

## REVIEW

## Exploiting Apoptosis Pathways for the Treatment of Pediatric Cancers

Simone Fulda\*

Resistance to apoptosis (programmed cell death) is a characteristic feature of human cancers including childhood malignancies. Further, evasion of apoptosis is a frequent cause of treatment resistance, since most anti-cancer therapies, for example chemo- or radiotherapy act primarily by inducing apoptosis in cancer cells. Over the last two decades, the dissection of apoptosis pathways in pediatric tumors has resulted in the identification of many key

molecules that may serve as molecular targets for drug discovery. Accordingly, components of the apoptotic cascade are currently exploited for the development of rationally designed molecular targeted therapies. This approach is expected to open new and more effective approaches for the treatment of childhood cancers. *Pediatr Blood Cancer* 2009;53:533–536. © 2009 Wiley-Liss, Inc.

**Key words:** apoptosis; Bcl-2; IAPs; pediatric cancers; TRAIL

## INTRODUCTION

Programmed cell death (apoptosis) is a genetically encoded program, which is evolutionally highly conserved from worm to man [1]. Apoptosis exerts a key regulatory function in many normal physiological processes during development as well as in adult life [1]. To give just one example, apoptosis is crucial to maintain tissue homeostasis, which is the sum of a subtle balance between proliferation and cell death [2]. Consequently, too little apoptosis can promote tumorigenesis also without an increased proliferation rate [3]. It is important to note that evasion of apoptosis is a characteristic feature of basically all human cancers including childhood malignancies [4]. Moreover, resistance to apoptosis can lead to treatment resistance, since the sensitivity of cancer cells to current treatment regimens, that is, chemo-, radio- or immunotherapy, depends to a large extent on intact intrinsic cell death pathways [5]. Therefore, a better understanding of the regulatory network that governs apoptosis in pediatric cancers is anticipated to provide new opportunities for the design of innovative, molecular targeted therapies.

## Apoptosis Signaling Pathways

Two principle apoptosis pathways can be distinguished, that is, the receptor (extrinsic) pathway and the mitochondrial (intrinsic) pathway, which both eventually lead to activation of caspases, a group of enzymes that function as death effector molecules in various forms of cell death (Fig. 1) [6,7]. In the extrinsic apoptosis pathway, stimulation of death receptors of the tumor necrosis factor (TNF) receptor superfamily, for example, CD95 (APO-1/Fas) or TNF-related apoptosis inducing ligand (TRAIL) receptors, leads to activation of the initiator caspase-8, which either directly cleaves effector caspase-3 or alternatively, links the receptor to the mitochondrial pathway by cleaving Bid [8,9]. Bid is a BH3-only protein of the Bcl-2 family, which translocates to mitochondria to initiate a mitochondrial amplification loop once it is cleaved [9]. In the intrinsic pathway, the release of mitochondrial protein including cytochrome *c*, apoptosis inducing factor (AIF), second mitochondria-derived activator of caspase (Smac)/direct IAP binding protein with Low PI (DIABLO) or Omi/high temperature requirement protein A (HtrA2) from the mitochondrial intermembrane space into the cytosol initiates a common effector phase during apoptosis [10]. To this end, cytochrome *c* triggers caspase-3 activation through formation of the cytochrome *c*/Apaf-1/caspase-9-containing apoptosome complex [10], while Smac/DIABLO promote activation of

caspase-3, -7, and -9 by neutralizing “inhibitor of apoptosis proteins” (IAPs) [10]. There are also various control points that tightly regulate cell death programs, since inappropriate activation of apoptosis may have detrimental effects on cell survival. These anti-apoptotic mechanisms, which serve to prevent accidental cell death under physiological conditions, are often aberrantly activated in cancers and have been implicated in drug resistance. Apart from apoptosis, several non-apoptotic modes of cell death have also been identified, for example, necrosis, autophagy, mitotic catastrophe, or paraptosis [11]. How these diverse forms of cell death function in pediatric cancers as tumor suppressor mechanisms, relate to treatment response or, alternatively, promote tumor progression under certain circumstances is far from being understood.

## Apoptosis and Cancer Therapy in Childhood Malignancies

Based on the rationale that evasion of apoptosis is a characteristic feature of childhood malignancies that contributes to carcinogenesis and also to treatment resistance, approaches that target defective apoptosis programs in tumor cells may overcome primary or secondary resistance [3,12]. To this end, apoptosis targeted therapies may either directly trigger cell death in cancer cells or may increase the responsiveness of pediatric tumors towards conventional treatments that are currently used in the clinic, for example, chemo- or radiotherapy as discussed in more detail below [7].

## Exploiting the Death Receptor Pathway Via TRAIL

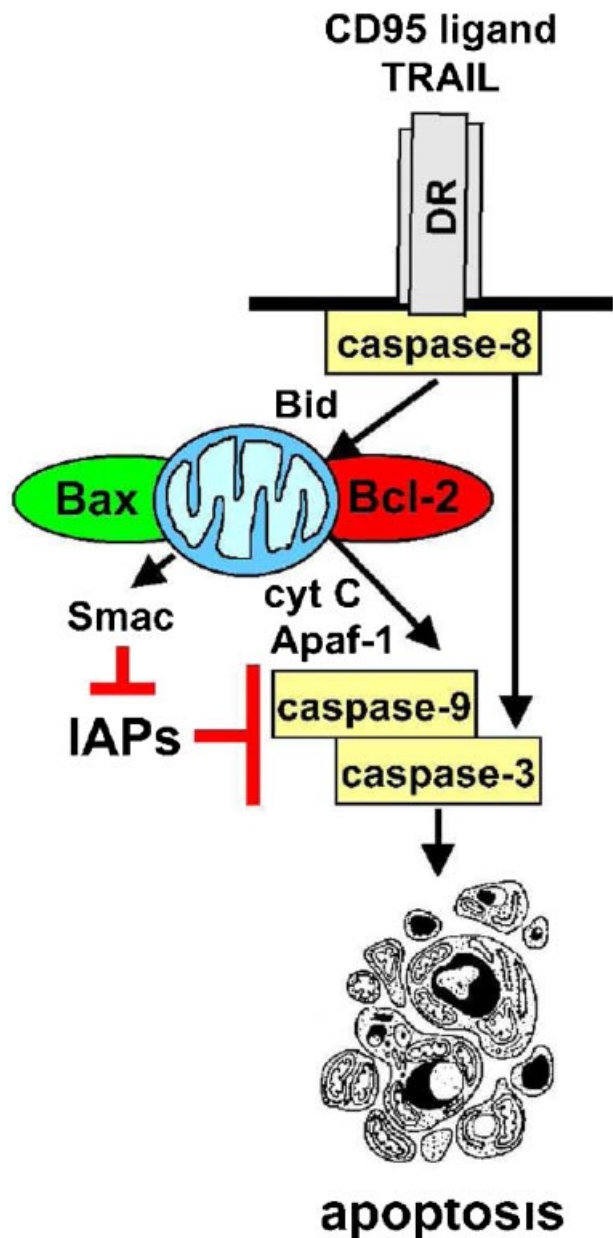
The idea to activate death receptors of the TNF receptor gene superfamily in order to induce cell death in cancer cells is attractive for the translation into medical application, since death receptors have a direct link to the cell's intrinsic death machinery [13]. For this

University Children's Hospital, Ulm, Germany

Grant sponsors: Deutsche Forschungsgemeinschaft; Deutsche Krebshilfe; Bundesministerium für Forschung und Technologie; Wilhelm-Sander-Stiftung; Else-Kröner-Fresenius Stiftung; IAP6/18; European Community (ApoTrain, APO-SYS).

\*Correspondence to: Simone Fulda, University Children's Hospital, Eythstr. 24, D-89075 Ulm, Germany.  
E-mail: simone.fulda@uniklinik-ulm.de

Received 30 October 2008; Accepted 1 December 2008



**Fig. 1.** Apoptosis pathways. In the death receptor (extrinsic) pathway, ligation of death receptors (DR) such as TRAIL receptors or CD95 by their respective ligands such as TRAIL and CD95 ligand leads to receptor trimerization, recruitment of adaptor molecules and activation of caspase-8. In the mitochondrial pathway, apoptogenic factors such as cytochrome *c* or Smac/DIABLO are released from mitochondria into the cytosol. The release of cytochrome *c* results in caspase activation via formation of the cytochrome *c*/Apaf-1/caspase-9-containing apoptosome complex. See text for more details. [Color figure can be viewed in the online issue, which is available at [www.interscience.wiley.com](http://www.interscience.wiley.com).]

approach, TRAIL is considered as a prime candidate, since TRAIL rather selectively induces apoptosis in cancer cells, while sparing normal cells [13]. The TRAIL receptor/ligand system is complex, since TRAIL can bind to five distinct receptors. Two agonistic receptors, TRAIL-R1 and TRAIL-R2, contain a conserved cytoplasmic death domain motif, which enables them to trigger

apoptosis upon binding of the ligand, while the antagonistic decoy receptors TRAIL-R3 to R-5 bind TRAIL, but do not transmit a death signal [13].

A number of strategies have been developed to make use of TRAIL in a therapeutic setting. For example, the recombinant natural ligand TRAIL has been reported to trigger apoptosis in a wide range of pediatric cancer cell lines including neuroblastoma, rhabdomyosarcoma, leukemia and Ewing's sarcoma [14–20]. Besides the recombinant ligand, monoclonal antibodies were specifically designed against one of the agonistic TRAIL receptors, that is, TRAIL-R1 or TRAIL-R2, which showed anti-tumor activity in cancer cell lines and xenograft-bearing mice [21,22]. However, there are so far only few studies that evaluated the anti-tumor activity of TRAIL or TRAIL receptor agonists on primary cells derived from pediatric tumor specimens. In one of such studies in childhood acute leukemia, half of the primary samples obtained before the onset of chemotherapy turned out to be refractory to TRAIL-induced apoptosis [17]. In primary neuroblastoma cells, TRAIL-mediated apoptosis was substantially potentiated by the concomitant neutralization of anti-apoptotic factors such as IAPs by Smac peptides [23]. Currently, soluble recombinant TRAIL as well as fully human monoclonal antibodies against TRAIL-R1 or -R2 are tested in phase I/II clinical trials [21].

### Exploiting Mitochondrial Apoptosis in Pediatric Oncology

An alternative approach to target apoptotic pathways for the treatment of childhood cancer is to neutralize anti-apoptotic proteins of the Bcl-2 family. There are two classes of Bcl-2 family proteins, that is, anti-apoptotic members such Bcl-2, Bcl-X<sub>L</sub>, Mcl-1, and pro-apoptotic proteins. The later includes the multidomain proteins Bax and Bak as well as BH3-domain only molecules such as Bim, Bid, Noxa, or Puma. Bcl-2 family proteins have a key function in the regulation of the intrinsic pathway of apoptosis, since they control mitochondrial outer membrane permeabilization. Accordingly, the dominance of anti- versus pro-apoptotic Bcl-2 proteins may tip the balance towards tumor cell survival instead of cell death and thus can contribute to cancer resistance.

Since elevated levels of anti-apoptotic Bcl-2 family proteins have been reported to confer resistance to chemotherapy or TRAIL, for example, in neuroblastoma cells [15,24], several approaches were developed in recent years to break the cytoprotective effect of Bcl-2 and related proteins. One of the prime examples is the design of the small molecule Bcl-2 family protein inhibitor ABT-737, which binds to the surface groove of Bcl-2, Bcl-X<sub>L</sub>, and Bcl-w and thus frees Bax and Bak from these anti-apoptotic Bcl-2 proteins to form pores in the outer mitochondrial membrane [25]. In childhood ALL cell lines and also in vivo in mouse xenografts derived from patients with ALL, ABT-737 showed synergistic cytotoxicity in combination with chemotherapeutic agents, including those that are part of current protocols in primary and relapsed childhood leukemia such as vincristine, glucocorticoids, and L-asparaginase amidohydase [26]. Notably, the orally bioavailable small molecule Bcl-2 family protein inhibitor ABT-263 showed the most prominent anti-tumor activity against cell lines derived from ALL in a recent evaluation of a panel of childhood tumor cell lines by the pediatric preclinical testing program [27]. Also in vivo in ALL xenografts in NOD/SCID mice, ABT-263 demonstrated potent anti-leukemic activity with complete remissions in three of six evaluable ALL

xenografts [27]. Moreover, BH3 peptidomimetics demonstrated single agent activity in neuroblastoma both in vitro and in vivo by inducing apoptosis [28]. Furthermore, the Bcl-2 antisense oligonucleotide G3139 was recently tested in a combination protocol with doxorubicin and cyclophosphamide in a phase I trial in children with relapsed solid tumors and was reported to show biologic effects [29]. Together, these findings suggest that targeting anti-apoptotic Bcl-2 family proteins may open new perspectives to reactivate the mitochondrial pathway of apoptosis in childhood tumors.

### “Inhibitor of Apoptosis” (IAP) Proteins as Therapeutic Target in Pediatric Cancers

Since “inhibitor of apoptosis” (IAP) proteins are expressed at high levels in many cancers including pediatric tumors and block apoptosis at a central point, they present promising targets for therapeutic intervention. There are eight human analogues of IAPs, including XIAP, c-IAP1, c-IAP2, survivin, apollon, livin/melanoma-IAP (ML-IAP), NAIP, and ILP-2 [30]. Among the IAPs, XIAP exerts the most potent anti-apoptotic properties and blocks apoptosis by inhibiting caspases such as caspase-3, -7, and -9 [30]. Compared to other IAP family proteins, the function of survivin is more complex, since survivin is involved not only in the regulation of apoptosis, but also in the control of mitosis [31]. Survivin is of particular interest for pediatric oncology, since of particular relevance for neuroblastoma, since the *survivin* gene is mapped to chromosome 17q25, a region, which is frequently gained in advanced stages of neuroblastoma [32]. Several independent studies showed that expression of survivin in primary neuroblastoma significantly correlated with tumors of high-risk and poor prognosis [32–36]. In pediatric precursor B-cell ALL, overexpression of survivin was reported to identify patients with a high-risk of early relapse [5]. To this end, higher expression of survivin was detected in relapse patients than those with a favorable outcome [5]. Furthermore, analysis of survivin splice variants in childhood ALL revealed an association between lower expression of survivin-2B, an isoform of survivin with pro-apoptotic properties, and affiliation to the high-risk group [37].

Several approaches have been developed to antagonize aberrant expression of IAPs in cancers including childhood malignancies. One strategy is based on the endogenous IAP antagonists Smac, a mitochondrial protein that is released from mitochondria upon the induction of apoptosis [10]. Smac promotes apoptosis by neutralizing IAPs [10]. To mimic the endogenous Smac protein, Smac peptides or small molecule Smac mimetics were developed that bind to the surface groove of the BIR3 domain of XIAP, which normally interacts with endogenous Smac once it is released from mitochondria into the cytosol [38]. Smac peptides were shown to increase TRAIL- or chemotherapy-mediated apoptosis in neuroblastoma and glioblastoma cells and even substantially increased the anti-tumor activity of TRAIL in vivo in an orthotopic glioblastoma xenograft model [23]. Further, ectopic expression of Smac enhanced TRAIL- or  $\gamma$ -irradiation-induced killing in neuroblastoma [23,39]. In addition, Smac overexpression was shown to inhibit clonogenic growth of neuroblastoma by blocking random migration and proliferation and by enhancing apoptosis in a cell density-dependent manner [40]. This indicates that Smac mimetics may also be used for tumor control of minimal residual disease [40]. Moreover, XIAP antisense oligonucleotides showed potent anti-tumor activity as single agent and also in combination with

anticancer drugs [41,42]. XIAP antisense oligonucleotides are under evaluation in early phase clinical trials in adults with cancer [43]. Further, survivin inhibition by antisense oligonucleotides was reported to induce cell death of neuroblastoma cells through caspase-independent and -dependent pathways and to cooperate with TRAIL to trigger apoptosis [44–47].

### CONCLUSIONS

Intact cell death pathways are required for the response of cancers to current treatment approaches, since most anticancer therapies including chemo- or radiotherapy primarily act by inducing apoptosis in tumor cells. The elucidation of key apoptosis signaling components in pediatric cancers has in recent years not only provided novel insights into mechanisms of cytotoxic drug action, but has also identified various molecular targets that could be exploited for therapeutic purposes. Consequently, strategies that either trigger apoptosis directly in cancer cells or enhance apoptosis sensitivity in combination protocols have been developed in preclinical models. Some of these approaches have already been translated into early clinical trials. Thus, apoptosis-based therapies may provide novel opportunities for the treatment of childhood cancers.

### ACKNOWLEDGMENT

Work in the author’s laboratory is supported by grants from the Deutsche Forschungsgemeinschaft, the Deutsche Krebshilfe, the Bundesministerium für Forschung und Technologie, Wilhelm-Sander-Stiftung, Else-Kröner-Fresenius Stiftung, IAP6/18, and the European Community (ApoTrain, APO-SYS).

### REFERENCES

- Hengartner MO. The biochemistry of apoptosis. *Nature* 2000; 407:770–776.
- Evan GI, Vousden KH. Proliferation, cell cycle, and apoptosis in cancer. *Nature* 2001;411:342–348.
- Lowe SW, Lin AW. Apoptosis in cancer. *Carcinogenesis* 2000;21: 485–495.
- Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell* 2000; 100:57–70.
- Troeger A, Siepermann M, Escherich G, et al. Survivin and its prognostic significance in pediatric acute B-cell precursor lymphoblastic leukemia. *Haematologica* 2007;92:1043–1050.
- Degterev A, Boyce M, Yuan J. A decade of caspases. *Oncogene* 2003;22:8543–8567.
- Fulda S, Debatin KM. Extrinsic versus intrinsic apoptosis pathways in anticancer chemotherapy. *Oncogene* 2006;25:4798–4811.
- Walczak H, Krammer PH. The CD95 (APO-1/Fas) and the TRAIL (APO-2L) apoptosis systems. *Exp Cell Res* 2000;256:58–66.
- Adams JM, Cory S. The Bcl-2 apoptotic switch in cancer development and therapy. *Oncogene* 2007;26:1324–1337.
- Saelens X, Festjens N, Vande Walle L, et al. Toxic proteins released from mitochondria in cell death. *Oncogene* 2004;23:2861–2874.
- Okada H, Mak TW. Pathways of apoptotic and non-apoptotic death in tumour cells. *Nat Rev Cancer* 2004;4:592–603.
- Johnstone RW, Ruefli AA, Lowe SW. Apoptosis: A link between cancer genetics and chemotherapy. *Cell* 2002;108:153–164.
- Ashkenazi A. Targeting the extrinsic apoptosis pathway in cancer. *Cytokine Growth Factor Rev* 2008;19:325–331.
- Yang X, Thiele CJ. Targeting the tumor necrosis factor-related apoptosis-inducing ligand path in neuroblastoma. *Cancer Lett* 2003;197:137–143.

15. Fulda S, Meyer E, Debatin KM. Inhibition of TRAIL-induced apoptosis by Bcl-2 overexpression. *Oncogene* 2002;21:2283–2294.
16. Petak I, Douglas L, Tillman DM, et al. Pediatric rhabdomyosarcoma cell lines are resistant to Fas-induced apoptosis and highly sensitive to TRAIL-induced apoptosis. *Clin Cancer Res* 2000;6:4119–4127.
17. Ehrhardt H, Fulda S, Schmid I, et al. TRAIL induced survival and proliferation in cancer cells resistant towards TRAIL-induced apoptosis mediated by NF-kappaB. *Oncogene* 2003;22:3842–3852.
18. Van Valen F, Fulda S, Truckenbrod B, et al. Apoptotic responsiveness of the Ewing's sarcoma family of tumours to tumour necrosis factor-related apoptosis-inducing ligand (TRAIL). *Int J Cancer* 2000;88:252–259.
19. Kontny HU, Hammerle K, Klein R, et al. Sensitivity of Ewing's sarcoma to TRAIL-induced apoptosis. *Cell Death Differ* 2001;8:506–514.
20. Fulda S, Wick W, Weller M, et al. Targeting inhibitor of apoptosis proteins (IAPs) for cancer therapy. *Anticancer Agents Med Chem* 2008;8:533–539.
21. Ashkenazi A, Herbst RS. To kill a tumor cell: The potential of proapoptotic receptor agonists. *J Clin Invest* 2008;118:1979–1990.
22. Humphreys RC, Halpern W. Trail receptors: Targets for cancer therapy. *Adv Exp Med Biol* 2008;615:127–158.
23. Fulda S, Wick W, Weller M, et al. Smac agonists sensitize for Apo2L/TRAIL- or anticancer drug-induced apoptosis and induce regression of malignant glioma in vivo. *Nat Med* 2002;8:808–815.
24. Dole M, Nunez G, Merchant AK, et al. Bcl-2 inhibits chemotherapy-induced apoptosis in neuroblastoma. *Cancer Res* 1994;54:3253–3259.
25. Oltersdorf T, Elmore SW, Shoemaker AR, et al. An inhibitor of Bcl-2 family proteins induces regression of solid tumours. *Nature* 2005;435:677–681.
26. Kang MH, Kang YH, Szymanska B, et al. Activity of vincristine, L-ASP, and dexamethasone against acute lymphoblastic leukemia is enhanced by the BH3-mimetic ABT-737 in vitro and in vivo. *Blood* 2007;110:2057–2066.
27. Lock R, Carol H, Houghton PJ, et al. Initial testing (stage 1) of the BH3 mimetic ABT-263 by the pediatric preclinical testing program. *Pediatr Blood Cancer* 2008;50:1181–1189.
28. Goldsmith KC, Liu X, Dam V, et al. BH3 peptidomimetics potently activate apoptosis and demonstrate single agent efficacy in neuroblastoma. *Oncogene* 2006;25:4525–4533.
29. Rheingold SR, Hogarty MD, Blaney SM, et al. Phase I Trial of G3139, a Bcl-2 antisense oligonucleotide, combined with doxorubicin and cyclophosphamide in children with relapsed solid tumors: A children's oncology group study. *J Clin Oncol* 2007;25:1512–1518.
30. Salvesen GS, Duckett CS. IAP proteins: Blocking the road to death's door. *Nat Rev Mol Cell Biol* 2002;3:401–410.
31. Altieri DC. Validating survivin as a cancer therapeutic target. *Nat Rev Cancer* 2003;3:46–54.
32. Islam A, Kageyama H, Takada N, et al. High expression of Survivin, mapped to 17q25, is significantly associated with poor prognostic factors and promotes cell survival in human neuroblastoma. *Oncogene* 2000;19:617–623.
33. Adida C, Berrebi D, Peuchmaur M, et al. Anti-apoptosis gene, survivin, and prognosis of neuroblastoma. *Lancet* 1998;351:882–883.
34. Azuhata T, Scott D, Takamizawa S, et al. The inhibitor of apoptosis protein survivin is associated with high-risk behavior of neuroblastoma. *J Pediatr Surg* 2001;36:1785–1791.
35. Ito R, Asami S, Motohashi S, et al. Significance of survivin mRNA expression in prognosis of neuroblastoma. *Biol Pharm Bull* 2005;28:565–568.
36. Miller MA, Ohashi K, Zhu X, et al. Survivin mRNA levels are associated with biology of disease and patient survival in neuroblastoma: A report from the children's oncology group. *J Pediatr Hematol Oncol* 2006;28:412–417.
37. Troger A, Siepermann M, Mahotka C, et al. Role of survivin splice variants in pediatric acute precursor B lymphoblastic leukemia. *Klin Padiatr* 2007;219:127–133.
38. Shiozaki EN, Shi Y. Caspases, IAPs and Smac/DIABLO: Mechanisms from structural biology. *Trends Biochem Sci* 2004;29:486–494.
39. Giagkousiklidis S, Vogler M, Westhoff MA, et al. Sensitization for gamma-irradiation-induced apoptosis by second mitochondria-derived activator of caspase. *Cancer Res* 2005;65:10502–10513.
40. Vogler M, Giagkousiklidis S, Genze F, et al. Inhibition of clonogenic tumor growth: A novel function of Smac contributing to its antitumor activity. *Oncogene* 2005;24:7190–7202.
41. LaCasse EC, Kandimalla ER, Winocour P, et al. Application of XIAP antisense to cancer and other proliferative disorders: Development of AEG35156/GEM640. *Ann NY Acad Sci* 2005;1058:215–234.
42. LaCasse EC, Cherton-Horvat GG, Hewitt KE, et al. Preclinical characterization of AEG35156/GEM 640, a second-generation antisense oligonucleotide targeting X-linked inhibitor of apoptosis. *Clin Cancer Res* 2006;12:5231–5241.
43. Jolivet J, Dean E, Ward T, et al. A phase I trial of AEG35156 (XIAP antisense) administered as 2-hour intravenous infusions in patients with advanced tumours. *J Clin Oncol (Meeting Abstr)* 2008;26:3541.
44. Kim S, Kang J, Qiao J, et al. Phosphatidylinositol 3-kinase inhibition down-regulates survivin and facilitates TRAIL-mediated apoptosis in neuroblastomas. *J Pediatr Surg* 2004;39:516–5521.
45. Schultze K, Bock B, Eckert A, et al. Troglitazone sensitizes tumor cells to TRAIL-induced apoptosis via down-regulation of FLIP and Survivin. *Apoptosis* 2006;11:1503–1512.
46. Fulda S, Debatin KM. Sensitization for tumor necrosis factor-related apoptosis-inducing ligand-induced apoptosis by the chemopreventive agent resveratrol. *Cancer Res* 2004;64:337–346.
47. Shankar SL, Mani S, O'Guin KN, et al. Survivin inhibition induces human neural tumor cell death through caspase-independent and -dependent pathways. *J Neurochem* 2001;79:426–436.