase-8 Pathway

Second, the alternative splicing machinery needs to be able to remove most of exon 2 (36). Previously, we have shown that the alternative splicing factors for BaxΔ2 are universal because cancer or noncancerous, human or murine fibroblast cells are all able to process the BaxΔ2 alternative splicing in a minigene assay (37). Thus, any Bax G7 allele, theoretically, is able to generate BaxΔ2. However, our current results showed that BaxΔ2 transcripts and proteins were only detected in cells harboring the Bax G7/G7 alleles (Fig. 1B). Furthermore, only less than 20% of total pre-mRNA from the Bax G7/G7 population went through exon 2 alternative splicing (Fig. 1B). Neither alternative splicing nor the BaxΔ2 protein was detected in all Bax G8/G7 subclones tested (Table 1). However, the underlying mechanism is not known. One possibility is that the amount of BaxΔ2 generated by Bax G8/G7 is too low to be detected. Another possibility is that there is a potential inhibitory mechanism for alternative splicing to occur in Bax G8/G7 cancer cells. Future studies are needed to test these possibilities.

Bax usually promotes apoptosis through activation of the intrinsic mitochondria death pathway (49, 50). Unlike BaxΔ2 and other known Bax isoforms, BaxΔ2 lacks exon 2, which is critical for the mitochondria targeting (33). Although ectopically expressed BaxΔ2 is able to activate the mitochondrial death pathway, it does not mean that BaxΔ2 directly targets mitochondria (36). Our results indicate that caspase-3 via caspase-8 is essential for the onset of BaxΔ2-induced apoptosis, and that mitochondria may act as an amplifier for the death process. Future studies are needed to explore whether this unique proapoptotic feature of BaxΔ2 is related to its ability to sensitize some “Bax-negative” MSI tumor cells to a subgroup of chemotherapeutic drugs.

Disclosure of Potential Conflicts of Interest
L. Ma and J. Xiang have ownership interest in a patent application on the anti-BaxΔ2 antibody in Mumetel LLC. No potential conflicts of interest were disclosed by the other authors.

Authors’ Contributions
Conception and design: Y. Lin, J. Xiang
Development of methodology: H. Zhang, Y. Lin, M.F. Denning, L. Ma, J. Xiang
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): H. Zhang, Y. Lin, A. Manas, Y. Zhao
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): H. Zhang, Y. Lin, A. Manas, Y. Zhao, J. Xiang
Writing, review, and/or revision of the manuscript: H. Zhang, J. Xiang
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): J. Xiang
Study supervision: J. Xiang

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