Abstract: Apoptosis (programmed cell death) is a systematic and coordinated cellular process that occurs in physiological and pathophysiological conditions. Sidestepping or resisting apoptosis is a distinct characteristic of human cancers including childhood malignancies. This review dissects the apoptosis pathways implicated in pediatric tumors. Understanding these pathways not only unraveled key molecules that may serve as potential targets for drug discovery, but also molecular nodes that integrate with other signaling networks involved in processes such as development. This review presents current knowledge of the complex regulatory system that governs apoptosis with respect to other processes in pediatric cancers, so that fresh insights may be derived regarding treatment resistance or for more effective treatment options.

Keywords: cancer; apoptosis; pediatric; development

1. Introduction

Apoptosis, a genetically coded programmed cell death, is a natural physiologic process evolutionarily conserved from worm to man [1]. Apoptosis occurs during embryonic development and is critical for maintaining tissue homeostasis. It also acts as a defense mechanism to eliminate unwanted cells such as those in immune reactions or cells with potentially harmful mutations damaged by disease or noxious agents [2,3]. Dysregulation of apoptosis is an important aspect of cancer pathogenesis as it disrupts the delicate balance between cell proliferation and cell death, and has been widely recognized as a hallmark of cancer [4,5].

For decades, defects in apoptosis during development have been implicated in the formation and progression of cancers including childhood malignancies [6], as many of these embryonal neoplasms and developmental processes share similar biological mechanisms. As conventional chemotherapy regimens primarily exert their anti-tumor activity by triggering the cells’ intrinsic cell death programs [7], this review provides insights on the interaction of apoptosis with other signaling pathways during pediatric cancer development, and how the apoptotic cascade can be further exploited for more targeted therapies in the treatment of these cancers.

2. Apoptosis Signaling Pathways

Two major apoptosis pathways have been widely described: (1) The extrinsic apoptotic pathway, which involved signaling from cell surface death receptors and (2) the intrinsic apoptotic pathway, which involved mitochondria [8] (Figure 1).
2.1. The Extrinsic Death Receptor Pathway

The extrinsic apoptotic pathway is activated when death ligands, which are predominantly expressed on immune cells such as activated T lymphocytes, natural killer cells, and macrophages, bind to its death receptors (DRs) \([9–11]\). Several DRs of the tumor necrosis factor (TNF) receptor superfamily have been widely described and are ubiquitously expressed on the surface of cells. These include CD95 (Fas), TNFR1, DR4, DR5.
(Fas/APO-1), TNF receptor 1 (TNFR1), DR3 (APO-3), DR4 (TNF-related apoptosis-inducing ligand (TRAIL) receptor 1, TRAIL R1), DR5 (TRAIL R2), and DR6 [12]. These DRs contain an intracellular death domain, which is induced to recruit adaptor proteins such as Fas-associated death domain (FADD) and TNF receptor-associated death domain (TRADD) upon binding of the death ligand to its DR. A multi-protein complex, known as the death-inducing signaling complex (DISC), is subsequently formed to initiate the assembly and activation of caspase-8. Activated caspase-8 then cleaves a chain of downstream caspases to execute apoptosis. [13]. Caspase-8 also cleaves BID, which then triggers the release of cytochrome c from the mitochondria and activates subsequent intrinsic apoptotic signaling [14].

2.2. The Intrinsic Mitochondrial Pathway

The intrinsic apoptotic pathway is activated when internal stimuli, such as growth factor deprivation, hypoxia, DNA damage, severe oxidative stress, and \( \text{Ca}^{2+} \) overload, are triggered within the cell [15]. BAX and BAK from the pro-apoptotic BCL-2 family of proteins are activated and form pores in the outer mitochondria membrane to trigger mitochondrial outer membrane permeabilization (MOMP). As a result, apoptogenic factors including cytochrome c, second mitochondria-derived activator of caspase/direct inhibitor of apoptosis protein-binding protein with low PI (Smac/DIABLO), apoptosis-inducing factor (AIF), and Omi/HtrA2 are released into the cytoplasm [16–18]. Cytoplasmic cytochrome c then interacts with Apaf-1 and caspase-9 to form apoptosome, a multiprotein complex that catalyzes effector caspase-3 activation, resulting in apoptosis [19]. Cytoplasmic Smac/DIABLO and Omi/HtrA2, on the other hand, bind to inhibitor of apoptosis proteins (IAPs) to disrupt the interaction of IAPs with caspase-3 or -9, thus releasing the caspases for subsequent activation and downstream apoptosis [17,20].

3. Dysregulation of Apoptosis and Apoptosis-Targeted Therapies in Childhood Cancers

Cancer is the second leading cause of death in children aged <14 years despite the advances in treatment over the years to increase the overall five-year pediatric cancer survival rate to approximately 80% [21,22]. The most common cancers in children include leukemias (acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML)), brain and central nervous system (CNS) tumors, neuroblastoma, Wilms’ tumor, lymphomas (non-Hodgkin lymphomas (NHL)), rhabdomyosarcoma, and bone cancers (osteosarcoma and Ewing’s sarcoma) [23].

Often, in pediatric oncology, it is not uncommon that many of the defects arise in the developmental signaling pathways such as Wnt, Hedgehog, Notch, and Hippo, all of which regulate cell fate, proliferation, migration, differentiation, apoptosis, and formation of organs/tissues [24–27]. These developmental defects at the embryonal level resulted in the accumulation of undifferentiated cells and the failure to actively remove these cells via apoptosis often predispose one to pediatric cancer development. In this review, two underlying mechanisms—(1) defective death receptor signaling and (2) imbalanced ratio of pro-apoptotic to anti-apoptotic proteins, both of which renders impaired apoptosis or development of resistance to it—will be discussed. Figure 2 summarizes the underlying the mechanisms that allow cells to escape from apoptosis in pediatric cancers and the therapeutic targets, which can reverse these mechanisms to restore apoptosis.
3.1. Defective Death Receptor Signaling

Different abnormalities in the death signaling pathways have been reported to cause the evasion of the extrinsic apoptotic pathway. As described in the following, these include decreased expressions of death receptors and the ligands, as well as loss-of-function of death receptors, all of which perturbed the transmission of death signaling downstream and hence a reduced apoptosis [28–48].

CD95/Fas signaling, one of the most widely studied death receptor signaling, plays a significant role in immune surveillance and normal lymphocytes/melanocytes homeostasis [28]. In fact, impaired CD95 signaling has been implicated in many pediatric haemopoietic malignancies, including childhood acute leukemias (ALL and AML) and B-cell non-Hodgkin lymphomas (NHLs) [29–31]. Reduced expression of CD95 and its ligand CD95-L were also found to play a role in various pediatric cancers such as T-cell ALL, treatment-resistant leukemia, or neuroblastoma cells [32–34]. Improved prognosis and chemotherapeutic response were observed in B-cell lymphomas and AML with higher CD95 expression, respectively [35,36]. Several cytotoxic drugs such as doxorubicin, methotrexate, cytarabine, etoposide, and cisplatin are used in treatment of leukemias. These drugs are able to induce the expression of CD95 and CD95-L, which subsequently increase the cancer cells’ sensitivity to CD95 death signals or provoke the cells’ intrinsic apoptosis capability [37]. However, despite therapeutic strategies to restore death signaling in cancer cells, most primary T-cell leukemias have been reported to be constitutively resistant against CD95-induced apoptosis [38–40]. The resistance against CD95-induced apoptosis...
can be attributed to the occurrence of alternative splicing in the tumor, which produces soluble CD95 (sCD95). sCD95 function by sequestering CD95 ligand to cell surface CD95 receptor, thus impairing downstream death signaling and causing resistance against cytotoxic drug-induced, CD95-mediated apoptosis [41]. In separate reports, sCD95 was detected in several human T-cell leukemia cell lines and primary leukemic cells from infants with ALL [42,43]. Increased expression of sCD95 was also observed in patients with lymphoid and not myeloid leukemias [44]. Moreover, a splicing variant of CD95, which resulted in a truncated receptor without its intracytoplasmic death-signaling domain, also provided resistance to CD95-mediated apoptosis [45]. Altered expression as a result from polymorphisms and deleterious mutations in the CD95 promoter region were identified in childhood T-cell ALL, AML, and NHL [46–48].

Fortunately, with the discovery and understanding of other death signaling pathways, various therapeutic strategies have been developed to activate the extrinsic apoptotic pathway. In addition to CD95-L, there are other TNF family members (e.g., TNF-α and TRAIL), which can directly trigger apoptosis with their respective receptors (TNFR1, TRAIL R1, and TRAIL R2). TNF is a proinflammatory cytokine that activates and elicits the effects of NF-κB and caspases, thus rendering it ineffective to be used as a therapeutic target for cancer treatment [49]. In contrast to the liver toxicity caused by CD95 antibodies, recombinant TRAIL ligands and specific antibodies are well-tolerated in mouse tumor xenografts [50,51]. Despite the success of using recombinant TRAIL ligand to trigger apoptosis in several pediatric cancers including leukemia, neuroblastoma, rhabdomyosarcoma, and Ewing’s sarcoma [52–56], numerous cases of TRAIL-mediated resistance have also been reported. For instance, in neuroblastoma, downregulation of TRAIL receptors as a result of promoter hypermethylation was shown to decrease the response and sensitivity to TRAIL ligand, thus weakening the downstream apoptosis signaling [57,58]. Hypermethylation or genomic deletions in CASP8 gene were also identified in neuroblastoma. This resulted in the loss or reduced expression of caspase-8, an effector caspase, which mediates the downstream death signaling, and further rendering neuroblastoma resistant to TRAIL-induced apoptosis [59–61]. Together, these imply the complexity of exploiting the death receptor pathway. Different approaches were used combinatorically with TRAIL to improve the sensitivity and response towards TRAIL-induced apoptosis. For example, in childhood ALL, XIAP inhibitors act synergistically with TRAIL in vitro and in vivo to enhance downstream apoptotic signaling. In high BCL-2-expressing acute leukemia cells with resistance to TRAIL, XIAP inhibitors were able to induce cleavage of BCL-2 proteins and promote MOMP through conformational change of BAK, further enhancing TRAIL-induced apoptosis [62].

In addition to the use of recombinant TRAIL ligand, monoclonal antibodies against TRAIL receptors, TRAIL R1 or TRAIL R2, were developed and evaluated in preclinical and clinical settings [63,64]. For instance, Mapatumumab (HGS-ETR1), a human monoclonal antibody that specifically targets TRAIL-R1, was evaluated in vitro and in vivo by the pediatric preclinical testing program [65]. While limited activity of Mapatumumab was demonstrated against a panel of pediatric cancer cell lines in vitro (e.g., leukemia, medulloblastoma, glioblastoma, neuroblastoma, Wilms’ tumor, rhabdomyosarcoma, osteosarcoma, and Ewing’s sarcoma), significant event-free survival (EFS) was only observed in Mapatumumab-treated patient-derived xenografts of glioblastoma, neuroblastoma, and osteosarcoma origins [65]. Lexatumumab (HGS-ETR2), a human monoclonal antibody against TRAIL-R2, was also recently evaluated in a pediatric Phase I trial in children with solid tumors including Ewing’s sarcoma, osteosarcoma, neuroblastoma, and rhabdomyosarcoma [66].

3.2. Imbalanced Ratio of Pro-Apoptotic to Anti-Apoptotic Proteins

The intrinsic apoptotic pathway is directly regulated by two groups of proteins, the BCL-2 family and the IAPs, both of which have been widely described to exert pro- or anti-apoptotic activity in the cell [67]. Of note, the initiation of an apoptotic response was dictated by the ratio of pro- to anti-apoptotic proteins and not the absolute quantities [68]. The expression of these apoptotic proteins is also influenced by many other survival signaling pathways such as the p53, STAT, and PI3K,
which indirectly affect apoptosis [69–71]. Often, a disrupted balance as a result of reduced expression of pro-apoptotic proteins and increased expression of anti-apoptotic proteins contributes to tumorigenesis by apoptosis evasion in cancer cells. Therefore, it is an attractive option to target these proteins as a treatment for childhood cancers.

3.2.1. The BCL-2 Family of Proteins

The BCL-2 family of proteins plays a pivotal role in the regulation of apoptosis, especially in the intrinsic pathway as BCL-2 family are usually localized at or near the mitochondria to facilitate or inhibit the activation of MOMP and subsequent cell death [67]. The BCL-2 family members can be categorized according to their functions and the presence of either one or multiple BCL-2 homology [72] domains: (1) Anti-apoptotic proteins with four BH domains (e.g., BCL-2, BCL-XL, BCL-w, and MCL-1), (2) pro-apoptotic proteins with four BH domains (e.g., BAX and BAK), and (3) pro-apoptotic proteins with only BH3-domain (e.g., BID, BIM, BAD, BIK, NOXA, and PUMA) [18]. BH3-only proteins are the sensors for apoptosis, which are triggered in response to cellular stresses such as growth factor withdrawal, DNA damage, and Ca^{2+} overload. They then trigger the activation and oligomerization of multi-BH-domain, BAX and BAK, which promote MOMP and apoptosis [18].

The chromosomal analysis from comparative genomic hybridization (CGH) of the BCL-2 family members identified frequent deletions of pro-apoptotic BAK, BID, and BAD loci, and overexpression of anti-apoptotic BCL-2 and BCL-XL in several tumors including childhood cancers—glioblastoma and acute leukemias [68]. In AML patients, high expression of BCL-2 was positively correlated with low disease remission rate while low expression of BCL-2 was associated with a favorable karyotypic t(8;21) translocation [73,74]. High levels of BCL-2 proteins were further observed in separate studies of childhood ALL [75,76]. Furthermore, a high BCL-2/BAX ratio was predicted to have a poorer overall survival in AML patients and be associated with relapse in childhood ALL [77,78]. Overexpression of BCL-2 was also commonly identified in many childhood solid tumors such as osteosarcoma [79], glioblastoma multiforme [80], Wilms’ tumor [81], and neuroblastoma [82], highlighting a significant BCL-2 involvement in the development of these cancers. Moreover, BCL-2 overexpression is reported to be an important mechanism by which apoptosis and drug resistance is altered in retinoic acid (RA)-induced differentiation of neuroblastoma cells [83]. In clinical practice, 13-cisRA (isotretinoin) is a well-established component of maintenance treatment for high-risk neuroblastoma. BCL-2 protein levels varied during differentiation of neuroblastoma cells, with well-differentiated subtypes containing higher BCL-2 expression [84]. Hence, combinatorial strategies of inducing differentiation in poorly differentiated neuroblastoma cells with RA, followed by pharmacologically altering BCL-2 expression could be useful to sensitize neuroblastoma to apoptosis.

In addition to alterations of the genetic loci of the BCL-2 family proteins, other mutations of genes and/or signaling pathways that can indirectly influence the expression levels of these BCL-2 family proteins were also identified in childhood malignancies. For example, p53, one of the best-known tumor suppressors, is a transcription factor that induces the expression of pro-apoptotic BAX protein [71,85]. Therefore, defects in the p53 tumor suppressor gene will lead to downregulation of BAX, hence inhibiting apoptosis. Overexpressing p53 through adenoviral-mediated transduction in childhood sarcomas (rhabdomyosarcoma, osteosarcoma, and Ewing’s sarcoma) has been observed to sensitize cells to chemotherapeutics [86–88]. Other than BAX, another BH3-only protein BIM was also found to be induced by the activation of p38/JNK-MAPK signaling, thus triggering apoptosis [89]. Although no direct evidence exists to link MAPK pathway in the pathophysiology of pediatric cancers, its activation or inhibition has been shown to improve drug sensitivity in these cancers. For instance, glucocorticoids (GC), which are commonly used for treatment of leukemias or lymphomas, trigger apoptosis through increased BIM expression following the activation of p38 MAPK [90]. In contrast, the inhibition of ERK1/2 MAPK in osteosarcoma cells prevented the paradoxical effect of doxorubicin-induced expression of BCL-2 and BCL-XL, thereby improving the sensitivity of doxorubicin-killing in these cells [91]. PI3K pathway, a key regulator of normal cellular processes involved in cell survival and
growth, is another example of an indirect regulator of apoptosis. Through the phosphorylation by kinases in this pathway, the BCL-2 family of proteins such as BAD or BCL-2 can be inactivated or activated to inhibit or promote apoptosis, respectively [70]. Moreover, molecules such as substance P, which are often overexpressed in neuroblastoma and childhood leukemias, act upstream of and activate MAPK and PI3K pathways to inhibit apoptosis [92–95]. Inhibition of PI3K and its downstream signaling was observed to enhance apoptosis in Ewing’s sarcoma, while treatment of PI3K inhibitors in neuroblastoma cells was found to target anti-apoptotic BCL-2 family proteins and further promote mitochondrial apoptosis through chemosensitization, thus emphasizing the association between PI3K and BCL-2 family-mediated apoptosis [96,97]. Furthermore, activation of hedgehog signaling, which governs cell growth and differentiation in a wide variety of tissues during embryonic development, has been identified in several pediatric malignancies including rhabdomyosarcoma, medulloblastoma, neuroblastoma, and Wilms’ tumor [98–101]. It is worth noting that Gli1, a transactivator downstream of Hh signaling, was found to upregulate the expression of BCL-2 [102,103]. However, whether the activation of Hh signaling with increased BCL-2 expression contributed to these pediatric tumors remains to be investigated. Similarly, Notch signaling was also found to contribute to oncogenesis in children. Notch pathway has been reported to regulate numerous downstream targets, most of which with oncogenic roles such as PI3K, p53, NF-κB, BCL-2, and IAPs [26]. Therefore, activation of Notch signaling either through mutations or amplification of Notch such as those identified in T-cell ALL, medulloblastoma, osteosarcoma, and Ewing’s sarcoma, ultimately promotes cell survival and inhibits apoptosis [104–107]. MicroRNAs have also been found to regulate the levels of BCL-2 family proteins. BCL-2 protein can be targeted by several microRNAs such as miR-143, whereby an overexpression of miR-143 in osteosarcoma promotes apoptosis by downregulating BCL-2 [108]. In rhabdomyosarcoma, STAT3 was observed to be constitutively activated to induce the expression of several target genes including anti-apoptotic BCL-2, BCL-XL, and Survivin, while transcription factors such as PAX3 and PAX3/FKHR were shown to induce BCL-XL mRNA expression, thus highlighting the involvement of BCL-2 family proteins in rhabdomyosarcoma tumorigenesis [69,109]. Accordingly, treatment with STAT3 inhibitor, XZH-5, caused suppression of BCL-2, BCL-XL, and Survivin, and resulted in increased apoptosis in rhabdomyosarcoma cells [110]. Taken together, the complexity surrounding the regulation of apoptosis conferred either directly by BCL-2 family proteins and IAPs or indirectly by several survival pathways expands the range of therapeutics strategies available to trigger apoptosis in pediatric cancers.

Since high expression of anti-apoptotic BCL-2 family proteins has been widely reported and highly associated with resistance towards chemotherapy and TRAIL in various pediatric cancers such as leukemias, rhabdomyosarcoma, and neuroblastoma [111–115], several approaches have been proposed over the years to inhibit the function these proteins. For instance, ABT-737, a BH3 mimic that selectively targets BCL-2, BCL-XL, and BCL-w, inhibits the activity of these pro-apoptotic proteins and releases BAX and BAK from their interaction, thus allowing the formation of pores in the outer mitochondrial membrane and activating apoptosis [116]. As ABT-737 selectively targets certain anti-apoptotic proteins, a method called BH3 profiling was developed to detect and analyze cancer cells’ dependency on BCL-2 and to predict the sensitivity of ABT-737 in these cells. Using this method, ALL primary and commercial cell lines were predicted to be responsive to ABT-737 treatment [117]. Indeed, in two separate studies conducted in childhood ALL, ABT-737 was shown to be effective as a single agent against pediatric ALL xenografts as well as synergistically potentiate the cytotoxic effects of clinically available chemotherapy drugs (e.g., topotecan, dexamethasone, etoposide) in both ALL cell lines and xenografts [118,119]. Similarly, BH3 profiling was used to stratify neuroblastomas with a specific type of BCL-2 family-mediated resistance and thus facilitate in the prediction of neuroblastomas’ sensitivity towards different BCL-2 inhibitors. The release of cytochrome c was measured as an output of BH3 profiling after different BH3-domain peptides were added to stimulate the mitochondria extracted from neuroblastoma cell lines. Three different subsets of neuroblastomas with different BCL-2 family proteins’ dependency were identified: Those that were (1) high in MCL-1 expression...
(reacted with NOXA-BH3 peptides and are sensitive to killing by MCL-1 inhibitor AT-101 and not ABT-737), (2) high in both/either BCL-XL or BCL-w expression (reacted with BIK-BH3 peptides and are sensitive to killing by ABT-737), and (3) relapsed cases, which are insensitive to both BH3 peptides and inhibitors [72]. Consistently, in another study, ABT-737 showed single-agent activity against only BIM:BCL-2 and not BIM:MCL-1-primed neuroblastoma-derived xenografts [120]. ABT-737 has a low binding affinity towards MCL-1 and several resistance cases of ABT-737 as a result of high MCL-1 expression have been described not only in neuroblastoma, but also in other cancers [121,122]. The silencing of MCL-1 was shown to sensitize or prime cancer cells to cytotoxic chemotherapy and BCL-2 family antagonists in ALL, rhabdomyosarcoma, and neuroblastoma [123–126].

Collectively, these findings suggest that combinatorial targeting of different components of the mitochondrial apoptotic pathway may be useful to overcome resistance and improve treatment outcome. Intriguingly, despite the development of another BH3 mimetic Obatoclax (GX15-070), which can also target MCL-1 in addition to BCL-2 and BCL-XL, and with its efficacy tested in various adult cancers [127], its effectiveness in childhood cancers was, nevertheless, not well-studied. Furthermore, even though ABT-737 performs efficiently as a single agent or combinatorially with cytotoxic drugs against AML [128,129] and lymphoma [130] cell lines, it has a poor oral bioavailability, which limits its usage in clinical setting. Therefore, a derivative, ABT-263 (navitoclax), which is orally bioavailable and with similar activity, was developed and evaluated in a pediatric preclinical testing program. [131]. The sensitivity of ABT-263 was tested in the program across a panel of 23 pediatric cancer cell lines and 44 mice xenografts. Of note, ABT-263 was most potent against ALL cell lines and xenografts with complete remissions in 50% (3/6) of ALL mouse xenografts [132]. Consistent with the above results, a separate study demonstrated a potent anti-tumorigenic activity of ABT-263 against different subtypes of pediatric ALL [133]. Collectively, these findings accelerated the clinical development of ABT-263, which is currently evaluated in Phase I clinical trial in combination with ABT-199 (Venetoclax) for children with relapsed/refractory ALL or lymphoblastic lymphoma (ClinicalTrials.gov Identifier: NCT03181126). There are also other several BH3 mimetics (e.g., TW-37, 73R) that were developed over the years. However, limited studies were available to investigate their efficacy against pediatric cancers [134,135].

In addition to BCL-2 family inhibitors, RNAi or antisense therapies are alternative strategies to antagonize anti-apoptotic BCL-2 family proteins. Several antisense oligonucleotides have been reported, but only Genasense (G3139) has been evaluated in Phase I clinical trial in combination with doxorubicin and cyclophosphamide for children with relapsed solid tumors (ClinicalTrials.gov Identifier: NCT00039481) [136,137]. Taken together, these results discussed the multiple strategies to target BCL-2 family proteins of the intrinsic mitochondrial apoptotic pathway and present new windows and opportunities for future therapeutic options in childhood cancers.

3.2.2. The IAPs

The IAPs are a family of eight structurally and functionally similar proteins characterized by the presence of baculoviral IAP repeat (BIR) domain. The IAPs include NAIP (BIRC1), c-IAP1 (BIRC2), c-IAP2 (BIRC3), X-linked IAP (XIAP, BIRC4), Survivin (BIRC5), Apollon (BRUCE, BIRC6), Livin/ML-IAP (BIRC7), and IAP-like protein 2 (ILP-2, BIRC8) [138]. The IAPs function by interacting directly with the caspases via their conserved BIR domains, thus inhibiting caspase activity and triggering caspases degradation [139]. Moreover, these IAPs can directly interact with pro-apoptotic proteins that are involved in the apoptotic pathway. Hence, together these IAPs help to moderate the extent of apoptosis within the signaling cascade. Therefore, the increase in expression of IAPs as a result of direct genetic alterations in IAPs’ loci or the decrease in pro-apoptotic proteins to inhibit IAPs can impair apoptotic signaling in favor cell survival and contribute to cancer development.

XIAP, the most potent anti-apoptotic IAP, blocks apoptosis by inhibiting the activation of caspase-3, -7, and -9 and its dysregulation is commonly found in pediatric cancers [138]. For instance, in two independent studies, XIAP is overexpressed in childhood AML associated with poor prognosis.
The high XIAP level is correlated with immature blast phenotype and cytogenetic profiles of high-risk groups, reduced relapse-free survival, and poor treatment response to induction chemotherapy [140,141]. Similarly, high XIAP expression is found in childhood T-cell ALL and its expression is associated with poor prednisone treatment response [142]. As the association was seen correlated to the levels of XIAP protein and not its mRNA, this highlights the presence of possible post-transcriptional or post-translational regulation on XIAP [142]. Indeed, an internal ribosomal entry site (IRES) element was found residing in XIAP mRNA to promote internal translation initiation in spite of conditions that terminate protein synthesis [143]. An example of a likely outcome of this is the implication of XIAP in mediating resistance to cell death induction by TRAIL in various resistant leukemia cell lines [144].

Survivin, the smallest member of IAPs with only one BIR domain, plays a pivotal role in mitosis regulation in addition to apoptosis [145]. It is involved in cytokinesis during early embryogenesis and is strongly expressed ubiquitously throughout embryonic development (e.g., thymocytes and brain development) [146,147]. As Survivin expression is found mainly in embryonic tissues and not adult tissues, it is particularly relevant to pediatric oncology [148]. Moreover, Survivin is a target gene of Yap, a core transcriptional co-activator in Hippo signaling pathway that is highly involved in embryonic organ development [149]. Intriguingly, dysregulation of Hippo pathway with overexpression of Yap has been reported in several pediatric cancers whereby Survivin is also overexpressed, suggesting possible implication of Hippo-Survivin signaling in these cancers [150–153]. Overexpression of Survivin is commonly found in pediatric cancers and has a correlation with the progression of disease, chemoresistance, metastasis, unfavorable prognosis, and survival. Such findings have been found in independent studies on AML, ALL, neuroblastoma, Wilms’ tumor, osteosarcoma, rhabdomyosarcoma, and Ewing’s sarcoma [154–160]. For example, Survivin gene maps to chromosome 17q25, a region that is frequently amplified in neuroblastoma [156]. Several risk factors for neuroblastoma have been identified to be significantly correlated with high Survivin expression and these include poor prognosis, reduced expression of TrkA, later age of onset, and advanced cancer stage [161–164]. Furthermore, other than Survivin itself, the Survivin gene locus also encodes for multiple genetic splice variants, which have unique properties and functions. These splice variants can also be dysregulated and play a role in tumorigenesis [165]. For instance, in childhood de novo AML, an increased ratio of splice variants, Survivin-2B/∆Ex2, was found to be associated with poorer survival outcome and treatment-resistant cases [154]. Intriguingly, Survivin-2B, one of the Survivin splice variants, has a pro-apoptotic function despite the canonical anti-apoptotic function of Survivin. For instance, low expression of Survivin-2B was found in pediatric precursor B-cell ALL and was associated with increased risk of early relapse and high-risk group [166]. Consistently with its pro-apoptotic role, low expression of Survivin-2B was also observed in some clinically unfavorable neuroblastomas while it is expressed at higher levels in clinically favorable tumors [156]. While most findings suggest poor prognosis with high Survivin expression in cancers, a study conducted on osteosarcoma conversely showed that prolonged survival was significantly associated with Survivin that was found in the nucleus and not cytoplasm [167]. Therefore, taken together, these findings demonstrate not only the role of Survivin in cancers, but also the complexity of response to Survivin in terms of its expression, localization in cells, and splice variants, which can be exploited as a useful tool for risk stratification, prognosis, as well as treatment evaluation in childhood cancers.

In addition to XIAP and Survivin, other IAPs were also shown to be involved in pediatric malignancies. For instance, high Apollon expression found in childhood AML was associated with unfavorable three-year relapse-free survival and day-7-induction chemotherapy response [168]. Interestingly, this is the first study that reported prognostic relevance of Apollon expression in human malignancies. Similarly, in a more recent study, it was shown that Apollon overexpression found in AML and ALL pediatric patients was significantly associated with poor prognosis with shorter overall and disease-free survival [169]. Another IAP, Livin, was also found to be overexpressed in osteosarcoma and its nuclear expression was significantly correlated with decreased overall survival [79]. However, in two independent studies on pediatric ALL, a high level of Livin was shown to correlate with favorable
rather than unfavorable prognosis [170,171]. Although the exact mechanism was not identified, Livin has been described in another study to exhibit pro-apoptotic activity. It was reported that the truncated form of Livin, generated upon its cleavage by caspases, promotes apoptosis [172].

Moreover, there are also pro-apoptotic proteins involved in pediatric cancers through their interaction and disruption of IAPs’ activity. Examples include Smac and XAF1 (XIAP-associated factor 1). Smac is an endogenous pro-apoptotic protein of the mitochondria, which is released during intrinsic apoptotic signaling. It inhibits the activity of IAPs such as XIAP, c-IAP1, and c-IAP2 either through direct binding to IAPs, thus interfering IAPs-caspase interactions, or promotes proteasome-mediated degradation of IAPs, both of which induces apoptosis [173]. High expression of Smac has been reported to be significantly associated with two promising clinical outcomes in AML patients—improved overall survival and complete remission rate, whereas low Smac level correlated with poor karyotype [174]. Furthermore, Smac overexpression was used to sensitize osteosarcoma cells to cytotoxic drug-induced apoptosis [175]. XAF1, on the other hand, is a potent antagonist of anti-apoptotic XIAP and Survivin [176]. XAF1 was reported to be upregulated during nerve growth factor (NGF)-withdrawal pathway, an apoptotic pathway that occurs during the neural crest development of sympathetic nervous system [177]. During development, NGF is synthesized and required for maintaining the survival and differentiation of neuronal progenitors. Neuronal progenitors that are locally deprived of NGF, on the other hand, were shown to undergo intrinsic mitochondrial apoptosis. [178–181]. Failure of this process has been shown to be implicated in pediatric malignancies including neuroblastoma [182,183]. Loss of XAF1 expression was observed in neuroblastoma and was associated with poor survival and disease status [184].

With the identification of key apoptotic regulators in childhood cancers, different strategies to oppose the anti-apoptotic effects of IAPs have been developed to restore death signaling. For example, Smac mimetics and peptides were developed to target XIAP and c-IAPs. Smac mimetics interfere with the XIAP-dependent inhibition of caspases and promote the auto-ubiquitylation of c-IAP1 and c-IAP2, thus facilitating their degradation via proteasome. This in turn induces the NF-κB signaling and the production of TNFα, resulting in the activation of caspase-8 and downstream apoptosis [185,186]. Smac mimetics were reported to enhance TRAIL- or chemotherapy-mediated apoptosis in glioblastoma and neuroblastoma [187–189]. An overexpression of Smac has also been shown to enhance the sensitivity of neuroblastoma and glioblastoma cells to radiation treatment with increased mitochondrial perturbation and caspases activation [190]. Furthermore, an increased Smac expression was observed to inhibit the growth of neuroblastoma cells by promoting apoptosis and suppressing the rate of migration and proliferation [191]. In a more recent study, Smac mimic LCL161, was evaluated as a single agent in a pediatric preclinical testing program. While LCL161 was found to be potent against several pediatric leukemia and lymphoma cell lines in vitro, LCL161 induced significant differences in event-free survival distribution only in one-third of solid tumor xenografts, such as osteosarcoma and glioblastoma, but not ALL xenografts [192]. On the contrary, another Smac mimic BV6 was reported to be potent in vitro and in vivo as a single agent, as well as enhancing the efficacy of conventional induction chemotherapy including vincristine, dexamethasone, and asparaginase, leading to prolonged remission in pediatric ALL [193]. BV6 was also shown to cooperate with demethylating agents to induce cell death in ALL [194]. Together, these findings suggest that there is varying potency among different derivatives of Smac mimetic, further increasing the complexity of apoptosis-based therapies in childhood cancers.

In addition to Smac mimetic, XIAP can also be targeted using antisense oligonucleotides (ASO), which was demonstrated to be potent against several cancers either as a single agent or in combination with conventional chemotherapeutics [195,196]. XIAP ASO, AEG35156, was reported to suppress XIAP expression and induce apoptosis in several pediatric cancer cell lines including osteosarcoma, neuroblastoma, rhabdomyosarcoma, and Ewing’s sarcoma cells. AEG35156 also helps in sensitizing these cells to clinically relevant cytotoxic agents [197]. Moreover, the effect of AEG35156 has also been evaluated in early Phase I/II clinical trials in combination with chemotherapy in AML patients.
A better outcome in patients with refractory AML was observed as compared to single induction regimen [198]. Despite the findings in adult AML cases, it may be a promising approach as an apoptosis-based therapy in childhood leukemias, though not yet tested in the clinic in children. Of note, XIAP inhibitors were also shown to enhance TRAIL-, CD95-, or chemotherapy-induced apoptosis in childhood ALL in vitro and in vivo [62,199,200]. Similarly, in AML, these XIAP inhibitors were found to suppress cell clonogenicity, promote cell differentiation, and synergistically enhance TRAIL-mediated apoptosis [201,202].

In addition to Smac mimetics and XIAP inhibitors, inhibitors targeting Survivin have been widely described and tested in childhood cancers. For instance, osteosarcoma cells treated with small molecule inhibitor YM155 potently reduced the expression of Survivin, suppressed cell growth, and induced apoptosis [203]. Survivin ASO, on the other hand, was shown to act synergistically with TRAIL to trigger apoptosis in neuroblastoma and also promote caspase-dependent and -independent signaling, resulting in cell death [204–206]. Furthermore, a Survivin minigene DNA vaccine was developed and was tested to be effective in suppressing the spread and growth of tumor in a syngeneic mouse model of neuroblastoma [207]. Taken together, several strategies have been presented to target intrinsic mitochondrial apoptotic pathway in pediatric cancers either through direct antagonizing of anti-apoptotic IAPs or act synergistically with conventional chemotherapeutics.

4. Conclusions

Understanding the apoptotic processes over the years has led to the advancement of new therapeutic strategies in the treatment for different cancers. Various approaches in attempting to induce apoptosis in pediatric cancers have been developed—cancers that evolved mainly during the early developmental stages due to the impairment of apoptosis for physiological culling during cell maturation and differentiation. Molecular targeted therapies that perturb developmental pathways dysregulated in the tumor may have devastating effects on normal tissues in a developmental stage and tissue-specific manner. Therefore, therapies centered around apoptosis remain as one of the most promising strategies for treating childhood malignancies. Future studies need to emphasize the identification of predictive biomarkers and rational design of combination therapies in cancer to better stratify patients who will achieve best clinical outcomes from these therapies.

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