Apoptotic Cell Death: Important Cellular Process as Chemotherapeutic Target

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Abstract

Apoptosis is a biological feature, which causes programmed cell death. It consists of two pathways, namely extrinsic and intrinsic, and mitochondria are the site of apoptotic process completion. An abnormality in the apoptotic process can make cells immoral, which is one of the major characteristics of cancer cell formation and cancer development. Chemotherapeutic molecules, which have been used as anticancer drugs, or drugs under investigations, have mostly designed in a way that they can revert apoptotic abnormalities or induce apoptosis. This book chapter discusses the apoptotic process and its abnormalities in cancer cells, and how chemotherapeutic drugs can induce apoptosis, with most advanced and updated findings on mechanisms of action.

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Keywords

Apoptosis · Intrinsic pathways · Cytochrome C · Extrinsic pathways · Death ligand · Caspase

4.1 Introduction

To grow and eliminate unnecessary or toxic materials, our body takes the support of apoptosis. Apoptosis is a type of cell death known as biological programmed cell death (PCD) in a controlled manner. The principal of apoptosis was first introduced in 1842 by Karl Vogt, a German scientist, which was later explained in detail by Walther Flemming in 1885 [1]. The number of cells is controlled by the contribution of both cell division and cell death. Intracellular cell death is activated when particular cells become useless. This technique is, therefore, referred to as programmed cell death, even though it is more commonly known as "apoptosis", a Greek word meaning "falling off." Billions of cells die in the bone marrow and intestine every hour in a healthy adult human [2]. Development of mouse paws, tadpole to frog, finger and toe formation of the fetus are all about apoptosis. If this were not so, the tissue would go through excess expansion and shrinkage, affected by antigen or limitless cell proliferation. Mainly there are two pathways in apoptosis: extrinsic pathway (via death receptor) is activated by extracellular pro-apoptotic stimuli; intrinsic pathway (mitochondrial) is initiated following mechanisms ingrained to the cell by itself. Stimulation of the caspases is the result of apoptotic pathways, which is crucial for this process [3]. The caspases change from inactive zymogen to active component during apoptosis [4].

Genome integrity and cellular homeostasis are processed through a complex system that proceeds following DNA damage, stimulating checkpoints of cell cycle and promoting DNA repair, or removing injured cells from the proliferation. Moreover, cell death regulates cell proliferation, such as the number of nerve cells to match the number of target cells entailed for innervations. Basically cell death controls cell division. So any stunt in the pathway can lead to heart failures, neurodegenerative diseases, immune-deficiencies, and more to say cancer, that is, uncontrolled cell proliferation [5, 6]. Accelerating apoptosis approach has been a novel way in the history of cancer treatment by the fact that abnormal cell death has seen to be the mainstay of tumor growth and anticancer drug resistance. The most effective anticancer drugs thus might target apoptosis pathway.

4.2 Basic Mechanism of Apoptosis

Approximately 50 to 70 billion cells go through apoptosis in adult people per day [7]. PCD, or more specifically, apoptosis, is a unique strategy for protecting a host from every possible pathogen. The apoptosis process is characterized by the accumulation of nuclear chromatin, condensation of cytoplasm, DNA damaging, formation of blebs, and dissolution of cell into small apoptotic bodies consumed by lysosomes of surrounding cells [8]. This PCD is stimulated by active caspase (cysteine-aspartic acid-specific proteases) protein, following intrinsic or extrinsic route. Extrinsic pathway worked by activating cell surface death receptor, while intrinsic pathway took place in mitochondria impairing the cytoskeletal protein and nuclear proteins which are crucial for cell surveillance [9]. Generally, the caspases remain as inactive zymogen form which develop into their active heterotetrameric forms in a consecutive proteolytic apoptotic stimulation process.

Mitochondrial proteins are involved in intrinsic pathways of apoptosis (Fig. 4.1). Cells with damaged DNA and/or overexpressed oncogenes influence this pathway. The overall pathway is governed by the B-cell lymphoma 2 (Bcl-2) family proteins [9]. The upregulation of Bcl-2 Homology 3 (BH3)-only proteins activates both Bcl-2 Associated X (BAX) and Bcl-2 antagonist/killer (BAK) [10]. BAX is regulated by tumor suppressor p53 [11]. BAK and BAX oligomerization results in forming mitochondrial outer membrane permeabilization (MOMP) after activation. MOMP is the significant event of intrinsic apoptosis and is taken as the point of no return

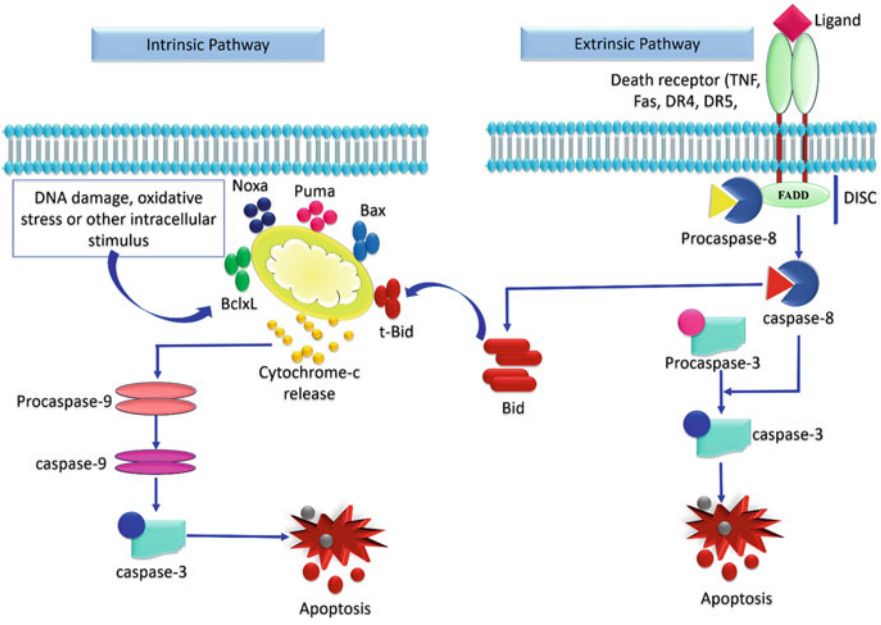

Fig. 4.1 Basic mechanism of apoptosis

[12]. Eventually upon the release of intermembrane protein cytochrome c, apoptosome forms, and apoptotic protease-activating factor-1 (APAF-1), deoxyadenosine triphosphate (dATP) activate procaspase-9 [13]. After that procaspase-9 is activated into caspase-9 that activates killer protein caspases-3 and -7 [14]. The executioner caspases immediately start to cleave proteins that leads to cell death. Additionally p53 has been demonstrated as crucial for the induction of apoptosis enabling activation of cell cycle checkpoints and DNA damage surveillance and p21 has appeared as down-regulator of p53, resulted in controlling apoptosis and cell cycle progression [6].

The extrinsic or death receptor pathway is mediated by death receptors (DR) activated by ligand binding (Fig. 4.1). DRs belong to tumor necrosis factor (TNF) receptor super family. Some death ligands possess TNF, TNF-related apoptosis-inducing ligand (TRAIL), and Fas ligand (Fas-L) [15]. The perforin/granzyme pathway is also involved in apoptosis, but mostly unclear. In this pathway, apoptosis is programmed via any of granzyme A or B. All these three apoptotic pathways coincide in the same terminal cellular pathway [15]. After ligand binding to receptor, intracellular death domain of DRs binds with some specific protein motifs like Fas-associated death domain (FADD) and TNF receptor-associated death domain (TRADD). These certain proteins are connected with other protein interaction domain, named death effector domain (DED). Pro-caspase-8 also has DED that is stimulated upon interaction with the DED of FADD [16]. At this phase, a death inducing signaling complex (DISC) is formed. This signal triggers auto-catalytic activation of procaspase-8 [17]. The active caspase-8 then activates effector caspases, which performs the execution of destruction. Moreover, there are other pathways of caspase activation too, including a principle role of caspase-2 or caspase-12 in apoptosis activation by endoplasmic reticulum (ER) stress [18]. Several of the inhibitor of apoptotic protein (IAP) family members have also been found to take part in pathological conditions, particularly neurodegenerative disorders and cancer by upsurging proliferation protein [19].

4.3 Apoptosis Dysregulation in Cancer Cells

Abnormal apoptosis has been found to be associated with human diseases whereas extreme apoptosis causes degenerative disorders, and inadequate apoptosis results in neoplastic diseases. Cancer involves the anomalous growth of cells due to the loss of balance between apoptosis and proliferation. The ratio of pro-apoptotic and anti-apoptotic proteins plays an important role in apoptosis regulation. In this respect, cancer cells evade apoptosis by deactivating the machinery of cell death through different mechanisms such as overexpression of Bcl-2 family proteins or inhibition of pro-apoptotic Bcl-2 proteins, thus acquisition of a higher survival benefit. Moreover, another well-known mechanism of cancer cell survival is tumor suppressor p53 inactivation [20]. Usually, cancer cells evade this apoptosis by following mechanisms (1) disruption of pro-apoptotic and anti-apoptotic protein balance

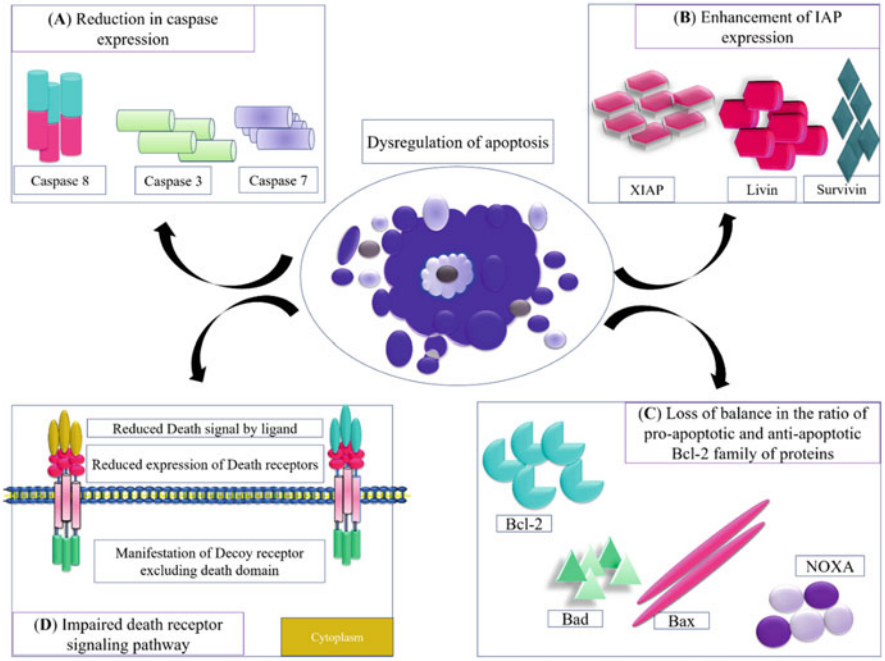

Fig. 4.2 Deregulation of apoptosis due to (**a**) Reduction in caspase activation; (**b**) Enhancement of IAP expression; (**c**) Imbalance in pro- and anti-apoptotic Bcl-2 ratio; (**d**) Impairment in death receptor signaling pathway mediated by reduced death signal, reduced death receptor expression, and decoy receptor expression without death domain

(2) Enhancement of IAP expression (3) inhibition of function of caspases, and (4) compromised signaling in DRs (Fig. 4.2).

Disruption of Pro-apoptotic and Anti-apoptotic Protein Balance

The Bcl-2 family of proteins are anti-apoptotic and pro-apoptotic, and they are involved in apoptosis regulation, particularly through the intrinsic pathway of caspase activation as they exist in upstream of cellular damage (irreversible) and function primarily in mitochondria. Based on the function and Bcl-2 homology (BH) domains, Bcl-2 family proteins are of three groups. (1) The anti-apoptotic proteins Bcl-2, Bcl-xtra large (Bcl-xL), myeloid cell leukemia 1 (Mcl-1), Bcl-w, A1/Bfl-1, and Bcl-B/Bcl-2-like protein 10 (Bcl-B/Bcl2L10) that comprise all of the four BH domains, and they defend cells from apoptotic signals. (2) The second group involves BH-3 proteins including Bcl-2 associated agonist of cell death (Bad), Bcl-2-modifying factor (Bmf), BH3 interacting domain death agonist (Bid), Noxa, Bcl-2-like protein 11 (Bim), BCL2 interacting killer (Bik), p53 upregulated modulator of apoptosis (Puma) and Harakiri, Bcl-2 interacting protein (Hrk).

These pro-apoptotic proteins being the initiator of apoptosis, become activated in response to deprivation of growth factors, DNA damage, and ER stress (3) A third group protein members including Bak, Bax, and Bcl-2 related ovarian killer/Mtd (Bok/Mtd) that contain all four BH domains, and they are pro-apoptotic too [21]. If there is an imbalance in the balance between pro-apoptotic and anti-apoptotic Bcl-2 family of proteins, the outcome is dysregulation in apoptosis process in the damaged cells.

Enhancement of IAP Expression

Apoptosis inhibitor c-IAP1 (BIRC2), NAIP (BIRC1), X-linked inhibitor of apoptosis protein (XIAP, BIRC4), IAP-like protein 2 (BIRC3), c-IAP2 (BIRC8), Apollon (BRUCE, BIRC6), Survivin (BIRC5), and Livin/MLIAP (BIRC7) are a group of functionally and structurally similar proteins, which regulate signal transduction, cytokinesis, and apoptosis. These inhibitors contain a characteristic baculovirus IAP repeat (BIR) protein domain and reduce the activity of caspase via binding BIR domain to caspase active site. IAPs promote degradation of active caspases by this mechanism or by keeping away the caspases from their target, thereby inhibit apoptosis [22].

Reduced Caspase Activity

The cellular machinery that mediates apoptosis includes a cysteine proteases family termed caspases. Therefore, it is rational to consider that Mammalian caspases are divided into 3 clusters functionally: initiator (caspase 2, 8, 9, and 10), executioner (caspase 3, 6, and 7), and inflammatory (caspase 1, 4, 5, 11, and 12) [23]. The binding of a death ligand to a DR initiates the extrinsic pathway of apoptosis, which then recruits, dimerizes, and activates the caspase-8 via TRADD/FADD adapter proteins. Activated caspase-8 later either stimulates apoptosis by cleaving directly and in that way activates the executioner caspases (3, 6, and 7), or stimulates intrinsic pathway of apoptosis via BID cleavage to persuade effective cell death. The mitochondrial or intrinsic or apoptosis pathway can be initiated through different cellular stresses that trigger to the freeing of cytochrome c from mitochondria, and apoptosome formation, consisted of apoptotic protease-activating factor 1 (APAF1), caspase-9, and cytochrome c, consequently activate caspase-9. Later the activated caspase-9 stimulates apoptosis by cleaving and activating executioner caspases [24]. Caspases become one of the key proteins in apoptosis initiation and execution. That is why, a reduced level of caspases or dysfunction of caspases is linked to decrease of apoptosis or cancer progression.

Impaired Death Receptor Signaling

DRs and DR-associated ligands are essential elements in extrinsic apoptotic pathway. DRs which are involved in this pathway are TNFR1 (also called DR 1), Fas (also known as APO-1 or DR2 or CD95), DR3 (also known as APO-3), DR4 (also known as TRAIL-1 or APO-2), DR5 (also known as TRAIL-2), DR 6, nerve growth factor receptor (NGFR) and ectodysplasin A receptor (EDAR). These receptors contain a death domain and triggered by death signaling, death domain attracted by numerous molecules that result in signaling cascade activation. But, when death ligands bind to decoy DRs excluding a death domain, it fails to generate signaling complexes, consequently fail to initiate signaling cascade. Different anomalies in this pathway, leading to avoidance of extrinsic apoptotic pathway have been characterized, for example, receptor downregulation or destruction of its function, as well as a reduction in death signal levels, which play role in the impairment of signaling and henceforth reduce apoptosis [25].

4.4 Chemotherapeutic Drugs and Apoptosis

Researchers developed numerous chemotherapeutics by targeting the intrinsic and extrinsic pathway regulating proteins of apoptosis. Fas and TRAIL induce the extrinsic pathway, and caspase 9 activation by MOMP and blocking of XIAP by second mitochondrial-derived activator of caspase/direct inhibitor of apoptosis protein binding protein with a low isoelectric point (SMAC/DIABLO) play role in the initiation of intrinsic apoptotic pathway [4].

Chemotherapeutics Targeting the Extrinsic Apoptotic Pathway

Pro-apoptotic Receptor Agonists (PARAs)

Activation of TRAIL stimulates apoptosis in cancer cells via TRAIL-R1 and TRAIL-R2 DRs. It is pre-clinically evident that agonistic antibodies against TRAIL-Rs induce apoptosis in different cancer types without affecting normal tissues, that made it an appropriate approach in targeting cancer [4].

Pan Recombinant Human TRAIL (rh-TRAIL) Antibodies: Dulanermin

Both of TRAIL-R1 and TRAIL-R2 are targeted by rh-TRAIL. In cancer cells, Dulanermin selectively induces apoptosis by activating caspase and leading to consequential cell death [26]. A number of studies reported its apoptotic function as a single chemotherapeutic agent or in combination with other agents in hematological cancer and solid tumor [4].

TRAIL-R1 Agonistic Monoclonal Antibodies: Mapatumumab

Mapatumumab, a human immunoglobulin G1 lambda (IgG1λ) targets TRAIL-R1. A number of studies (mainly pre-clinical) revealed that mapatumumab inhibits tumor

progression in mice indicating established human tumor xenografts expressing TRAIL-R1. Mapatumumab is competent to improve the anticancer potential of cytotoxic compounds in numerous cancer cell lines as a single agent, with those resilient to chemotherapy [27]. Its activity also evaluated in combination with other chemotherapeutics by many studies. A phase I clinical trial investigated mapatumumab activity with paclitaxel and carboplatin in advanced solid tumor patients, where 44% of patients acquired stable disease (SD) [28]. Again, mapatumumab was used in combination with gemcitabine and cisplatin, and 25 gained SD with an average length of 6 months [29]. Another study combined mapatumumab and sorafenib in patients with progressive hepatocellular carcinoma (HCC), and reported a PR in 2 patients out of 19, with 4 SD patients [30].

TRAIL-R2 Agonistic Monoclonal Antibodies

Lexatumumab Lexatumumab is a fully recombinant human IgG1λ mAb, which efficiently binds with and triggers TRAIL-R2. Its activity against ovarian, breast, renal, colorectal cancer (CRC), and hematological cells and animal model by activating caspase 8 and caspase 9 is well-evident [31].

Conatumumab Conatumumab (AMG 655), another mAb found to stimulate the caspases in human cancers by targeting specifically TRAIL-R2 [32]. Though there is no data of overall survival (OS) or progression free survival (PFS) advantage with doxorubicin in refractory soft tissue sarcoma or carboplatin and paclitaxel in non-small-cell lung carcinoma (NSCLC) [33, 34], in combination with gemcitabine in randomized phase II study resulted in a non-significant upgrading [35].

Other Agonistic TRAIL-R2 Antibodies: Tigatuzumab, Drozitumab, and LBY135

Tigatuzumab, drozitumab, and LBY135 are agonist antibodies to TRAIL-R2, which have been tested in phase I/II trials. During the study, minor responses were found for drozitumab in 3 patients suffered from CRC, chondrosarcoma, and granulosa cell tumor, whereas 14 patients out of 41 got SD [36]. In case of tigatuzumab phase I trial, 7 patients out of 17 got SD [37]. LBY135 testing reports revealed that clinical activity was restricted to SD, when used as single agent, though 2 PRs (CRC, breast) were attained in combination with capecitabine [38].

Chemotherapeutics Targeting the Intrinsic Apoptotic Pathway

Bcl-2 Inhibitors

Anti-apoptotic Bcl-2 proteins, named Bcl-XL, Bcl-2, Mcl-1, and Bcl-w are overexpressed in different cancers, including hematological malignancies, small-cell lung cancer (SCLC) and B-cell lymphoma [39]. Inhibitors are of different types as follows:

Antisense Oligonucleotides as Bcl-2 Inhibitors: Oblimersen Sodium The 18-antisense oligonucleotide "oblimersen sodium" (Genasense, G3139) targets Bcl-2 m RNA of intrinsic pathway. G3139 exerts pro-apoptotic effects by increasing Bax, discharging cytochrome c from mitochondria to stimulate caspases, and eventually releasing Smac/DIABLO to suppress IAPs, which causes caspase 3 and 9 activation, triggering the initiation of apoptosis [40]. Also, Bcl-2 downregulated by oblimersen in the non-apoptotic pathway where stimulation by Bcl-2 caused the release of Beclin-1 to mediate cell death by autophagy [41]. Furthermore, oblimersen has been found to boost tumor immunity via triggering polyclonal antibody production, and stimulating dendritic cell maturation [42].

Small Molecule Downregulating Bcl-2 Gene or Protein Expression Several small molecules are established for regulating upstream factors of anti-apoptotic Bcl-2 proteins that caused their reduced expression [43]. Sodium butyrate (NaB), Depsipeptide and Vorinostat are the inhibitors of class-I histone deacetylase (HDAC), which expression is positively correlated with Bcl-2 expression. Inhibition of HDAC1 causes the downregulation of the Bcl-2, Bcl-XL, and Mcl-1 in multiple myeloma (MM) and mesothelioma cells [44].

Synthetic Retinoid Synthetic retinoids were documented to decline the expression of Mcl-1 through phosphorylating the c-Jun kinase (JNK) in malignant cells without affecting non-cancerous cells [45]. The upregulation of Mcl-1 is generally linked with several antitumor drugs resistance, so Mcl-1reduced expression should augment cytotoxicity of the cancer cell targeting drugs.

BH3 Mimetics Targeting BH3 Domain of Bcl-2

BH3 mimetics small molecules can target BH3 domain of Bcl-2. These BH3 mimetics make interaction with anti-apoptotic Bcl-2 proteins via binding to their BH-3 binding groove. Some of the BH3 mimetics are discussed below:

Gossypol Gossypol (AT-101, Ascenta) isolated from cotton seeds and roots. This BH3 mimetic natural polyphenolic compound suppressed Bcl-2 by disrupting the Bcl-2 and pro-apoptotic protein hetero dimerization [46]. Levo gossypol with higher affinity binds with hydrophobic groove of Bcl-2, Bcl-XL, and Mcl-1 and mediates apoptosis more competently compared to dextro gossypol [47]. It can also bind to Bak directly, consequently form oligomer by activating the Bak [48]. Moreover, levo gossypol also upsurges the sensitivity of chemotherapy and radiation therapy via activating the signaling pathway of stress-activated protein kinases (SAPK/JNK) that is regulating mitochondrial pro- and anti-apoptotic proteins [47]. Subsequently, levo gossypol is verified in a clinical trial in combination with other chemotherapeutic agents, such as with docetaxel in hormone refractory prostate cancer and with rituximab in treating chronic lymphocytic leukemia (CLL) [43].

Obatoclax Obatoclax (also identified as GX15-070) is an indole bi pyrrole small molecule that can inhibit Bcl-2. It prevents BAK to bind with MCL-1 and upregulates BIM expression [49].

ABT-263 (Navitoclax) and ABT-737 (A-779024) ABT-737 (A-779024) mimics BH3 domain of BAD protein and specifically binds with higher affinity to Bcl-XL, Bcl-2, and Bcl-w, but not to Bcl-B, Mcl-1, and A1 proteins [50]. ABT-263 (navitoclax) shows parallel anti-Bcl-2 activity with its antecedent, and reveals higher affinity for Bcl-2, Bcl-w, and Bcl-XL, but not for protein A1 or Mcl-1 [51]. ABT-737 displays strong antitumor activity as single agent in vitro against small-cell carcinoma cells and lymphoma, and similarly in mouse xenograft models with elevated upregulation of Bcl-XL or Bcl-2 [52]. Phase I and II clinical trials disclosed that both ABT-737 and ABT-263 were efficient in SCLC and CLL. Besides their activity as single agent, ABT-737 and ABT-263 have noteworthy effects in triggering apoptosis as combination therapy with other anticancer drugs. ABT-263 has been found to increase the effectiveness of chemotherapy and radiation therapy for CLL, SCLC, follicular lymphoma, and so on [51], while ABT-737 prompts sensitization of cancer cells to arsenic trioxide, flavopiridol, or fenretinide [53]. Further studies exhibited that ABT-263 promotes sensitization of many solid tumors to conventional agents, such as cyclophosphamide, fludarabine, and rituximab [51, 54]. Nevertheless, both ABT-263 and ABT-737 can decrease platelet for pointing Bcl-xl, which is essential in upholding the life expectancy of circulating platelet, demanding the improvement of Bcl-2 inhibitors selectively [52]. Several other BH3-mimetic compounds developed, that shares similar features like ABT-263 and ABT-737's inhibiting Bcl-xl and Bcl-2; these compounds include S44563, BM-1198, AZD4320, and Bcl2-32 [3].

ABT-199 (Venetoclax) ABT-199 (GDC-0199) showed its inhibitory effect against Non-Hodgkin's lymphoma (NHL) cell lines, comprising those resultant from follicular lymphoma (FL), diffuse large B-cell lymphoma (DLBCL), or mantle cell lymphoma (MCL), along with its activity in clinical trials against acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) cell lines [55]. Due to its specific inhibitory function to Bcl-2, it was approved to treat CLL by FDA in 2015 [56]. ABT-199 was designed to circumvent the nonselective interaction of ABT-263 with Bcl-xl inducing the antagonistic effect of thrombocytopenia [57]. Research studies also exposed that ABT-199 had a substantial sensitizing role in combination therapy with other anticancer drugs, like obinutuzumab, rituximab, in AML and CLL patients [52, 58, 59].

S55746 (Bcl201, Servier-1) This orally available chemotherapeutic agent showed effective killing of cancer cells overexpressing Bcl-2 in vitro and in vivo, and it was tested in refractory CLL patients in a phase I trial. S55746 also tested as a sensitizing agent in combination with phosphoinositide 3-kinase delta (PI3Kδ) inhibitor in follicular lymphoma (FL) and mantle cell lymphoma [52].

Selective Inhibitors Targeting Bcl-XL Agent (A-1155463, A-1331852, and WEHI-539) These therapeutic agents mimic BH3-only proteins and do not bind to Bcl-2, instead they bind strongly at p4 and p2 hotspots of Bcl-XL [60]. In colorectal cancer, Bcl-XL plays vital role, and study showed that these inhibitors are efficient against solid tumors. WEHI-539 was developed based on A-1155463 or A-1331852 and possesses the greatest selectivity for Bcl-XL, signifying its promising role as a single agent for some solid tumors [61].

Selective Anti-Mcl-1 Agents (UMI-177, A-1210477, and AMG176) UMI-77 precludes Mcl-1from binding with Bak and Bax, which stimulate apoptosis for many tumor cells. Though, UMI-77 had a rational selectivity for Mcl-1, demanding additional optimization. Consequently, A-1210477 was created and revealed high selectivity and binding affinity for p3 and p4 hotspots of Mcl-1. Through a sub nanomolar affinity, A-1210477 can be employed as a single agent and could also combine with ABT-263 to kill more cell lines [62].

AMG176, the recognized Mcl-1 inhibitor, also tested for clinical acceptability, antitumor response, pharmacokinetics in combination therapy for refractory multiple myeloma, Burkitt Lymphoma (BL), and AML where it induces apoptosis by altering the expression of anti-apoptotic and pro-apoptotic Bcl-2 proteins [52, 63, 64].

Maritoclax Maritoclax (also called marinopyrrole A) was isolated from marine-dwelling *Streptomyces* species that can directly target MCL-1, and marks it for proteasomal degradation; thus effectively mediating apoptosis. Also it can stimulate apoptosis in MM cell lines through interfering with MCL-1 [3].

ML311/EU-5346, S63845, S64315 (MIK665) ML311/EU-5346 has optimal strength for MCL-1 suppression in MCL-1 dependent cell lines. A threefold to fourfold lower efficacy for Bcl-2 inhibition and negligible effect on BCL-XL inhibition [3]. S63845 revealed effectiveness against MCL-1 reliant cell lines equally in vitro and in vivo, which were resilient to both venetoclax and navitoclax, as like A1210477, but S63845's effectiveness against MCL-1-reliant cell lines was above 1000 times superior. S64315 (MIK665) was derived from S63845, and is currently employing patients for two phase I studies: in myelodysplastic syndrome and refractory/relapsed AML (clinical trial ref.#NCT02979366), and another in patients with lymphoma or relapsed/refractory MM (clinical trial ref.# NCT02992483) [3].

AZD5991 AZD5991 is comparatively newly described. It is macrocyclic structurally and lucidly designed compound demonstrating higher selectivity for MCL-1. It binds directly to MCL-1, promptly enabling the detachment of BAK from the BAK/MCL-1 [3].

Targeting Inhibitors of Apoptosis (IAPs) by SMCS

Smac-Mimetic Compounds (SMCS) [SH-130, JP1201, Compound A (CA), AT-406, LCL-161, GDC-0152, Birinapant, HGS-1029, BV6 XIAP]

A Smac-mimetic SH-130 compound, as a radio sensitizer has revealed activity in prostate cancer cells. JP1201 was found effective against pancreatic cancer model. An unique and smac-mimetic molecule, "compound A" (CA), was found synergistically effective with TRAIL in primary CLL cells as an inhibitor of XIAP to promote effective apoptosis [4].

AT-406, another inhibitor of cellular inhibitor of apoptosis protein 1 (cIAP1), cIAP2, XIAP play inhibitory role towards solid tumors. It is also utilized synergistically with Carboplatin, cisplatin, Bcl-2, paclitaxel, radiation therapy, TRAIL, and BRAF inhibitors [65]. LCL-161 destroys cIAP1 and cIAP2 and has potential action against solid tumors, multiple myelofibrosis, esophageal squamous cell carcinoma, and NSCLC. It is used in combination with TNF-α/TRAIL, paclitaxel, and radiation therapy [65, 66]. GDC-0152 is an inhibitor of cIAP1, cIAP2, XIAP and ML-lAP, and it has been used against breast cancer and glioblastoma [65, 67].

Birinapant was found to degrade cIAP1 and cIAP2 in solid tumors and melanoma. It is used in combination therapy by combining with Carboplatin, TRAIL, TNF-α [65, 68]. HGS-1029 causes XIAP inhibition, and loss of cIAP expression [69] in colon cancer and adenocarcinoma [65]. BV6 XIAP, degrade cIAP1 and cIAP2 [70] playing role against breast cancer, AML, and childhood ALL in combination with different chemotherapeutics, such as Drozitumab, 5-azacytidine, and dexamethasone [65]. Table 4.1, represents a bird eye view of various chemotherapeutic agents that are known to target apoptotic cell death of cancer.

Targeting Survivin and XIAP

Upregulation of XIAP via apoptotic stimuli is associated with tumor cell death resistance [77]. Some agents targeting XIAP and survivin are discussed below.

AEG35156 This has been tested in early phase clinical trials. Pre-clinical studies displayed the efficacy of AEG35156 in triggering XIAP downregulation and therefore boost apoptosis [71].

YM155 This small imadazolium-based YM155 (sepantronium bromide) compound was recognized against anti-apoptotic protein survivin. YM155 showed pre-clinical success regarding survivin inhibition at both of mRNA and protein levels [72].

LY2181308 This molecule can bind to survivin complementarily and suppress its expression in cancerous cells. As a radio sensitizer, it showed potential effect in cancer cell lines with an inhibition of survivin expression [73, 78], along with substantial suppression of human xenograft growth while directed intravenously.

Table 4.1 Apoptosis inducing chemotherapeutics in pre-clinical and clinical trial and their mode of action for triggering apoptosis

Drug inducing apoptosis	Molecular mechanism	References
Dulanermin	Caspase activation	[31]
Mapatumumab	Enhance the anticancer activities of cytotoxic compounds	[31]
Lexatumumab	Activating caspase 8 and caspase 9	[31]
Conatumumab	Activating intracellular caspases by stimulating DR5	[32]
Drozitumab	Stimulate death receptor DR5	[36]
Tigatuzumab	Stimulate death receptor DR5	[37]
LBY135	Stimulate death receptor DR5	[38]
Oblimersen sodium (Genasense, G3139)	Increasing Bax, discharging cytochrome c from mitochondria to stimulate caspases and eventually releasing Smac/DIABLO to suppress IAPs, and activation of caspase-3 and caspase-9	[40]
Sodium butyrate (NaB), Depsipeptide, and Vorinostat	Downregulation of the anti-apoptotic proteins Bcl-2, Bcl-XL, and Mcl-1	[44]
Synthetic retinoid	Reduce the expression of Mcl-1 through phosphorylating the c-Jun kinase (JNK)	[45]
Gossypol	Suppressed Bcl-2 by disrupting the Bcl-2 and pro-apoptotic proteins hetero dimerization, activate the Bak,	[46, 48]
Obatoclax	Prevents the binding of BAK to MCL-1, and increases BIM expression	[49]
ABT-199, ABT-263, and ABT-737 (navitoclax)	Inhibit Bcl-2, Bcl-XL proteins; but not of BCL-w protein	[50, 51, 55, 56]
S55746 (Bcl201, Servier-1)	Inhibit anti-apoptotic Bcl-2	[52]
A-1155463, A-1331852, and WEHI-539	Inhibit anti-apoptotic Bcl-XL	[60, 61]
UMI-177	Precludes Mcl-1 from binding with Bak and Bax, which stimulate apoptosis	[52, 62–64]
A-1210477 and AMG176	Inhibit anti-apoptotic Mcl-1	[52]
Maritoclax (marinopyrrole A)	Binds to Mcl-1 and induces proteasomal degradation	[3]
ML311/EU-5346, S63845, S64315 (MIK665)	Inhibit anti-apoptotic Mcl-1	[3]
AZD5991	Inhibit anti-apoptotic Mcl-1	[3]
SH-130 compound	Enhance radiation-induced activation of caspase and induction of apoptosis	[4]
JP1201	Inhibit IAPs	[4]
Compound A (CA)	Inhibit XIAP	[4]
AT-406	Inhibit cIAP1, XIAP, cIAP2	[65]
LCL-161	Destroys cIAP1 and cIAP2	[66, 65]
GDC-0152	Inhibit XIAP, cIAP1, cIAP2, and ML-lAP	[65, 67]
Birinapant	Degrade cIAP1 and cIAP2	[65, 68]

(continued)

Table 4.1 (continued)

Drug inducing apoptosis	Molecular mechanism	References
HGS-1029	Inhibition of XIAP inhibition, and loss of cIAP expression	[65, 69]
BV6 XIAP	Degrade cIAP1 and cIAP2	[65, 70]
AEG35156	Down regulation of XIAP	[71]
YM155	Inhibit survivin	[72]
LY2181308	Inhibit survivin	[73]
Thymoquinone	Regulation of p53 pathway, generation of ROS, and interference with NF-κB pathway	[74]
Cordycepin	Increased ROS generation	[75]
Resveratrol	Upregulation of the expression and enzymatic activity of SOD, CAT, and GAP	[76]

LY2181308 also made tumor susceptible to cytotoxics such as paclitaxel, gemcitabine, and docetaxel [73].

Other Molecules

Thymoquinone Thymoquinone (TQ), a compound from black cumin was found to induce apoptosis in cervical cancer cells (CaSki and SiHa). In those cell lines, not by affecting the expression of poly A polymerase (PARP), Bcl-2, Bax, caspase 3 and 9, indicating other possible mechanisms involved in apoptosis induction, such as regulation of p53 pathway, NF-κB pathway, reactive oxygen species (ROS) generation, etc. [74].

Cordycepin Cordycepin treatment was found to enhance apoptotic cell death in SiHa and HeLa cervical cancer cell lines. Its mode of action indicated that apoptotic activity was might be due to the increased ROS generation in the tested cancer cell lines as no remarkable changes were detected for anti-apoptotic or pro-apoptotic proteins [75].

Resveratrol Resveratrol treatment in a low concentration remarkably elevated the activity of superoxide dismutase (SOD) in PC-3, MCF-7, and HepG-2 cells, and upregulated the expression of SOD, Catalase, and glutathione peroxidase disproportionally in cancer cells that leads to H_2O_2 accumulation in mitochondria, which in turn stimulated apoptotic death of cancer cells [76].

Role of Redox Potential of Anticancer Molecules in Apoptosis Induction

ROSs are reactive biochemical components, for example, superoxide anion ($O_2^{\bullet -}$), hydroxyl radical ($^{\bullet}OH$), hydrogen peroxide (H_2O_2), or nitroperoxide (ONOOH). Upon produced by eukaryotic cells cellular aerobic metabolism plays major role in signaling pathway and apoptosis. Oxidative stress by ROS and associated signaling pathways offer a critical challenge towards anticancer therapies because of its both pro- and antitumor dual roles. Cancer cell requires moderate oxidative stress for its proliferation and invasion, whereas increased oxidative exposure to cancer cell could induce its apoptosis. Highly effective redox system makes cancer cell resistant to oxidative stress. Thus targeting the redox system in cancer cells by using oxidants or antioxidants is an important approach in current cancer therapeutic research [79, 80].

Antioxidant Enzymes: Regulator of Apoptosis

SOD, catalase, glutathione peroxidase (GPx), and thioredoxin reductases (Trx) are important antioxidant enzyme systems. These enzymatic antioxidants possess the ability to destroy ROS that provide highly effective protection against vigorous and substantial oxidative damage.

Studies corroborated that the mitochondria are the key generators of ROS as well as the leading target of generated ROS. Enormous accumulation of ROSs in mitochondria triggers Mn-SOD overexpression to suppress oxidative injury in mitochondria. Besides, this accumulated ROS in mitochondria can promote the transition of mitochondrial permeability, hence distort the stability of mitochondrial membrane. Mitochondrial outer membrane damage eventually causes the cytochrome c release along with pro-apoptotic factors, namely apoptosis inducing factor (AIF), OMI/HtrA2, Smac/Diablo, and endonuclease G, finally prompts caspase activation and apoptosis [81]. GSH used as reductant by GPx to catalyze the conversion of organic hydroperoxides or H_2O_2 into water or the analogous alcohols. GPxs members have anti-oxidative role at diverse cellular organelles, such as cytosol and mitochondria (GPx1), cytosol and nucleus (GPx2), plasma (GPx3), and in membrane (GPx4). The endogenous Trx antioxidant system includes NADPH and Trx, which play very significant role against oxidative insults. These antioxidants repair DNA and protein via reducing methionine sulfoxide reductases and ribonucleotide reductase. Trx antioxidants and its binding proteins (TBP2 and ASK1) regulate apoptosis or metabolism of lipids and carbohydrates. Both Trx and GSH system can defend oxidative attack by removing different ROS effectively [81, 82].

For example, resveratrol, a natural anticancer polyphenol mediates the accumulation of H_2O_2 in mitochondria through antioxidant enzymes regulation, which in turn, stimulated apoptosis in different cancer cells [76]. Resveratrol also plays suppressive role in colorectal cancer in rats by inhibiting oxidative stress. Investigational results demonstrated that resveratrol supplementation (entire-period) considerably elevated the enzymatic (SOD, glutathione reductase, catalase, GST, and GPx)

and non-enzymatic (decreased vitamin C, beta-carotene, vitamin E, and glutathione) antioxidant status along with a concomitant alleviation in the level of lipid peroxidation markers. Taurine upsurges the expression of catalase, SOD, and GPx gene and hence, it was found potent against melanoma [80].

ROS Trigger Apoptosis by Modulating Different Cellular Pathways

Initiation of cell apoptosis originates from intracellular and extracellular signals by the DRs and the mitochondria-mediated extrinsic and intrinsic pathways. After the initiation of cellular apoptosis, disruption of the homeostasis of intracellular redox system and consistent oxidative alterations of DNA, lipid, and protein enhance ROS concentration that influences oxidative stress mediated signaling of apoptosis. ROS stimulate the cancer cell apoptosis through TRAIL, and increase CD95 expression and TRAIL DRs via instigating NF-κB [83]. Further, ROS-induced activation of JNK plays an important role in mitochondrial dysfunction with consecutive apoptosis initiation. Instigation of ROS/JNK can also uplift and withstand p53 activity that further leads to robust apoptotic effect in cancer cells [84]. The mitogen-activated protein kinase (MAPK) that is sensitive to redox and the apoptosis signal-regulating kinase 1 (ASK1) are the upstream proteins of ROS/JNK. The activity of ASK1 is inhibited due to its interactions with redox proteins (Trx1 and Grx). ROS induce the dissociation of Trx1 from the Trx1-ASK1 complex, and also recruit tumor necrosis factor receptor-associated factors (TRAF2/TRAF6) to the Trx1-ASK1 complex. Stimulated ASK1 later provide signals to activate JNK, and persuades apoptosis either by signaling to mitochondria or by AP-1-dependent pro-apoptotic gene transcription. Moreover, ROS-induced distraction of the Trx2/ASK1/ASK2 complex of the mitochondria mediates cytochrome c release. ROS can also be increased due to the ER stress and stimulate the adjacent mitochondria for initiating the intrinsic apoptosis signaling pathway [85].

Anticancer molecules found to play significant role in ROS mediated apoptosis by activating different molecular pathways. Evidence have shown that thymoquinone mediates apoptosis by ROS generation through various molecular signaling pathways, like inducing Akt activation and stimulating Bax protein's conformational changes that eventually leads to the damage of membrane potential of mitochondria and cytochrome c release and next, initiation of the caspase-dependent apoptotic pathway. Also, ginsenosides apply their anticancer potentials through ROS mediated signaling cascades [86]. Figure 4.3 presents a simplified diagram showing ROS mediated apoptotic mechanisms.

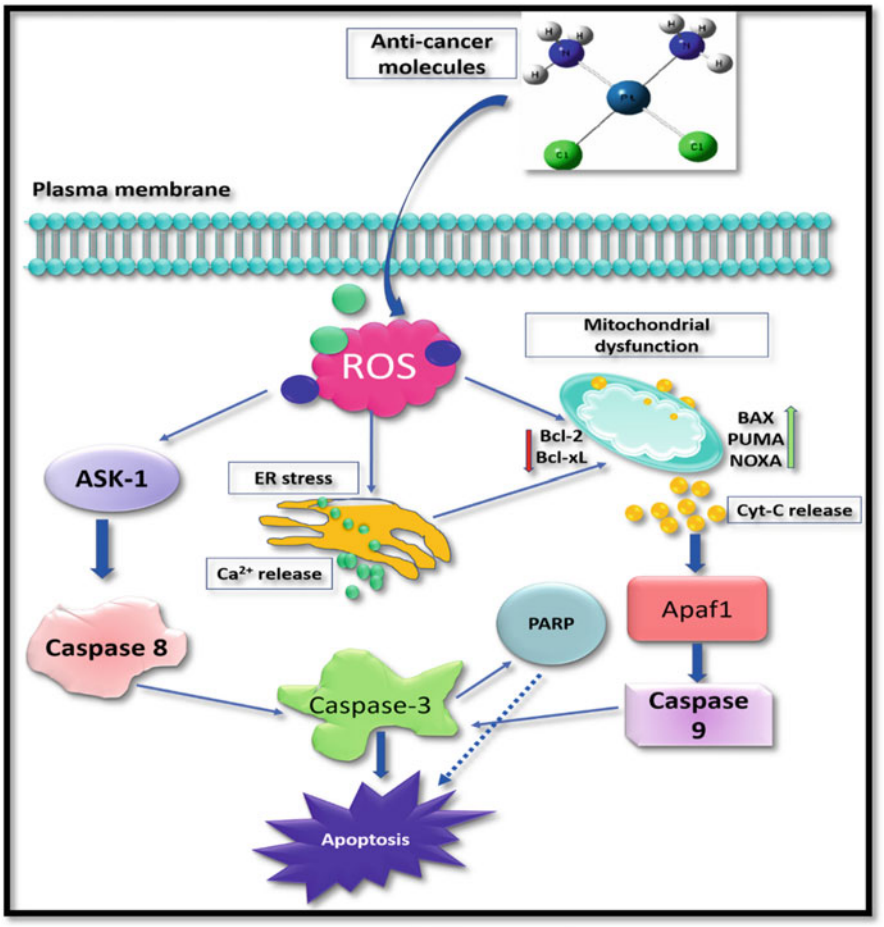

Fig. 4.3 ROS mediated signaling of apoptosis through caspase activation via the release of cytochrome c and ASK-1 activation

Mechanism of Balancing Antioxidant/Oxidant Mechanism by Chemotherapeutic Molecules to Protect Cells and Induce Apoptosis

Cancer cells are capable of adopting to new environments easily because of their highly compatible redox mechanisms that allow them to mediate a new redox balance for promoting cancer cell's growth.

There are different anticancer molecules mimicking antioxidant enzymes, targeting anti-apoptotic Bcl-2 proteins, caspase activation, and IAP. Mangafodipir is a potent SOD mimic possessing a combination of catalase-, SOD, and glutathione reductase-like functions. Hence, it can modulate different ROS cascade steps by neutralizing H_2O_2, $O2^{\bullet-}$ and by reestablishing GSH enzymes actions [87]. Niclosamide has proved as a powerful radiosensitizer that sensitize cells to

H_2O_2, via activating p38 MAPK-c-Jun axis, thus increasing apoptosis [88]. Organotellurides are well designated catalyst of redox with unique prooxidative role. Tellurium and selenium-based compounds convert the oxidizing redox milieu (existed in particular cancer cells) into a deadly accumulation of ROS that force these cells towards an acute redox threshold, and finally destroy these cells via apoptosis [89]. Allicin from garlic is a reactive sulfur species that has oxidizing properties, and is capable to oxidize thiols groups in cells, for example, cysteine residues in glutathione. This organosulfur stimulates apoptosis by elevating the cytochrome c level of mitochondria and release of Bax [90]. Quercetin provides anti-oxidant activity as metal chelator and ROS scavenger. It also exerted anticancer functions in cancer cells mainly via activating apoptosis [91].

4.5 Limitation of Apoptosis Targeting Chemotherapeutics

Chemotherapeutic Dulanermin did not show any maximum tolerated dose (MTD) and dose-limiting toxicity (DLT) in patients. Again, phase I studies reported that Mapatumumab is safe. However, the most recurrent side effects were nausea, hypotension, fatigue, transaminitis, pyrexia, thrombocytopenia, and neutropenic fever found for mapatumumab. In case of Lexatumumab, the DLTs were transaminitis, hyperamylasaemia, and hyperbilirubinaemia. Phase I clinical study of AMG655/Conatumumab displayed fatigue, and elevated lipase level in patients. Study of antisense oligonucleotide Oblimersen revealed fatigue, and LFTs elevation. ABT-263 caused nausea, thrombocytopenia, fatigue, and elevated ALT, grade 4 thrombocytopenia, and bronchitis as dose-limiting toxicity (DLT). Grade III thrombocytopenia in some patients was observed by ABT-199, tumor lysis syndrome (TLS), neutropenia, or infections as adverse effects in patients [92]. Obatoclax showed neurological symptoms including dizziness, gait disturbance, somnolence, euphoric mood, QTc prolongation. AEG35156 showed DLT such as hypophosphatemia, asymptomatic reversible transaminitis, and thrombocytopenia. Another apoptosis inducing therapeutic YM155 showed nausea, stomatitis, pyrexia, and thrombocytopenia. LY2181308 showed flu-like symptoms, prolonged prothrombin time, thrombocytopenia, fatigue, and grade III transaminitis [4]. One of the established chemotherapeutic Levo gossypol affects male reproduction, causes fatigue, diarrhea, lymphopenia, neutropenia, hypophosphatemia, and mediates gastrointestinal (GI) toxicity in patients [93], necessitating the improvement of analogs with less toxicity. This caused the current advancement of apogossypol, which does not possess two reactive aldehydes that have been recommended to be accountable for the levo gossypol toxicity [4]. Conversely, for AZD4320, BCL2-32, BM-1197, S44563, WEHI-539, A-1155463, A-1331852, A1210477, Maritoclax, ML311/EU5346, S63845, and UMI-77, no pre-clinical or active clinical trial done and not assessed in humans for toxicity. Furthermore, S55746 (BCL201, Servier-1), S64315/MIK665, AZD5991, and AMG176 are in clinical trial but no adverse effect has been reported yet [3]. Figure 4.4 summarizes the adverse effects of apoptosis.

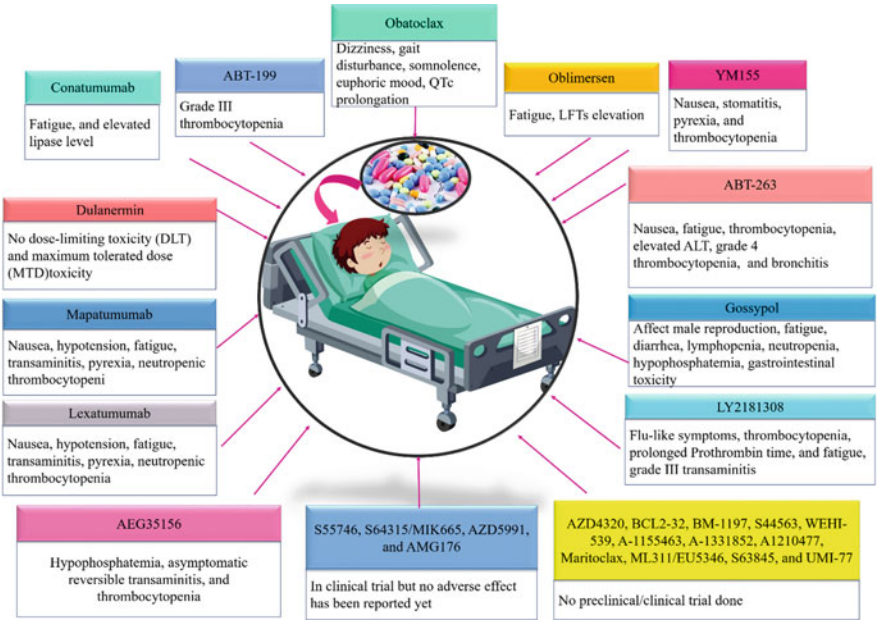

Fig. 4.4 Adverse effect of apoptosis inducing chemotherapeutics in patients

4.6 Conclusion

Apoptosis is one of the vital biological processes of life, and lack of cellular apoptosis is one of the major events in carcinogenesis Targeting the defective regulatory system of apoptosis is thus one of the most important approaches in chemotherapies. Drugs inducing apoptosis by targeting its different events have always received special consideration, and there are ongoing processes in scientific research to develop cancer treatments, especially chemotherapeutics on the basis of targeting apoptosis.

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