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REVIEW

Major apoptotic mechanisms and genes involved in apoptosis

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Abstract As much as the cellular viability is important for the living organisms, the elimination of unnecessary or damaged cells has the opposite necessity for the maintenance of homeostasis in tissues, organs and the whole organism. Apoptosis, a type of cell death mechanism, is controlled by the interactions between several molecules and responsible for the elimination of unwanted cells from the body. Apoptosis can be triggered by intrinsically or extrinsically through death signals from the outside of the cell. Any abnormality in apoptosis process can cause various types of diseases from cancer to auto-immune diseases. Different gene families such as caspases, inhibitor of apoptosis proteins, B cell lymphoma (Bcl)-2 family of genes, tumor necrosis factor (TNF) receptor gene superfamily, or p53 gene are involved and/or collaborate in the process of apoptosis. In this review, we discuss the basic features of apoptosis and have focused on the gene families playing critical roles, activation/inactivation mechanisms, upstream/downstream effectors, and signaling pathways in apoptosis on the basis of cancer studies. In addition, novel apoptotic players such as miRNAs and sphingolipid family members in various kind of cancer are discussed.

Keywords Intrinsic/extrinsic pathway · Bcl-2 · Caspase · TNF · TRAIL · p53

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Introduction

The main goal for a cell is to stay alive during the lifetime. Holding the key of proliferation events as much as death mechanism has vital importance for the cells to keep the balance between living and death cells in the body. As one of the cellular death mechanisms, apoptosis, also known as programmed cell death, can be defined as the process of a proper death of any cell under certain or necessary conditions. Apoptosis is a part of natural homeostatic mechanism to keep the number of the cells constant in an organism and helps the tissue to eliminate increasing number of unwanted/unneeded cells that are damaged or no longer manageable during development, growth or aging [1]. It also plays crucial roles in early development and differentiation of the embryo in order to generate a full and decent organism. Although the term of "apoptosis" was firstly used by Kerr et al. in 1972 to identify a distinguished type of cell death, the first description and understanding of the programmed mechanisms of apoptosis was derived from the studies on the development of the nematode *Caenorhabtidis elegans* in 1999 [2, 3].

Apoptosis is a defense mechanism against damaged, stressed, or stimulated cells by any agents to prevent accumulation of non-functional cells in the tissues. If apoptosis is not be mediated properly in unwanted cells, the mutations could continue to accumulate in the cells that eventually could lead to generation of cancer and other diseases such as autoimmune diseases, AIDS, or some of neurodegenerative disorders [4]. However, apoptosis is the best defined and well-understood "programmed" type of cell death; there are many different types of cellular death mechanisms such as pyroptosis, necrosis, or autophagy, and some others might not yet be discovered [5, 6]. Apoptosis regulatory pathways, including a number of gene families, orchestrate the specific morphological and biochemical changes in the cells during the

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process. We touched on these significant chances briefly and focused on the genes involved in apoptosis with details.

Morphological and biochemical processes of apoptosis

Many biochemical events and a series of morphological changes occur at the early stage and increasingly continue till the end of apoptosis process. Some of the changes such as cell shrinkage, chromatin condensation, or nuclear differences could be observed by microscopic techniques [1, 7]. Morphological event cascade including cytoplasmic filament aggregation, nuclear condensation, cellular fragmentation, and plasma membrane blebbing finally results in the formation of apoptotic bodies. All the morphological hallmarks of apoptosis can be gathered under three headings; (i) the changes occur in nucleus; (ii) cell membrane and cytosolic changes; (iii) those happen in mitochondria [1, 8]. Chromatin condensation, DNA fragmentation, and nuclear fragmentation are the nuclear changes that could be observed with light and fluorescence microscopy during apoptosis. The apoptotic cell loses its association with other cells at initiation stage of the process by different signals breaking the connection. This separation followed by apoptotic body formation and resulted in blocking the inflammatory reaction of the cells, since they package their ingredient and do not release any contaminant outside the cell. Then, the buddies are rapidly phagocytized by other neighbor cells and these absorber cells do not produce any signal that causes any inflammatory response. Also, mitochondria play an important role by interacting with many different apoptotic/anti-apoptotic proteins and releasing signal molecules [9, 10].

Chromatin condensation and nuclear fragmentation are the major modifications observed in the nucleus, eventually resulting in pyknosis (chromatin condensates irreversibly that signs cell death) and followed by karyorrexis (nuclear fragmentation, the last event in nucleus during apoptosis) [11]. The fragmentation of double-stranded DNA into 180–200 bp sequences in length by the help of caspase proteins is also another essential hallmark of nuclear events during apoptosis. Caspases are responsible for DNA repair during replication as well as the termination stage of apoptosis. They are also involved in the fragmentation in apoptosis together with DNA fragmentation factors (DFFs) and endonucleases. Most of the nuclear changes of apoptosis observed by electron microscopy or even light microscopy make the apoptosis process to be determined easily [11, 12].

As soon as the apoptosis is initiated in the cells, they lose the connection between the neighboring cells; membrane shrinks and the cell packs its cytosolic ingredients into apoptotic buddies. The apoptotic buddies will be eliminated by phagocytotic cells that recognize phosphatidylserine, which is normally located in the inner side of the cell membrane and flips to the outer membrane during apoptosis [13]. At the same time, the cytoplasmic scaffold proteins and cell junction proteins such as actin, β-catenin, spectrin, or Gas2 are deactivated by cleavage and the cell loses its integrity by the function of caspases [14].

Mitochondria have complex and important roles by providing various pro-apoptotic signals, creating a downstream cascade of apoptosis activation. The balance between the pro-apoptotic and anti-apoptotic molecules keeps the cellular homeostasis stable and determines the cell fate, which is either apoptosis or proliferation. Mitochondria have a leading role in releasing a number of important apoptosis inducing molecules including cytochrome c, SMAC, apoptosis-inducing factor, or endonuclease G as a result of permeabilization of mitochondrial membrane. Permeabilization is triggered by pro-apoptotic B cell lymphoma (Bcl)-2 family proteins, while the integrity of mitochondrial membrane is maintained by anti-apoptotic members of Bcl-2 family [10, 15, 16].

Several biochemical changes such as protein modifications/degradations, DNA and chromatin deteriorations, and synthesis of cell surface markers form morphological process during apoptosis. Caspases are mainly responsible for these changes with their extensive capabilities to cleave certain molecules from one or more specific points, causing degradation and inactivation of target protein. Moreover, they can also inhibit the negative regulatory domains of specific proteins, which leads to the activation of subjected molecule [17, 18]. They are also involved in DNA fragmentation process.

Intrinsic and extrinsic apoptotic pathways

Apoptosis can be stimulated by two different pathways: (i) intrinsic pathway (or mitochondria) that mainly occurs via release of cytochrome c from the mitochondria, which activates different caspases as downstream signals, and (ii) extrinsic pathway when Fas death receptor is activated by a signal coming from the outside of the cell. After the activation of different intermediate molecules by signaling cascade, both of the pathways meet up at the final caspase activation step and commonly lead to cleavage of different proteins [19] (Fig. 1).

Intrinsic pathway

The intrinsic pathway of apoptosis is independent from a receptor signaling, and mitochondria-associated stimuli create an intracellular signaling. The inner activation of this pathway makes the cell undergo apoptosis in either a positive or negative manner. The positive stimuli (e.g., toxic materials, viral infections, and radiations) directly activate all the mediators for apoptosis, whereas negative stimuli (loss of growth factors, different cytokines, or certain type of hormones) work in contrast to positive one and eliminate the factors that suppress apoptosis in the cells and cause apoptotic activation [1, 20]. In addition to different infections or cytokine-mediated intrinsic

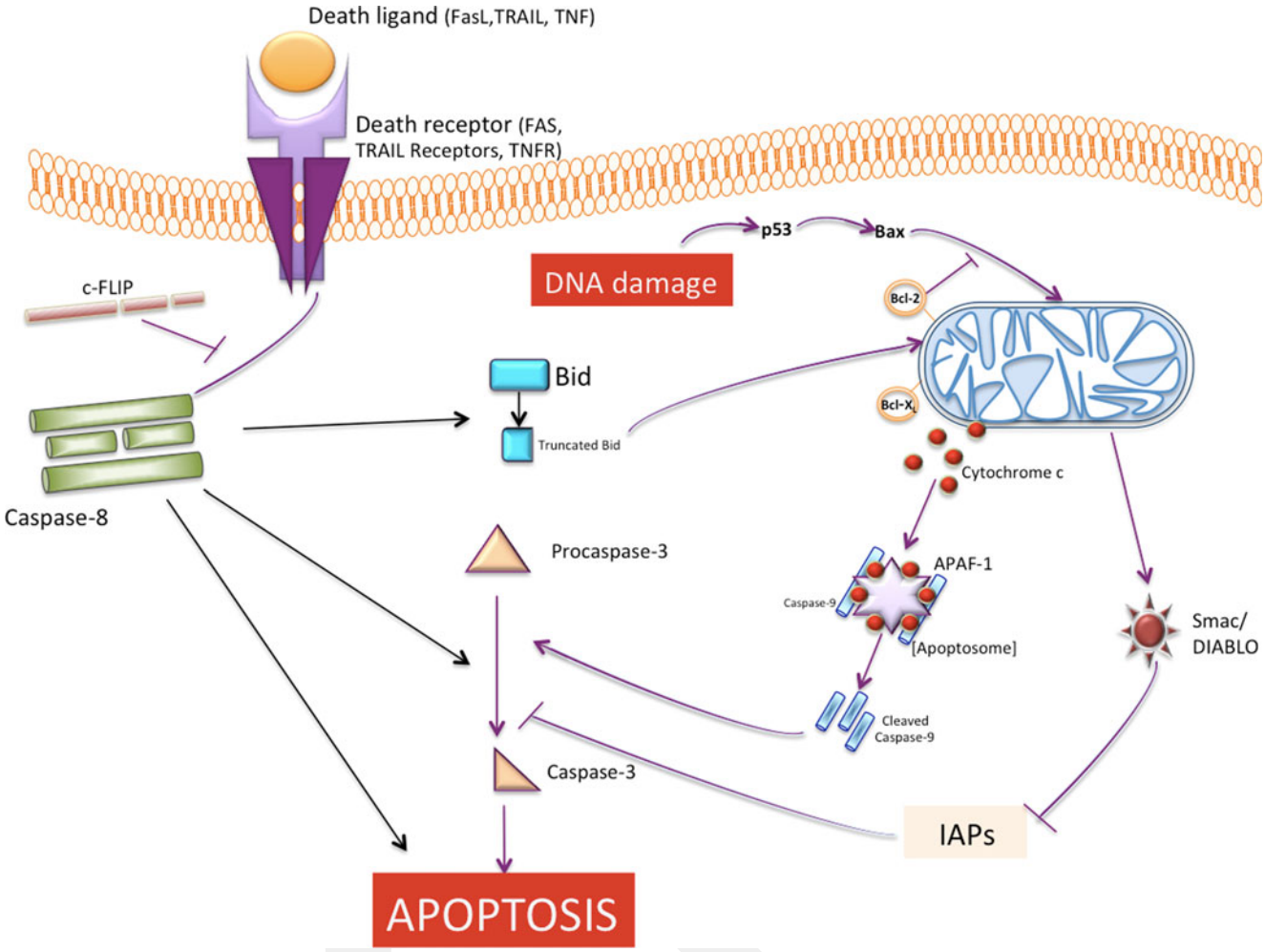

Fig. 1 Two main apoptotic pathways; intrinsic and extrinsic pathways. In the extrinsic pathway, interaction between the death receptors and their ligands initiates the pathway, resulting in caspase 8 activation. This activation can be inhibited by cFLIP. Caspase 8 can directly induce apoptosis or activates caspase 3 or Bid, which lead to apoptosis. On the other hand, intrinsic pathway can be initiated by DNA damage. As a response, the cells can trigger apoptosis through mitochondrial pathway, which starts with the activation of the pro-apoptotic member of the Bcl-2 family, Bax. Anti-apoptotic proteins inhibiting the action of Bax are located on the membrane of the mitochondria such as Bcl-2 and Bcl-X_L. Release cytochrome c, APAF-1 complex, and pro-caspase9 can be gathered in the cytosol, which is called apoptosome. The formation of this complex will result in the activation of caspase 9 followed by the transformation of pro-caspase-3 to caspase 3, which is the last step for apoptosis. The cross talk between extrinsic and intrinsic pathways of apoptosis is regulated by Bid, a pro-apoptotic member of Bcl-2 family. The cleavage of Bid is mediated by caspase 8, which induces apoptosis by releasing cytochrome c release from the mitochondria

apoptosis activation, DNA damage also majorly induces apoptosis as a protection mechanism of the cells that do not let self to continue proliferation with an imperfect DNA sequence. DNA damage or any other type of apoptosis stimuli basically causes the changes in the trans-membrane potential of mitochondria, which result in the release of pro-apoptotic proteins into the cytoplasm.

Cytochrome c, Smad, or high-temperature requirement protein A2 (HtrA2)/Omi are a group of pro-apoptotic molecules released from mitochondria and cause the activation of caspase protein cascade [21, 22]. Cytochrome c interacts with Apaf-1, resulting in the formation of "apoptosome" complex, which activates pro-caspase-9. After active caspase-9 activates caspase-3, the final cascade is become activated and nucleus will be fragmented together with the breaking of nuclear membrane [23]. This stage is the initial event for extrinsic and intrinsic pathways of apoptosis, where caspase-3 cleaves the different proteins such as kinases, DNA control proteins, cytoskeletal proteins, or inhibitor of endonucleases. DNA condensation, membrane blebbing, and all the morphological changes are regulated by caspases as a common mechanism for both intrinsic and extrinsic trigger [17].

On the other hand, another group of molecules released by mitochondria including endonuclease G or AIF are also pro-apoptotic proteins but involved in the process at the later stages. These molecules are trans-located into the nucleus where they first cause an elementarily DNA fragmentation and chromatin condensation which is defined as "stage 1,"

and an advanced condensation and DNA fragmentation by the help of caspase-3 at later stage is called as "stage 2" [24].

All the intrinsic apoptosis events are primarily controlled by Bcl-2 family of proteins and p53 tumor suppressor protein which is majorly involved in the activation of Bcl-2 family proteins. The members of Bcl-2 protein family can act as either pro-apoptotic (Bax, Bak, Bid, Bim, Puma, Noxa, Bad, and Blk) or anti-apoptotic (Bcl-2, Bcl-XL, Bcl-X, and BAG) and also determine the membrane integrity of mitochondria and are involved in the process of cytochrome c release [1, 25].

Extrinsic pathway

Apoptosis triggered by extrinsic pathway is primarily mediated by signaling through membrane-bound death receptors that belong to tumor necrosis factor (TNF) gene superfamily. The initial signal is provided by the interactions between the ligands and cell membrane death receptors such as Fas ligand/FasR, TNF/TNF R1, Apo2L/DR4, or TNF-related apoptosis-inducing ligand (TRAIL) R1, which is resulted in ligation of death domains of these receptors [26]. Binding of Fas ligand to its receptor induces the binding of adaptor protein, Fas-associated death domain (FADD), while TNF/tumor necrosis factor receptor (TNFR) interaction causes the binding of TNFR-associated death domain (TRADD), which is resulted in pro-caspase-8 activation. Pro-caspase-8 is activated autocatalytically by the help of death-inducing signaling complex (DISC). Active caspase-8 either induce Bid, thus intrinsic pathway also become involved and activated with an outside signal, or caspase-3 and caspase-7 and the activation process of apoptosis is terminated with the same final pathway as intrinsic stimuli does [1, 19, 27, 28]. Bid is the pro-apoptotic member of Bcl-2 family, exhibiting a common molecule between intrinsic and extrinsic pathways of apoptosis. Caspase-8 causes the cleavage and myristoylation of cytoplasmic Bid protein, leading to its movement through mitochondria. Then, apoptosome formation is induced by cytochrome release via Bak and Bax molecules [29].

The extrinsic activation of apoptosis can also be inhibited via two different ways. The one is binding of FLICE-like inhibitory protein (cFLIP) to FADD and pro-capase-8 and blocking their activity, and the other way is inhibition of caspase-8 biogenesis by a protein named Toso which is firstly described in T cells [30, 31].

In the next sessions, we will discuss all the gene families involved in intrinsic or extrinsic pathways of apoptosis with their main player and their functions.

Caspase family members

Caspase family comprise conserved cysteine aspartic-specific proteases, and members of caspase family are considerably crucial in the regulation of apoptosis [32]. In *C. elegans*, Ced-3 was reported to be essential for the cell death, which is mainly conducted by the caspase Ced-3, Ced-4 activating Ced-3, and Ced-9 inhibiting apoptosis [33, 34]. There are 14 different caspases in mammals, and they are basically classified as the initiators including caspase-2, -8, -9, and -10; and the effectors including caspase-3, -6, -7, and -14; and also the cytokine activators including caspase-1, -4, -5, -11, -12, and -13 [35, 36]. Structurally, while initiator caspases have long N-terminal pro-domain known as caspase recruitment domains (CARDs) including more than 90 amino acids, effector caspases have shorter sequences known as death effector domain (DED) including 20–30 amino acids. Since caspases firstly synthesized as zymogens, they are subsequently activated during the apoptotic process. While initiator caspases are self-activated, effector caspases are activated by initiator caspases via internal cleavages [37]. Rather than apoptosis, most of caspase family members are functional in cellular proliferation, survival, and inflammation, whereas some of them are essential for apoptosis [38, 39].

Caspase-1, the first identified caspase, is interleukin-1b processing enzyme (ICE), and it is known as Ced-3 homologue [40]. Caspase-1 is involved in cytokine activator group of caspase family since inflammatory cytokines, pro-IL-1b and pro-IL-18, are the main substrates for caspase-1 [39]. While caspase-1 is not essential for apoptotic signaling, it is essential in inflammation process [41].

The second identified caspase, caspase-2, containing CARD, plays important roles in DNA damage-, metabolic abnormality-, and ER stress-induced apoptosis [42]. Caspase-2 is known as a substrate for both caspase-3 and caspase-8 [43, 44]. Caspase-2 activation comprises the formation of PIDDosome complex including RIP-associated ICH-1/ECD3 homologous protein with death domain (RAIDD) that have CARD and death domain (DD) and p53-induced protein with death domain (PIDD) [45]. Functional properties of caspase-2 have still not been clarified thoroughly.

Caspases-3, -6, and -7 are involved in the effector caspase group, and they act in a similar manner in the apoptotic process [46]. Caspase-3 is activated via both extrinsic and intrinsic apoptotic pathways [47]. Despite there are limited information about caspase-6 and -7 rather than caspase-3, it is known that while caspase-3 suppression results in the inhibition of apoptosis, suppression of caspase-6 and -7 do not significantly affect the apoptotic process [48]. Furthermore, caspase-3 was reported to be crucial for PARP cleavage and DNA fragmentation which are hallmarks of apoptosis [49]. However, some studies showed that under the conditions that both caspase-3 and caspase-7 were knocked out, the cells could undergo cell death in an alternative manner via necrosis. Studies with caspase-3/caspase-7 double-knockout thymocytes and mouse embryonic fibroblasts showed that thymocytes remain sensitive to Fas-mediated apoptosis, whereas fibroblasts become resistant [49, 50].

Functionally well-known member of caspase family, caspase-8, is crucial factor for TNF-induced extrinsic apoptotic pathway [51]. Pro-caspase-8 is recruited to DISC by FADD, and dimerization or trimerization triggers pro-caspase-8 activation via reciprocal cleavage. Caspase-8, in turn, cleaves and activates caspase-3, -7, Bid, and also NF-kB [52, 53]. Caspase-8 activation is regulated by cFLIP that is structurally homologous to caspase-8 but does not have caspase activity [54]. $FLIP_S$, the short isoform of cFLIP, controls the DISC formation in a negative manner. However, the long isoform of cFLIP, $FLIP_L$, has reciprocal effect on DISC formation and caspase-8 activity. While some studies reported $FLIP_L$ to be inducer of DISC formation, some reports showed that it could be an inhibitor [55, 56]. Inhibitor and inducer effects of $FLIP_L$ on caspase-8 activity depend on $FLIP_L$ levels. At low concentrations, generation of heterodimers between $FLIP_L$ and pro-caspase-8 or pro-caspase-10 induces their activity. However, at higher levels of $FLIP_L$, caspase-8 activation diminishes and NF-kB activation increases [57, 58].

Caspase-9, the initiator caspase, is an important factor for the generation of apoptosome complex in the mitochondrial pathway. Once cytochrome c is released from the mitochondria, it binds to Apaf1 which is the receptor for cytochrome c in the cytoplasm [59]. Cytochrome-c and Apaf-1 generate apoptosome, and then, pro-caspase-9 binds to Apaf-1. Afterward, pro-caspase-9 is activated via reciprocal cleavage, and by this way, apoptosome complex also become activated. Then, caspase-3 is cleaved and activated via caspase-9 found in the active apoptosome complex [60].

Inhibitors of apoptosis proteins and inhibitors of apoptosis protein antagonists

Inhibitors of apoptosis proteins (IAPs) were firstly discovered in *Baculovirus* as gene products. All IAPs have baculovirus IAP repeats (BIRs) that composed of one or more zinc finger motifs [61]. The first identified IAP, OpIAP, inhibits pro-caspase cleavage and activation rather than direct inhibition of caspase activity [62]. NAIP, the first identified mammalian IAP, was reported to be related to the generation of immune response against bacterial infection. Hence, it is not directly correlated with caspase inhibition [63].

Survivin/BIRC5, another identified mammalian IAP, was firstly reported as caspase inhibitor, but now, it is known that survivin does not directly inhibit caspase activity. Mechanism of action of survivin, bearing one BIR domain, is to assemble with centromeres and p21Waf1 at the beginning of mitosis [64, 65].

XIAP/BIRC4, the mostly clarified mammalian IAP, has three BIR domains and a RING domain; it is located on X chromosome; and also, it is quite effective in apoptosis inhibition via inhibiting caspase activity [66]. Mainly, caspase-3 and caspase-7 can be inhibited by XIAP via inserting a residue of aspartic acid into the caspase active region [67]. Additionally, XIAP can also inhibit caspase-9 activity via binding the third BIR to the N-terminus of pro-caspase-9, resulting in the prevention of caspase-9 dimerization [68].

Similarly, another mammalian IAPs, cIAP1/BIRC2, and cIAP2/BIRC3 have three BIR domains and a RING domain in their structures [63]. Rather than inhibiting directly caspase activity, they inhibit apoptosis indirectly. They can bind IAP inhibitor, second mitochondrion-derived activator of caspase/direct inhibitor of apoptosis-binding protein with low pI (SMAC/DIABLO), trigger NF-kB and MAPK activity, and also they can trigger proteasomal degradation of caspases [69].

Bruce/BIRC6, a member of IAP family, is mainly found in secretory organs, testis, lymphatic cells, and brain. Similar to survivin, Bruce located at the outer membrane of the trans-Golgi network bears only one BIR domain. Bruce can inhibit caspase-3, -6, -7, -8, and -9, and also, it can trigger the proteasomal degradation of SMAC/DIABLO. Many studies showed that Bruce is upregulated in ovarian and brain cancer cell lines, resulting in development of resistance against apoptotic agent [70].

IAP antagonists, SMAC/DIABLO, HtrA2/Omi, and XIAP-associated factor 1 (XAF1) are known as potent inhibitors of IAPs. SMAC/DIABLO bears a mitochondrial targeting signal (MTS) at N-teminus, and it become mature after the MTS cleavage [21]. Once apoptosis is triggered, SMAC/DIABLO is delivered into the cytosol, and SMAC/DIABLO homodimers bind to IAPs via its Ala-Val-Pro-Ile sequence in N-terminal domain [71]. Interaction of SMAC/DIABLO with XIAP via the second and third BIRs results in caspase-3 and caspase-9 release [72]. It was reported that SMAC is overexpressed in several types of solid tumors such as colon, stomach, prostate, ovary, and lung cancers [73]. Likewise, HtrA2/Omi delivery is triggered via apoptotic induction and then, it binds to IAPs through its IAP-binding motif (IBM) [74]. XAF1, the other potent IAP antagonist, binds to BIR domains of IAPs such as XIAP, cIAP1, and cIAP2 and by this way, promotes apoptosis [75]. A study suggesting XAF1 as a prognostic marker for colon cancer showed that XAF1 is overexpressed in colon cancer cells as compared to adenoma cells which are benign [76].

Bcl-2 family members

Bcl-2 family members, which play important roles in regulating apoptotic signaling, are divided into three subfamilies including (i) pro-survival subfamily members (Bcl-2, Bcl_{XL}, Bcl_W, MCL1, and BFL1/A1), (ii) BH3-only subfamily members (Bad, Bim, Noxa, and Puma9), and (iii) pro-apoptotic mediator subfamily members (Bax and Bak) [77, 78]. Basically, all of the members of Bcl-2 family share typical characteristic functions; (i) they dimerize with other members

of Bcl-2 family, (ii) they contribute to the regulation of mitochondrial homeostasis by binding proteins, and (iii) they contribute to outer mitochondrial membrane pore formation [79].

The members of BH3-only subfamily members become activated under stress conditions like growth factor deprivation and DNA damage. Active BH3-only proteins, in turn, inactivate members of pro-survival subfamily via binding that promotes the activation of the members of pro-apoptotic subfamily members. Active pro-apoptotic subfamily members, Bak and Bax, then provide cytochrome c release from the mitochondria through permeabilizing the outer mitochondrial membrane [80].

In several types of human malignancies, the balance between the expression levels of Bcl-2 family genes is broken down, and the equilibrium changes to the pro-survival subfamily member direction. In this case, cancer cells can escape from apoptotic signals and therefore develop resistance against therapeutic agents [81, 82]. Additionally, Bcl-2 family members are also considered in cancer therapy due to their therapeutic potentials [83]. In the clinical trials, the BH3-only mimetic agents targeting Bcl-2 are being investigated in order to find alternative potent therapeutic approaches in several types of malignancies [84–86].

TNF gene superfamily

One of the most important ways of triggering apoptosis is mediated through death receptors (DRs), which are classified in TNF superfamily including also ligands such as TNF, TRAIL, and Fas ligand (FasL) [87]. The induction of apoptosis by these ligands is initiated by binding to their specific membrane receptors [88, 89]. TNF superfamily is known to comprise 19 ligands and 29 receptors that function in highly different processes in the body including inflammation, apoptosis, proliferation, and invasion [90]. Even though all members of TNFR superfamily are generally trimeric type I transmembrane proteins and possess cysteine-rich extracellular subdomains, they are actually different in their primary structure, which make them unique to recognize their ligand in a specific and exclusive manner [91]. These DRs also contain a homologous cytoplasmic cysteine-rich "DD" which is responsible for transmission of apoptotic signals from cell surface to intracellular signaling pathways [92]. Because, adapter molecules such as FADD and TRADD have these death domains as well to interact with DRs. The ligands included in TNF superfamily share a common extracellular TNF homology domain (THD), which is involved in the formation of homotrimers via non-covalent bonding [93]. The extracellular domain of most TNF ligands undergoes proteolytic cleavage to make a soluble ligand, even though they are synthesized as type II trans-membrane proteins [93].

CD95 (DR2/Fas/APO-1), TNF receptor 1 (DR1/TNFR1), TRAIL-R1 (DR4), and TRAIL-R2 (DR5) are the best characterized DRs of which ligands are CD95 ligand (CD95L/FasL), TNFα, lymphotoxin-α (these two bind to TNFRI), and TRAIL (these two bind to TRAIL-R1 and TRAIL-R2), respectively [94]. The genes encoding TNF superfamily receptors and ligands are the scope of this part together with their significant contribution to apoptosis specifically.

Fas cell surface death receptor and Fas ligand

The proteins encoded by Fas cell surface death receptor (FAS) and Fas ligand (FASLG) genes are members of the TNF superfamily. Fas also known as CD95/APO-1/DR2 is one of the best studied DRs with molecular weight of 48 kDa. Fas gene occupies about 25 kb on human chromosome 10 with nine exons in which exon 6 codes for trans-membrane domain [95]. This receptor containing a DD plays a crucial role in the regulation of programmed cell death and is involved in the pathogenesis of various malignancies such as cancer. On the other hand, FasLG gene is located on human chromosome 1, which encodes a type II trans-membrane protein called FasL (CD95L) present at the surface of activated immune cells such as T cells and natural killer cells [93]. Therefore, Fas/FasL interaction results in the elimination of infected and transformed cells, which is generally used by immune cells to avoid cancer development.

The interaction of Fas with FasL results in changes in the conformation and aggregation of receptor on the plasma membrane and triggers an initial signaling event through protein-protein interactions. The main structural changes take place in DD of receptor, which recruits FADD through its DD. Then, FADD interacts with pro-caspase-8 and -10. After activation of these caspases via auto-cleavage, they are released in the cytosol as active caspases resulting in the apoptotic cell death. The complex CD95/FADD/caspase-8/-10 is called DISC stands for "death-inducing signaling complex" [96].

In the literature, there are various kinds of studies identifying the roles of Fas/FasL in order to induce apoptosis in several malignancies especially in cancer. Escaping from apoptotic stimuli is a very well known feature of cancer cells which might develop multiple strategies to inhibit apoptotis mediated by CD95. In a majority of cancer types from different origins, somatic mutations in CD95 gene were found to be a common way to trigger the development of resistance toward apoptosis. For instance, 5′ region of CD95 gene was analyzed in terms of somatic mutations in nodal diffuse large B cell lymphoma and defined mutations were shown to be included in progression of diseases due to inhibition of apoptosis [97]. Another common way to become resistant to CD95-induced apoptosis is the regulation of surface expression of receptor [98]. In a study performed by Ivanow et al. (2006) [98], the treatment of melanoma cells having increased surface expression of Fas receptor with soluble FasL resulted in the induction of apoptosis.

CD95-mediated apoptosis can also be blocked by decreasing expression of FADD or caspase-8 [99, 100].

In a recent study, the effect of a toxic steroid on human bladder cancer cells was found to be related to increased expressions of Fas and FasL in vitro and in vivo at both messenger RNA (mRNA) and protein levels [101]. Zhong et al. (2015) [102] showed that the mechanism of resistance to Fas-mediated apoptosis in human hepatocellular carcinoma cells (HCCs) and overexpression of oxysterol-binding protein-related protein 8 (ORP8) which is normally downregulated in HCC was found to induce apoptosis by upregulating FasL.

TRAIL

TRAIL (also called as APO2 ligand) is a type II transmembrane protein and processed proteolytically at the cell surface to form a soluble ligand. TRAIL is grouped into the TNF cytokine family, which was discovered based on its extracellular domain sequence homology with CD95L and TNF [103]. TRAIL-mediated apoptosis takes place after its binding to its DD containing receptors, TRAIL receptor 1 (death receptor 4, DR4), and TRAIL receptor 2 (death receptor 5, DR5) [104]. There are also three other TRAIL receptors, which do not possess apoptotic ability and function as decoys. Decoy receptors 1 (DcR1) and 2 (DcR2) are expressed on the cell surface similar to DR4 and DR5. Even though their extracellular and ligand-binding domains show significant homology to DR4 and DR5 and are fully functional, they lack of functional intracellular DD [105]. Therefore, increased expression of either DcR1 or DcR2 provides resistance against TRAIL-induced apoptosis [104]. In a recent study, it was shown that coexpression of DcR1 and DcR2 with DR4/DR5 on the same cell can block apoptosis. However, TRAIL was engineered in order to escape from binding to DcRs, which were found to still exert trans-cellular regulation originating from stromal cells and affect tumor cells. Therefore, it is important to target these decoy receptors selectively to gain maximum efficacy [106]. Another recent study showed that DR4 and DR5 were upregulated while DcR1 and DcR2 downregulated in colon cancer cells after their treatment with a non-steroidal anti-inflammatory drug. Therefore, colon cancer cells became sensitive to TRAIL-induced apoptosis [107]. The fifth TRAIL receptor is osteoprotegerin (OPG), which is a secreted receptor for TRAIL with low affinity. OPG has sequence homology to TNF receptor superfamily, however but lacks of a transmembrane domain. OPG can bind to TRAIL and prevents its interaction with death receptors, thus preventing Jurkat cells from undergoing TRAIL-mediated apoptosis [108]. Therefore, OPG has an anti-apoptotic effect by preventing interaction between TRAIL and the death receptors [109]. Lane et al. (2012) [110] showed the relationship between higher OPG expression and resistance of ovarian cancer cells to TRAIL-induced apoptosis.

Similar to apoptosis induction by Fas/FasL, binding of TRAIL to its DRs, DR4 and DR5 recruits the adaptor protein FADD and inactive versions of caspase-8 and -10 to form the DISC complex in which caspase-8 and -10 become activated by auto-proteolytic cleavage and released in the cytosol to activate effector caspases [111].

The size of human TRAIL gene is ~20 kb, containing five exons and four introns. The first exon encodes for the transmembrane domain and the cytoplasmic domain, whereas exons 4 and 5 code for the extracellular domain responsible for the interaction of TRAIL with its receptors. Exon 5 also encodes for the C-terminal amino acids along with containing the 3′-untranslated region (3′-UTR) and poly-A tail [112]. There are various variants of TRAIL; however, only one specific isoform of TRAIL (TRAILα) is responsible for its cancer-selective apoptosis induction potential [113].

TRAIL gene is subjected to strict regulation due to its critical role in the induction apoptosis. Altered expression of TRAIL gene has been found in various kind of disease. Multiple studies have been performed to analyze single-nucleotide polymorphisms (SNPs) in various patient populations. In a recent study, peripheral blood samples were analyzed in terms of SNPs in TRAIL promoter and substitution of C to T at position -723 was found to be significantly associated with sporadic breast cancer and decreased TRAIL mRNA levels due to transcriptional repression [114]. Bos et al. (2009) found that TRAIL mRNA expression decreased in breast cancer patients with brain metastasis [115].

TRAIL has been considered as a potential therapeutic target due to its selective apoptosis inducing action in cancer cells as compared normal cells [116]. There are several ongoing and completed clinical phase studies targeting TRAIL apoptotic pathway by using agents including monoclonal antibodies and recombinant human proteins, and these studies are giving promising results with no significant toxic effects [117].

TNF and TNF receptor 1–2 (TNFR1 AND TNFR2)

TNF is expressed as a trans-membrane 26-kDa protein and then undergoes proteolytic cleavage to form 17-kDa trimeric soluble cytokine. The resulting TNF functions by binding to two different receptors, TNRF1 and TNFR2, which are also trimeric trans-membrane proteins [90]. TNF is produced by immune cells including activated natural killer cells, T cells, and activated monocytes/macrophages and a wide range of non-immune cells such as fibroblasts [118]. TNF/TNFR signaling is well known to be involved in various cellular functions such as apoptosis, cell proliferation, and differentiation [119].

TNF-mediated apoptosis is generally carried out by TNFR1 (DR1), since only TNFR1 is known to contain DD [87]. Binding of TNF to TNFR1 triggers the recruitment of TRADD protein through its DD. Then, TRADD interacts with FADD resulting in the recruitment of pro-caspase-8, which is proteolytically cleaved to active caspase-8. Caspase-8 then activates caspase-3 responsible for apoptotic cell death [120]. The activity of caspase-8 is strictly regulated by a negative inhibitor protein cFLIP that contains DED instead of DD. cFLIP interacts with pro-caspase-8 to prevent its continuous recruitment to the TNFR1 DISC [121]. TNF-induced cell death occurs only under stress conditions such as altered cell metabolism, inhibition of cell cycle progression, and protein synthesis [120]. Therefore, it only induces apoptosis in transformed cells (cancer cells), virus-infected cells, or stressed cells, not in normal healthy cells. Interestingly, TNFR2 has been shown to function in cell proliferation unlike TNFR1 [122].

In a recent study, IL32-α, a novel cytokine, was found to inhibit colon cancer cell growth in an experimentally generated colon cancer model by increasing TNFR1-induced cell death signaling, which was evidenced by increased expression of TNFR1. Yu et al. [123, 124] found a new way of apoptosis induction in adenosine-treated colon cancer cells, which included increased expression of TNFR1. TNFα secretion increased in acute myeloid leukemia cells after treatment with SMAC mimetic and IFNα combination [125]. Inhibition of TNFα and TNFR1 by a pharmacological inhibitor and genetic silencing, respectively, reduced SMAC mimetic/IFNα-triggered apoptosis. Tao et al. suggested that survivin, an inhibitor of apoptosis, inhibitor induced programmed cell death in Wilms tumor cells by increasing expression of TNFR1 signaling [126].

As well as TNFα, lymphotoxin α (LTα) which is secreted as a homotrimer binds to TNRF1 [127]. Even though both TNFα and LTα bind signal via TNFR1, LTα was found to possess less ability to induce TNRF-mediated cell death in an early study [128]. However, a recent research by Etemadi et al. displayed that LTα has same potential to induce apoptosis via TNFR1 signaling [129].

Genetic changes in the promoter region of TNFα have been well studied in several human diseases to find correlation between apoptosis induction and TNFα expression. De Oliveria et al. studied the effect of a polymorphism, TNF-α-857 C/T, on gastric cancer patients, found that this mutation decreased mRNA level of TNFα resulting in resistance to apoptosis [130].

Death receptor 3

Death receptor 3, also known as APO-3, has four characteristic cysteine-rich motifs with molecular weight of 53.5 kDa. Death receptor 3 (DR3) gene is localized on chromosome 1 and encodes a type II trans-membrane protein. Similar to other TNFR family members, DR3 has a DD in its cytoplasmic part and initiates apoptotic signaling [87].

Most of the studies have been related to DR3 involvement in immune system modulation due to its frequent expression on lymphoid tissues such as the spleen, thymus, and peripheral blood lymphocytes [131]. The role of DR3 in the development of some human malignancies has also been enlightened. DR3 expression was shown to be increased in human colon cancer cells treated with cordycepin, a deoxy form of adenosine, resulting in apoptosis induction [132]. A specific phenolic compound triggered apoptosis in human non-small-cell lung cancer (NSCLC) cells by increasing the expression of DR3 [133]. Silencing of DR3 via siRNA approach reversed its growth inhibitory effect.

p53

p53 is encoded by human TP53 gene localized on the short arm of chromosome 17 with a molecular mass of 43.7 kDa [134]. It occupies 19.200 bp including 11 exons. There are various p53 isoforms based on alternative splicing of TP53 gene; some of which play opposite roles as compared to p53, while others have similar functions like full-length p53 [135].

Human p53 protein is composed of the following three different domains with important functions: the DNA-binding domain, the N-terminal trans-activational domain, and the C-terminal oligomerization domain [136]. DNA-binding domain binds to response elements of target genes, whereas the N-terminal trans-activation domain forms binding sites for several negative or positive regulators. The C-terminal domain undergoes alternative splicing and posttranslational modifications [137].

p53 acts as tetrameric transcription factor, which controls the expression of a large set of genes involved in significant cellular processes including DNA damage detection, cell cycle arrest, apoptosis, DNA repair, and senescence [134]. It is both involved in intrinsic and extrinsic pathways of apoptosis by inducing transcription of several proteins like PUMA, Bid, Bax, TRAILR2, and CD95, which is called transcription-dependent apoptotic pathway of p53 taking place in the nucleus [138]. In this pathway of p53-dependent apoptosis, the trans-activation domain of p53 interacts with the players of basal transcription machinery such as the transcriptional coactivator p300/CBP [139]. In normal healthy cells, p53 levels are very low due to its rapid turnover; however, if a damage is detected in the cells, p53 becomes stabilized resulting increased p53 level [140]. p53 stability is controlled by mouse double minute 2 (MDM2) gene, which is an E3 ubiquitin ligase that negatively regulates p53 stability through ubiquitination, thus proteosomal degradation. MDM2 also inhibits the interaction between the p53 trans-activation domain and the components of transcription machinery [141]. MDM2

has been found to be overexpressed in many cancer cells, which leads to neutralization of interaction between p53 and transcription machinery components, thus impairing transcription-dependent apoptosis [142, 143].

p53 has the ability to activate intrinsic pathway of apoptosis by inducing the transcription of especially apoptotic Bcl-2 family genes such as PUMA [144]. p53 induces PUMA mRNA expression immediately in response to DNA damage by binding the two p53-responsive elements in the *PUMA* promoter. p53 binding results in the acetylation of core histones, H3 and H4, which is responsible for chromosome decondensation and transcriptional activation [145]. Similarly, TRAIL-R2 expression is induced by p53, which binds to a p53-responsive element in TRAIL-R2 promoter in order to induce extrinsic pathway of apoptosis [140]. As an alternative mechanism, p53 functions a transcriptional repressor of certain anti-apoptotic genes including survivin which promotes caspase activation [146]. p53 is also directly involved in apoptosome formation by activating transcription of Apaf-1 gene including a p53 response element in its promoter [54]. In response to DNA damage, p53 is also displayed to activate caspase-6 cleaving nuclear envelope protein lamin A and various transcription factors through a response element within the third intron of the gene [147].

As well as transcription-dependent functions of p53, its transcription-independent functions in terms of apoptosis have been defined. p53 induces apoptosis by acting directly at mitochondria. p53 trans-locates to the mitochondria in response to apoptotic signal, where it forms inhibitory complexes with Bcl-XL and Bcl-2 causing the permeabilization of the mitochondrial membrane and cytochrome c release [148]. The interaction between p53 and Bcl-XL and Bcl-2 is mediated by p53 trans-activation domain like p300/CBP binding, even though these two modes of p53-dependent apoptosis induction occur in different cellular compartments [149]. Moreover, cytosolic p53 might induce the activation of pro-apoptotic Bax via direct protein-protein interactions [150]. Leu et al. displayed that p53 interacts with pro-apoptotic mitochondrial membrane protein Bak, which makes Bak undergo oligomerization and releases cytochrome c from mitochondria. Binding of p53 to Bak damages interaction between Bak and anti-apoptotic Mcl-1 [151].

Mutations in p53 gene have been considered most common genetic changes in cancer. Mutant p53 proteins can both lose their native tumor suppressor activity and provide active tumor development [152]. Missense mutations form the majority of alterations in p53 gene, which commonly occur in the DNA binding domain of p53, thus preventing p53 from promoting target gene expression [153]. Recently, Saleem et al. analyzed loss of function mutations in p53 gene in the patients of oral squamous cell carcinoma and found that AGT to ACT missense mutation in DNA binding domain may result in impaired p53 function. In chronic lymphocytic leukemia patients, missense mutations in DNA binding motif were correlated with poor survival [154, 155].

p53 has been considered as a significant player of apoptosis in many studies, and there is a growing accumulation of articles revealing its role in many malignancies. In a recent study by Wang et al., acute pro-myelocytic leukemia cells were subjected to apoptosis when treated with combination of tetraarsenictetrasulfide and arsenic trioxide through upregulation of p53 and its target gene Bax. Transcription-independent role of p53 in apoptosis induction was identified in a study in which p53 trans-located to mitochondria and induced mitochondrial membrane depolarization in HUVEC cells exposed to heat stress [156, 157].

There is an increasing attention to develop different strategies that can modulate p53-dependent apoptotic pathways such as inhibition of p53-MDM2 interaction using MDM2 inhibitors, restoring mutated p53 back to its wild-type form and p53 vaccines [158–160].

MicroRNAs in extrinsic and intrinsic apoptotic pathways

Changes in the apoptotic response in cancer can result in tumor initiation, progression, and treatment resistance [3]. There are numerous studies including the roles of microRNAs in the control of apoptosis, and these microRNAs display their effects by directly targeting genes involved in both extrinsic and intrinsic pathways of apoptosis [161]. These microRNAs (miRNAs) can be classified as oncogenic and tumor suppressive miRNAs [162]. One of these miRNAs is miR-130a that was found to reduce drug resistance in non-small-cell lung cancer by targeting MET proto-oncogene and to sensitize this cancer cells to TRAIL-induced apoptosis by inhibiting miR-221 and miR-222, which are upregulated by MET and involved in TRAIL resistance [163]. Therefore, miR-221 and miR-222 are oncogenic miRNAs that induce drug resistance and block apoptosis in several cancer types such as gliomas [164]. Another oncogenic miRNA exerting its anti-apoptotic effects by inhibiting FasL directly is miR-21, which is found to be upregulated in advanced pancreatic cancer patients [165]. miR-24 regulates apoptosis by binding to coding sequence of Fas-associated factor 1 (FAF1) mRNA and induced apoptosis of several different types of cancer [166]. miR-21 also negatively regulates PTEN, a tumor suppressor gene, involved in the apoptotic pathway through the formation of DISC complex in many tumors such as breast and gastric cancers [167, 168]. In gastric cells, miR-21 upregulated and decreased PTEN expression, resulting in significant suppression of trastuzumab-induced apoptosis [168]. miR-200c sensitized cells to apoptosis by directly targeting Fas-associated phosphatase-1 (FAP-1), which is an inhibitor of Fas-induced apoptosis [169]. miRNA-886-5p inhibited apoptosis of human cervical cancer cells by downregulating Bax [170]. Zhou et al. (2010) [171] found that miR-125b was upregulated

in taxol-resistant breast cancer cells and suppressed apoptosis. Bak1, pro-apoptotic Bcl-2 antagonist killer 1, was identified as the direct target of miR-125b. miR-25 and miR-32 could directly target the pro-apoptotic function of Bim, therefore suppressing apoptosis in ovarian and human myeloid leukemia cells, respectively [172, 173]. In addition, miR-483-3p might target PUMA, whose enforced expression protects cells from apoptosis [174]. miR-34 decreases the expression of Bcl-2, resulting in an increase in apoptosis [175]. In this study, downregulation of miR-34 induced cell proliferation and invasion in malignant mesothelioma. miR-15b/16 could downregulate Bcl-2, thereby triggering apoptosis [176]. miR-153 induced apoptosis of glioblastoma cells by targeting 3′-UTR of Bcl-2 and Mcl-1 [177]. miR-491 could induce apoptosis in colorectal cancer cells by downregulating anti-apoptotic Bcl-XL [178]. There are several studies showing the roles of miRNAs in the regulation of caspase expression. miR-23a and miR-24a blocked mitochondrial apoptosis by inhibiting the expression of caspase-9 [179, 180]. The downregulation of miR-23a increased the 5-FU-induced apoptosis in colon cancer cells [179]. Wu et al. (2014) [181] displayed that miR-421 upregulated in human gastric cell lines and tissues. miR-421 downregulated the expression of caspase-3 and blocked the apoptosis of cancer cells. miR-106b-25 was shown to be upregulated in human prostate cancer and functions by partly inhibiting of caspase-7 expression [182]. Floyd et al. (2014) [183] demonstrated that miR-582-5p and miR-363 inhibit apoptosis by directly targeting caspase-3 and caspase-9 in glioblastoma. Caspase-3 has been shown to be a target of miR let-7a in human squamous carcinoma cells and hepatocellular carcinoma cells [184]. Curcumin induced apoptosis of NSCLC by the downregulation of miR-186, whose direct target is caspase-10 [185]. Based on all this evidence discussed in this particular review, miRNAs play key regulatory roles in apoptosis and could be important therapeutic targets in cancer (Fig. 2).

Bioactive sphingolipids in apoptotic pathways

Bioactive sphingolipids are a family of membrane lipids that have many regulatory roles in several cellular events such as cell proliferation, senescence, adhesion, migration, and also apoptosis [186]. Ceramide, the central molecule of bioactive sphingolipid metabolism, was reported as a regulator of apoptosis in many studies. Treatment of cancer cells with radiation and chemotherapeutics such as vincristine, daunorubicin, gemcitabine, and etoposide result in ceramide accumulation in the cells as a secondary effect of these therapeutics [187]. Decreased ceramide levels result in the development of drug resistance in cancer cells [188]. In vitro studies indicated that treatment of cancer cells with ceramide triggers the release of cytochrome-c from the mitochondria [189]. Additionally, it was reported that anti-apoptotic Bcl-2 blocks ceramide channels in an independent manner from Bak and Bax [190]. Furthermore, ceramide also induces Bax-mediated apoptosis in several types of cancer including, breast, prostate, and colon cancers [191]. Ceramide was reported that it activates cathepsin D, an inducer of apoptosis in a form of lysosomal aspartyl protease, in response to gemcitabine treatment [192]. Unlike ceramide, the other member of sphingolipid family, sphingosine 1-P (S1P) is related to cell proliferation, survival, and also inhibition of apoptosis [193]. S1P was reported to cause

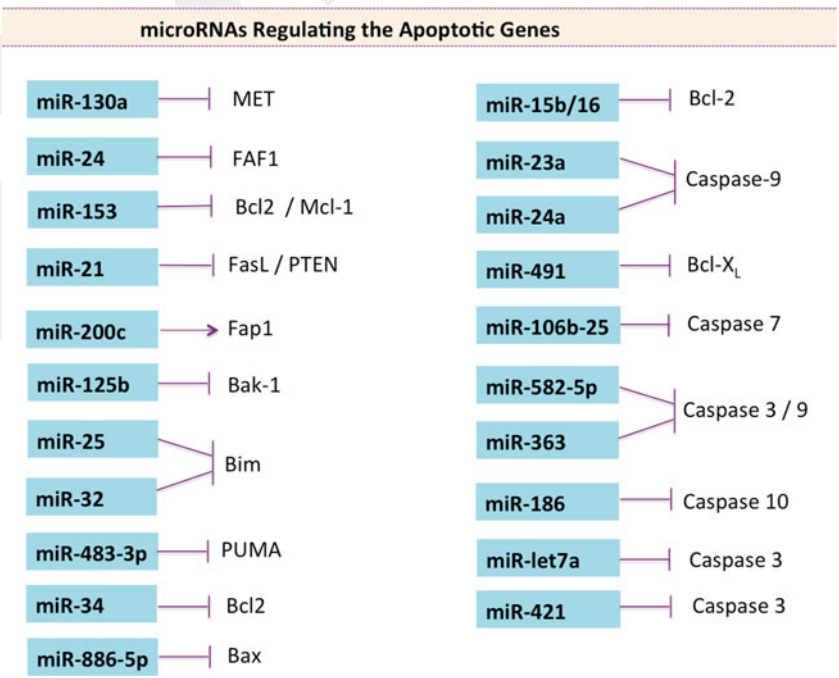

Fig. 2 miRNAs involved in the apoptotic pathways. Some of the miRNAs can inhibit apoptosis by targeting the death-receptor pathway including miR-21, miR-24, and miR-200c. In the mitochondrial pathway, various miRNAs could target Bcl-2 family proteins and caspases including miRNA-886-5p, miR-125b, miR-25, miR-32, miR-483-3p, miR-34, miR-15b/16, miR-153, miR-491, miR-23a and miR-24a, miR-421, miR-106b-25, miR-582-5p and miR-363, miR let-7a, and miR-186

angiogenesis by VEGF signaling, which then triggers RAS and MAPK signaling in cancer cells. By this way, S1P causes cytoskeleton reconstruction and apoptosis inhibition [194]. While increased ceramide levels cause induction of apoptosis, increased S1P levels cause inhibition of apoptosis [195]. S1P leads to drug resistance due to its anti-apoptotic function. Sphingosine kinase-1 (SK1), which catalyzes the conversion of apoptotic ceramide to anti-apoptotic S1P, was also reported to decrease apoptotic effects of chemotherapeutic agents in prostate cancer [196]. Like S1P, glucosylceramide (GC), the other important member of sphingolipid family, is also known as a powerful anti-apoptotic molecule [193]. Glucosylceramide synthase (GCS), the enzyme catalyzing the conversion of apoptotic ceramide to anti-apoptotic GC, was found to be increased in drug-resistant cancer cells [193]. Treatment of pancreatic cancer cells with a ceramide analogue causes the accumulation of ceramide in the mitochondria, and by this way, ceramide reduces drug resistance and triggers apoptosis [197]. Additionally, treatment of pancreatic cancer cells with ceramide in combination with a GCS inhibitor, PDMP, decreases tumor growth in vivo [198]. Moreover, GC degradation increases ceramide generation resulting in the trigger of apoptosis and also reduced tumor growth in melanoma xenografts [199]. Reduced tumor growth was also reported in mouse models with breast cancer treated with C6:ceramide [200]. Furthermore, a type of sphingosine kinase inhibitor, SK2, was reported to trigger apoptosis, inhibit cell proliferation, and decrease tumor size in mouse models bearing hepatoma, mammary adenocarcinoma, and kidney carcinoma [201, 202]. Safingol, the first agent used in the clinic due to its ability in sphingosine kinase inhibition, increases apoptotic effects of chemotherapeutics used in cancer therapy [203]. An FDA-approved drug that has inhibitory effects on SK1 and SK2 activities, FTY720, inhibits cell proliferation and leads to apoptosis in mice bearing breast cancer or melanoma [204]. In chronic myeloid leukemia (CML) cell lines, increase in serine palmitoyltransferase levels via BCR/ABL inhibition result in activation of apoptotic signals [205]. GCS inhibition in CML cell lines bearing T315I mutation results in apoptosis via activating GSK-3 [206]. Another study reported that ceramide triggers apoptosis via activating p38, caspase-8, and c-Jun N-terminal kinase (JNK) in K562 CML cells [207]. Many studies from our laboratory also showed effects of bioactive sphingolipids on apoptosis. In K562 CML cell lines resistant to nilotinib, apoptotic ceramide synthase-1 and Bax genes were found to be downregulated while anti-apoptotic SK1 and GCS genes were overexpressed, and inhibition of these overexpressed genes sensitized the cells against nilotinib treatment [208]. Our studies also showed that when we overexpress GCS in imatinib-sensitive K562 cells, the cells developed resistance against the drug via inhibiting apoptosis. Inhibition of GCS and SK1 triggered apoptosis via leading to ceramide accumulation in imatinib-resistant and imatinib-sensitive K562 CML cell lines [209]. Additionally, when we treated K562 human CML cell lines and HL60 human acute pro-myelocytic leukemia cell lines with resveratrol in combination with ceramide analog, GCS inhibitor, or SK1 inhibitor, apoptotic effects of resveratrol increased synergistically in the cells treated with resveratrol and ceramide combination, while the cells treated with resveratrol and GCS or SK1 combinations resisted to apoptosis as compared to the control group [210, 211]. Furthermore, our studies also showed that GCS and SK1 inhibition in combination with nilotinib or dasatinib treatment synergistically triggers apoptosis in K562 and Meg01 human CML cell lines [212, 213].

Briefly, while ceramide is a powerful apoptotic molecule, glucosylceramide and S1P generated from ceramide by GCS and SK1 activities, respectively, are powerful anti-apoptotic molecules, and therefore, alterations in intracellular levels of these sphingolipids could be a novel approach for cancer therapy.

Summary and perspectives

Apoptosis is highly regulated way of cell death, which is crucial for all higher-level organisms to balance tissues homeostasis and control cell proliferation as well as remove damaged or unnecessary cells. Apoptosis has its own morphological and biochemical properties where caspases play a central role at the end. Here, we have focused on aspects of apoptosis in terms of critical genes and their products together with their role in both intrinsic and extrinsic pathways of apoptosis. In this specific vital process, diverse groups of molecules function compatibly for strict regulation. Any alterations or abnormalities occurring in apoptotic processes contribute to development of human diseases and malignancies especially cancer. Deep understanding of apoptotic signaling mechanisms, individual players, and genes involved in apoptosis have provided a great opportunity to develop novel agents that make apoptosis deficient cells sensitive to apoptosis.

Compliance with ethical standards

Conflict of interest None

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