Noxa: Role in Cancer Pathogenesis and Treatment

Rami Z. Morsi¹, Rouba Hage-Sleiman², Hadile Kobeissy⁴ and Ghassan Dbaibo³⁴⁵,*

¹Faculty of Medicine, American University of Beirut, P.O. Box 11-0236, Riad El Solh, Beirut, Lebanon; ²Department of Biology, Faculty of Sciences, Lebanese University, Hadath, Lebanon; ³Department of Pediatrics and Adolescent Medicine, Division of Pediatric Infectious Diseases, Faculty of Medicine, American University of Beirut, P.O. Box 11-0236, Riad El Solh, Beirut, Lebanon; ⁴Department of Biochemistry and Molecular Genetics, Faculty of Medicine, American University of Beirut, P.O. Box 11-0236, Riad El Solh, Beirut, Lebanon; ⁵Center for Infectious Diseases Research, American University of Beirut, Beirut, Lebanon

**Abstract:** The B-cell lymphoma 2 (Bcl-2) family proteins play an important role in regulating apoptosis, or programmed cell death, in response to several extracellular and intracellular signals. These proteins are either pro-apoptotic or anti-apoptotic. The pro-apoptotic Noxa is a Bcl-2 family protein that belongs to a subclass of BH3-only proteins. Noxa induces apoptosis via p53-dependent and/or p53-independent mechanisms. While Noxa may play a limited role in apoptosis, it is a crucial player that interacts with several proteins in the apoptosis pathway, highlighting its importance in the pathogenesis and treatment of certain cancers. In this review, we will elucidate the mechanisms by which Noxa regulates apoptosis and review the roles of chemotherapeutic drugs in relation to Noxa.

**Keywords:** Bcl-2, apoptosis, Noxa, cancer, B-cell lymphoma 2, p53.

1. INTRODUCTION

1.1. Overview of the Bcl-2 Family

Apoptosis, also known as programmed cell death or cell suicide, is a cellular process of controlled death without any significant inflammation [1, 2]. Members of the cysteiny1 aspartate-specific protease (caspase) family are involved in several proteolytic steps in the two main signaling pathways that lead to apoptosis, the extrinsic pathway and the intrinsic pathway [3, 4]. The extrinsic pathway initiates apoptosis by activating transmembrane death receptors of the tumor necrosis factor (TNF) receptor gene family. The TNF receptors (TNFR) share a cytoplasmic domain, known as the “death domain,” which plays an important role in transmitting the death signal intracellularly. Two ligands, FasL and TNF-α, can bind to their corresponding receptors Fas and TNFR, respectively. Once the ligand binds, an adapter protein, Fas-associated death domain (FADD) associates with procaspase-8, eventually leading to autocatalytic activation of procaspase-8. Activated caspase-8 ultimately leads to death-receptor mediated apoptosis [5]. In the intrinsic pathway, certain stimuli, such as hypoxia, radiation, and toxins, induce internal cellular damage that triggers different stress-response pathways that converge on the mitochondria where they result in a change in the proportions of the pro- and anti-apoptotic factors. This change increases the mitochondrial permeability and induces the release of cytochrome c [8]. Cytochrome c is necessary for the activation of caspase-9, a member of a family of proteases necessary for the activation and execution of apoptosis after it interacts with the adapter protein Apaf-1.

Once the initiator caspases caspase-8 and caspase-9 are activated, they cleave and activate caspase-3 [9]. These pathways are highly dependent on the B-cell lymphoma 2 (Bcl-2) family, which contains several members that play either pro-apoptotic or anti-apoptotic roles in response to several intracellular and extracellular signals, including DNA damage, hypoxia, cytokine deprivation, certain drugs and many other stressors [10, 11]. The Bcl-2 protein itself is anti-apoptotic as it inhibits the release of cytochrome c, subsequently activating caspase 9 and Apaf-1 apoptosome complex. Additional caspases seem to be in operation to convey Bcl-2’s anti-apoptotic mechanism [12]. Bcl-2 protein also inhibits the activation of both caspase-2 and caspase-3, preventing apoptosis via a caspase-3 dependent cascade at a downstream level from cytochrome c release [9]. Moreover, the BH3 interacting-domain death agonist (Bid) functions to link both the extrinsic and intrinsic pathways as its N-terminal region is cleaved by death receptor-activated caspase-8 generating a truncated Bid (tBid) [13, 14], which then activates the intrinsic pathway via Bcl-2 associated X protein (Bax) activation [15]. Therefore, the Bcl-2 family proteins seem to play an important role in regulating this caspase cascade in both the extrinsic and intrinsic pathways [16].

These Bcl-2 family proteins are either pro-apoptotic or anti-apoptotic, and their function depends largely on the presence of conserved BH domains. The anti-apoptotic proteins, such as Bcl-2, B-cell lymphoma-extra large (Bcl-xL), induced myeloid leukemia cell differentiation protein (Mcl-1), Bcl-2-like protein 2 (BCL2L2/Bcl-W), and A1, have four BH domains in common (BH1-4). However, the pro-
apoptotic proteins either share three BH domains (BH1-3) such as Bax, Bcl-2 homologous antagonist/killer (Bak), or Bcl-2 related ovarian killer (Bok), or only possess the BH3 domain, such as p53-upregulated modulator of apoptosis (Puma), Noxa, Bid, Bcl-2-interacting mediator of cell death (Bim), Bcl-2-interacting killer (Bik), Bcl-2-modifying factor (Bmf), and harakiri (Hrk) [3, 10, 11, 17-21].

The pro-apoptotic activities of BH3-only proteins are induced by several mechanisms. For instance, some BH3-only proteins are upstream of Bax and Bak, and the BH3-only proteins Bim and Bid function as direct activators of Bax and Bak [22-24]. Once the effectors Bax and Bak are activated, they form an oligomer and increase the mitochondrial outer membrane permeability via pore formation. After pore formation, cytochrome c and second mitochondria-derived activator caspase (SMAC) are released into the cytosol where they initiate the caspase-cascade process of apoptosis [10, 11, 17, 19, 22]. Not only can BH3-only proteins directly activate the effectors Bax and Bak, but another subclass of the BH3-only proteins known as sensitizers/de-repressors inhibit the anti-apoptotic network [23, 24]. This subclass of BH3-only proteins may undergo to post-translational modifications leading to their sequestration, which restrains them from interaction with anti-apoptotic Bcl-2 family members [25]. Sensitizers/de-repressors like Puma, Bid, and Bim appear to have the ability to bind all the anti-apoptotic Bcl-2 members, whereas Bad, Noxa, and Bmf can bind certain members only [26-30]. The mechanisms of function of the BH3-only protein Noxa have not been fully elucidated. This review aims to summarize our current understanding of Noxa, its role in the pathogenesis of cancer, and its potential as a therapeutic target in treating several types of cancer.

1.2. Overview of Noxa and its Interaction with other Bcl-2 Family Members

It has been established that Noxa belongs to a subclass of BH3-only proteins called the sensitizers/de-repressors. Noxa binds to the anti-apoptotic Bcl-2 members at their hydrophobic grooves, and this binding prevents any further interactions between anti-apoptotic members and pro-apoptotic members [10]. When pro-apoptotic conditions develop, Noxa competes with, and displaces, Bax and Bak bound to anti-apoptotic members. Noxa can also interact with Mcl-1 and, to a lesser extent, A1 where its pro-apoptotic actions result from promoting their degradation [31-33]. Thus, when Noxa displaces Bax from the Bak-Mcl-1 complex, for example, activation occurs due to the degradation of Mcl-1 [26, 34]. Therefore, Noxa’s specificity for Mcl-1 and A1 makes it a potentially useful target when these proteins are pathologically overexpressed.

1.3. Splicing Variants of Noxa

Noxa’s specificity is determined by alternative splicing of its exons. Human Noxa, also previously referred to as phorbol-12-myristate-13-acetate-induced protein 1 (PMAIP1), is made up of 54 amino acids and one BH3 domain. However, mouse Noxa is made up of 103 amino acids and two BH3-only domains [26]. There are three different splicing variants of human Noxa: Noxa (PMAIP1), Noxa splicing variant-1 (NSV-1 or/and Noxa splicing variant-2 (NSV-2). NSV-1 and NSV-2 do not contain a BH3 domain [35]. These two splicing variants, when induced by the p53-inducing agent etoposide, and a proteasome inhibitor, PS341, fail to induce p53-mediated apoptosis. Therefore, NSV-1 and NSV-2 have no pro-apoptotic activity, because these variants lack the pro-apoptotic BH3 domain. Moreover, due to the short half-lives of their mRNAs, these variants’ expression could not be detected at a protein level under normal physiologic conditions or even after stress was induced. Also, because of their low expression levels, it is difficult to determine whether these two Noxa variants affect negatively Noxa’s activity [35]. Therefore, the role of these two Noxa variants remains unclear.

2. REGULATION OF NOXA EXPRESSION

2.1. p53-dependent Regulation

Noxa activity was initially thought to be due to p53-induced apoptosis. Oda, et al. (2000) determined that Noxa mRNA levels rapidly increase after introducing p53 via adenovirus into p53-/- or wild type mouse embryonic fibroblasts (MEF) or in wild type thymocytes exposed to γ-irradiation but not in p53-/- thymocytes [26]. Oda, et al. (2000) also determined that expression of Noxa gene involves direct activation of its promoter by p53. After isolation of the mouse Noxa gene, it was found to have three exons with the BH3 motifs A and B encoded by exons 2 and 3, respectively. The mouse Noxa gene contains one potential p53-recognition sequence located in the promoter region at -155 to -174 [26]. This finding led to the discovery of a similar p53-response element in human Noxa, located upstream of the transcription start site, consisting of 195 base pairs [26, 36].

The discovery of the p53-response element led to the investigation of a role for Noxa in p53-induced apoptosis. Oda, et al. (2000) used human Saos2 cells that do not normally express p53. Screening for human homolog of Noxa cDNA was performed, and the cloned cDNA was found to be nearly identical to adult T cell leukemia-derived PMA-responsive (APR), also known as human Noxa. It was also found that APR contains a BH3 motif of 8-9 amino acids. This motif is equivalent to the mouse Noxa’s motif B, given the fact that the human Noxa gene lacks a DNA segment corresponding to exon 2 in mouse Noxa [26]. It was established that increased levels of human Noxa expression induce apoptosis in several cells, including Saos2, which lack p53, in a BH3-dependent manner. The human Noxa gene contains a promoter region with one p53-response element. The authors support this finding by infecting Saos2 cells with adenovirus expressing p53, leading to increased human Noxa mRNA expression levels. When Noxa expression is inhibited by antisense oligonucleotide, apoptosis is abrogated. Similar results were obtained in the hematopoietic cell line BAF-3 that contains p53 [26]. Put together, these findings support a role for human Noxa as a target of p53 and a mediator of p53-induced apoptosis [26].

2.2. p53-independent Regulation

While it has been established that Noxa is a mediator of p53-induced apoptosis, Noxa induction can also occur in the absence of p53 [37]. Noxa has been found as a downstream target for p53-target gene growth differentiation factor-15 (GDF15) [38]. GDF15, also known as macrophage inhibitory
cytokine-1 (MIC-1), is a divergent member of the transforming growth factor beta (TGF-beta) superfamily [39, 40]. A microRNA (miRNA), embedded in the intron of GDF15 gene, identified as miR-3189, is found to have tumor suppressive function by upregulating the expression of p53 targets, including Noxa, in a p53-independent manner both in vitro and in vivo [38].

Additionally, oncogenic stress can induce Noxa expression in a p53-independent fashion [41]. This finding is highlighted in one study, where E1A overexpressed in p53-deficient Saos2 cells caused oncogenic stress, and this stress resulted in Noxa induction. Investigation of this finding led to the discovery that adenovirus E1A protein induces Noxa expression independently of p53 by activating p73, a member of the p53 family of proteins [41]. p53-independent Noxa expression also increases when HDM2 or E3 ubiquitin-protein ligase Mdm2 — a negative regulator of p53 — is inhibited [41].

3.1. Interrelation with Bax/Bak and Cytochrome c Release from Mitochondria

Noxa’s role in apoptosis is associated with its mitochondrial localization. This finding is particularly highlighted in Oda, et al. (2000) study with HeLa cervical carcinoma cells overexpressing Noxa [26]. Noxa requires its functional BH3 domain in order to perform its apoptotic function. Once apoptosis is triggered, cytochrome c is released followed by caspase activation. Some studies did not report any direct binding of Noxa to pro-apoptotic Bax or Bak, and it was originally thought that Noxa promoted Bax-mediated mitochondrial dysfunction without any direct binding but rather indirectly via inhibition of anti-apoptotic Bcl-2 family members [26, 49]. However, more recent studies have shown that direct interaction of Noxa with either Bax or Bak occurs during apoptosis induction [50-52]. Using bimolecular fluorescence complementation (BiFC), it was found that Noxa not only forms complexes with Mcl-1 and Bcl-xL, but it also forms complexes with Bax and Bak. Both Bax and Bak share similar homology and are considered “effectors” for mitochondrial outer membrane permeabilization. However, they differ in their interactions with BH3-only proteins, including Noxa. Bax interaction with BH3-only proteins involves H1α, which has a proposed function as a “receptor” for activator BH3-only proteins. Interactions between Bax and Noxa could be prevented if binding of anti-apoptotic proteins to an H1α-inclusive region occurs [52]. On the other hand, two conditions are required for Noxa’s interaction with Bak. Both the H1α and the BH3 domains of Bak are necessary to facilitate binding of Noxa. Using time-lapse microscopy, it was also established that direct association of Noxa to Bax and Bak subsequently leads to programmed cell death. It is also important to note that these direct associations occur exclusively at the mitochondrial level [52].

3.2. Relationship between Noxa and Bad

While it has been shown that Noxa can directly bind Bak and Bax to promote apoptosis, this can also occur by inhibiting anti-apoptotic proteins from performing their actions. One of the major mechanisms of inhibiting anti-apoptotic proteins involves Noxa’s relationship with Bad. Chen, et al. (2005) demonstrate that Noxa could associate with Bad, which targets Bcl-2, Bcl-xL, and Bcl-w. When both Noxa and Bad are overexpressed, they both act like the non-selective BH3-only proteins, Puma or Bim, in the inhibition of all anti-apoptotic Bcl-2 family proteins [31]. Therefore, when Noxa and Bad are simultaneously overexpressed, they have a more potent apoptotic effect.
Bad can also work synergistically with Noxa to induce apoptosis. Bcl-xL can prevent apoptosis induced by Noxa. Noxa can directly bind to Bak. Noxa can target Mcl-1 indirectly or directly, possibly via ubiquitylation, thus releasing Bim and Puma from their sequestration by Mcl-1, subsequently exerting a pro-apoptotic effect. Bad can also work synergistically with Noxa to induce apoptosis. Bcl-xL can prevent apoptosis induced by Noxa (not illustrated in figure). Noxa can also interact with Bfl-1/A1 covalently regulated via an intramolecular disulfide bridge [1]. Also illustrated in the figure is how p53 can directly translocate to the mitochondria and alter mitochondrial permeability [6].

3.3. Selectivity for Mcl-1 and Bfl1/A1

It has been noted that while Bim and Puma among others can non-selectively bind all pro-survival Bcl-2 family proteins, Noxa, on the other hand, selectively binds Mcl-1 and to Bfl1/A1 [31]. The selectivity for Mcl-1 and Bfl1/A1 is based on specific amino acid residues residing in the BH3 domain, which contact residues in the hydrophobic groove of the anti-apoptotic Bcl-2 family proteins. It was discovered that if only two amino acids are changed in the human Noxa BH3 domain – K35E and F32I – Noxa’s binding to Bcl-xL decreases more than 20-fold and 100-fold, respectively [31]. To further determine Noxa’s specificity for Mcl-1, Chen, et al. (2005) analyzed the binding specificity of Bimiso, an isoform of Bim in which the BH3 domain is replaced with that of Noxa. They discovered that the Bimiso chimeric protein behaves like Noxa, and binds Mcl-1. Thus, it was inferred that Noxa’s limited apoptotic potential is based on its BH3 domain, and consequently, its inability to bind all the pro-survival Bcl-2 family proteins except for mainly Mcl-1 and Bfl1/A1.

Earlier studies reached a consensus about how Noxa interacts with Mcl-1 with a much greater affinity than with Bfl1/A1 [31, 53, 54]. However, a recent study found that human Noxa interacts with Mcl-1 with higher affinity compared to human Mcl-1; this study hypothesized that human Noxa and Bfl1 interaction is regulated by an intramolecular disulfide bridge with a cysteine residue at position 25 in the BH3 domain and a cysteine residue at position 51 in the transmembrane helix domain of human Noxa, allowing for a more covalent and potent interaction between the two proteins [1]. Such an interaction may drive research towards designing drugs targeting Bfl1/A1 and Noxa interaction. Nevertheless, the selectivity for Mcl-1 and Bfl1/A1 may be advantageous when targeting Mcl-1 or Bfl1/A1 but when a broader inhibitory activity against pro-survival Bcl-2 family members is needed, co-expression of other pro-apoptotic proteins, especially the BH3-only protein Bad, would be required [31].

While Noxa selectively binds to Mcl-1 and Bfl1/A1, the events following this binding are still not clearly understood. Some studies found that Noxa targets Mcl-1 for proteasomal degradation, subsequently inducing apoptosis. Noxa expression tends to decrease Mcl-1 expression by first ubiquitinating its own lysine residues independently of Mcl-1 and then translocating Mcl-1 from the cytosol into the mitochondria by binding to the Noxa BH3 domain. Mcl-1 subsequently gets phosphorylated and ubiquitinated, eventually leading to its degradation by the proteasome [55]. The fate of Noxa/Mcl-1 complex is encoded in the C-terminal tail of the Noxa BH3 domain [32, 56]. It was recently discovered that Noxa is degraded by the proteasome in a ubiquitin-independent manner that does not require any kind of direct association with Mcl-1. Moreover, it was found that single amino acid mutations in each of the three lysine residues at positions 35, 41 and 48 do not alter the ubiquitylation process. Therefore, polyubiquitylation is not a requirement for Noxa degradation [56]. However, the C-terminal sequence of the Noxa BH3 domain is necessary to initiate Mcl-1 degradation.

Czabotar, et al. (2007) discovered that replacing the C-terminal amino acids in Noxa BH3 domain with those in the corresponding position in the Bim BH3 domain (-AYYARR-) leads to Mcl-1 stabilization rather than degradation [32]. Strikingly, however, a chimeric Bad with the Noxa BH3
domain does not initiate Mcl-1 degradation, which means that non-BH3 regions might play a role in Noxa/Mcl-1 degradation [32]. Interestingly, it is possible that a process dependent on Mcl-1 ubiquitylation is responsible for Noxa/Mcl-1 complex degradation. However, this possibility remains unclear [56]. Nevertheless, Mcl-1 degradation is a key initiation step in apoptosis induction by Noxa in the context of histone deacetylase inhibitors, anticancer agents like arsenic trioxide, cytokine deprivation, metabolic stress and UV irradiation [57-61].

3.4. Interplay with Bim and Puma

Noxa, Puma and Bim are all BH3-only pro-apoptotic members of the Bcl-2 family, and their interactions with other proteins and with each other are crucial in apoptosis. One study, for instance, explores the interplay between Bim and Noxa in mediating Bax/Bak-dependent mitochondrial apoptosis. When there are no apoptotic stimuli, Noxa and Bim are sequesters Bim, preventing Bim from performing its apoptotic functions. Either Noxa or Bim can bind to Mcl-1, meaning that upon Noxa induction, Bim is displaced from Mcl-1 sequesteration. Upon Noxa induction, Bim is now Mcl-1-free and can perform its Bax/Bak-mediated apoptotic role. Therefore, Noxa cooperates with Bim by acting upstream of Bim and by displacing Bim from Mcl-1 sequestration [62].

Not only does the interplay between Bim and Noxa help mediate apoptosis, but Puma also appears to act as a functional link with Noxa [63-65]. Much like Bim/Noxa interactions, Puma/Noxa interactions are also associated with Mcl-1. Puma is released from its association with Mcl-1 after Noxa sensitization post-DNA damage. While Noxa itself is a weak stimulus of apoptosis, E1A-expressing MEFs appear to be more sensitive to Noxa’s apoptotic activity. This effect is due to E1A-induced Puma and the resulting synergistic induction of Puma and Noxa in p53-mediated apoptosis [63, 64]. Thus, oncogene-expressing cells become more susceptible to apoptosis due to accumulated Puma sequestering Mcl-1 in mitochondria and to subsequent DNA damage-induced Noxa release of Puma from Mcl-1 [63]. One possible reason as to why this “catch and release” mechanism occurs is due to the different binding capacities of the BH3 domains of Noxa and Puma to p53. Park, et al. (2012) confirm that both Puma and Noxa interact with p53 in vitro, but the DNA binding domain of p53 is found to have a higher affinity towards the BH3 domain of Noxa than that of Puma [65]. p53 also binds to Noxa and Puma at different sites, altering electrostatic interactions, and allowing Noxa or Puma complexes to form with p53, possibly after Puma has been “released” from Mcl-1 [65]. These different Puma/Noxa and p53 interactions, and the different binding affinity of Noxa to p53 could contribute to resolving the relative importance of Puma and Noxa in pro-apoptotic pathways.

3.5. Chromatin Interactions

Noxa has proven to be a key player in apoptosis despite its limited pro-apoptotic function. It is a key facilitator be-
tween pro-apoptotic and anti-apoptotic proteins both indirectly and directly. Its expression is highly regulated so that there is a balance between pro-apoptotic proteins and anti-apoptotic proteins. This balance determines the cell’s fate. In an effort to explore how this balance is regulated, Yang, et al. (2015) hypothesized that special AT-rich binding protein 1 (SATB1) – a mediator of higher-order chromatin organization – is involved in the regulation of cooperative expression of the Bcl-2 and Noxa genes and in altering the apoptotic response by changing the balance between Bcl-2 and Noxa [66]. Bio-informatic analysis shows that the Noxa gene promoter contains SATB1 binding sequences, and this finding suggests that Noxa expression might be regulated via SATB1. It was also demonstrated that the major breakpoint region (mbr) enhancer physically targets the Noxa gene just 3.4 Mb downstream of the Bcl-2 gene on chromosome 18. The Noxa gene promoter might actually be involved in forming a complex with the Bcl-2 gene promoter and the mbr enhancer in the nucleus. Yang, et al. (2015) actually demonstrated that cleavage of SATB1 at an early apoptotic stage results in a switch of the Bcl-2 chromatin loop structure toward the Noxa chromatin loop structure. The reduced binding of SATB1 results in an increase in mbr-Noxa interactions and a decrease in mbr-Bcl-2 interactions [66]. Therefore, the function of SATB1 in co-regulating anti-apoptotic Bcl-2 and pro-apoptotic Noxa on a higher chromatin order is crucial in determining the fate of a cell, and consequently, whether or not cancer might develop.

3.6. Necroptosis

Several studies show how Noxa is involved in apoptosis. Recent studies also describe a role for Noxa in necrosis, or rather, necroptosis, of cancer cells. Necroptosis is a regulated form of cell necrosis, whereby stimulation is mainly induced via the TNF signaling pathway [67, 68]. Receptor-interacting protein kinase (RIPK) 1 and RIPK3 are two important kinases involved in mediating this TNF-induced necroptosis [67, 68]. Seo, et al. (2009) demonstrate how the mitochondrial-targeting domain (MTD) of Noxa is a pro-death domain that induces necrosis in vitro through the release of mitochondrial calcium into the cytosol [69]. This pro-necrotic peptide domain is also found to induce necrosis of cancer cells in vitro after fusing it with neuropilin-1 (NRP-1) peptide [70]. Therefore, this MTD peptide in Noxa could potentially serve as a target for anti-tumor therapies [69, 70].

4. ROLE OF NOXA IN CANCER

Although Noxa plays a restricted role in apoptosis, its ability to interact with several players in the apoptosis pathway, both directly and indirectly, is an integral determinant of a cell’s fate. Therefore, Noxa’s properties make it an important player in oncogenesis and a putative target for chemotherapy. In fact, studies have demonstrated that Noxa has direct and indirect roles in the pathogenesis of certain cancers, including, but not limited to, lung cancer [55, 71-73], leukemias [74-82], rhabdomyosarcoma [83-86], prostate cancer [87], ovarian cancer [88], colorectal cancer [89-91], melanoma [92-95], and multiple myeloma [96, 97]. Treatment of these cancers may involve Noxa-specific drugs, or perhaps BH3 mimetics.
4.1. Lung Cancer

4.1.1. Pathogenesis

Lung cancer is still the most common cancer-related cause of death in the world, despite many advances in diagnosis and treatment. Prognosis for non-small cell lung cancer (NSCLC) remains poor. Therefore, further research on the pathogenesis of the disease is needed in order to improve our understanding and devise new therapeutic approaches. One mechanism for cancer development is when apoptosis is inhibited or deregulated. The mitochondria-mediated intrinsic pathway of apoptosis plays an important role in the pathogenesis of NSCLC, particularly the BH3-only proteins. One study determined that there is a link between low Bim expression and squamous cell carcinoma (SCC) histology and tumor aggression or proliferation [72]. However, there is no significant link observed between Noxa expression and disease stage, histology and survival outcomes. In another study, Noxa induction is actually found to promote apoptosis of human lung cancer cells and consequently, inhibit cancer development. It appears that Mcl-1 provides an escape route for the cells from apoptosis, and is overexpressed in several human cancers, including NSCLC [73].

The stability of Noxa/Mcl-1 complex is crucial in the pathogenesis of NSCLC. However, this stability, or rather its instability, is also highlighted in the development of small cell lung cancer (SCLC) [55, 71]. Therefore, Noxa’s role in preventing Mcl-1 from being overexpressed in normal cell growth conditions is crucial. Because of this complex formed between Noxa and Mcl-1, several therapeutic approaches have been developed to treat both types of lung cancer.

4.1.2. Treatment

SCLC is more responsive to chemotherapy than NSCLC. Based on this information, there have been several therapeutic approaches to treat SCLC. Sensitivity to BH3 mimetic drugs like ABT-737, and its orally available derivative ABT-263, is highly determined by Noxa expression [55, 71]. ABT-737 functions by binding Bcl-xL and Bcl-2 and releasing Bak from them to translocate to the mitochondria. Under conditions where Mcl-1 is expressed, the released Bak is bound by Mcl-1 and cannot be activated. ABT-737 resistance actually increases upon increased Mcl-1 expression levels. Under these circumstances, Noxa expression can bind Mcl-1 and release Bak so that it can be activated at the mitochondria [71]. Thus, upregulating Noxa expression potentially increases ABT-737 efficacy [55, 71]. One study demonstrated that inhibiting mitochondrial E3 ubiquitin-protein ligase (MARCH5), known as membrane-associated ring finger CH, attenuates ABT-737 resistance [98]. MARCH5 is the only member of the MARCH immunomodulation family to be localized and regulated within the mitochondria. Inhibition of MARCH5 activates the Noxa/Mcl-1 axis and potentially increases the efficacy of ABT-737 in a Bax-dependent but Bak-independent manner [98]. In addition, MARCH5 and Mcl-1 share part of an apoptotic pathway, as both seem to decrease ABT-737 resistance when separately downregulated. Interestingly, Mcl-1 is upregulated when MARCH5 is inhibited despite the decreased resistance of ABT-737, possibly because MARCH5 controls Mcl-1 stability in a Noxa-dependent mechanism [98]. Therefore, Noxa seems to play a direct and an indirect role in ABT-737 sensitivity. Other BH3 mimetics like negative enantiomer (-) gossypol and its derivatives – apogossypol, TM-106 and TW-37 – have shown great promise in promoting apoptosis. The (-) gossypol has been shown to bind to Bcl-2, Bcl-xL, and Mcl-1 with lower affinities than those of BH3-only proteins and of its derivatives, and exhibits greater toxicity than its derivatives [99-101]. Histone deacetylase inhibitors (HDACis) are also newly introduced antitumor agents, which induce apoptosis [102, 103]. They appear to upregulate Noxa and Bim, and the upregulation of Noxa seems to cause an increase in Mcl-1 immunoprecipitation because of an increased binding to Mcl-1 [104]. Vorinostat, an HDACi, in combination with ABT-263, induces apoptosis in SCLC cell lines, including those that are resistant to ABT-263 [104]. Thus, therapeutic agents capable of binding anti-apoptotic Bcl-2 family proteins coupled with the potential sensitizing role of Noxa hold promise in future therapeutic advances in treating SCLC.

NSCLC is less responsive to chemotherapy. While Noxa can potentially increase ABT-737 efficacy, Noxa does not seem to be involved in cisplatin-induced apoptosis of NSCLC cell lines in synergy with ABT-263 [105]. On the other hand, pemetrexed, a folate antimetabolite, induces apoptosis via Noxa upregulation and is used as a first-line therapy with cisplatin for locally advanced or metastatic NSCLC [73]. Yan, et al. (2014) found that pemetrexed increases Noxa expression by upregulating activating transcription factor 3 (ATF3) and activating transcription factor 4 (ATF4). Induction of Noxa results in Usp9x deubiquitinase downregulation thus leading to Mcl-1 ubiquitination and its subsequent degradation. Thus, Yan et al. (2014) demonstrate that pemetrexed induces apoptosis by regulating the Noxa-Usp9x-Mcl-1 pathway. Another therapeutic regimen showing promise in treating NSCLC includes the pan-Bcl-2 family inhibitor molecule called GX15-070, a BH3 mimetic drug, combined with cytotoxic chemotherapy such as cisplatin – cisplatin-resistant NSCLC cells via Noxa and Bak [107]. The Noxa-related activity of GX15-070, which is still in Phase II clinical trials, involves displacing Bak from Mcl-1 [108]. Table 1 shows Noxa-mimetics and BH3-mimetics, and the cancers they can potentially treat, including NSCLC.

4.2. Leukemia

4.2.1. Pathogenesis of Acute Myeloid Leukemia

Leukemias have diverse biological profiles but studies have shown that Bcl-2 family proteins play a role in various leukemia types [74]. Bcl-2 family proteins are overexpressed in acute myeloid leukemia (AML), especially Bcl-2, Bcl-xL, and Bad [75]. High Bcl-2 protein levels are considered to be a poor prognostic factor for AML patients with favorable or intermediate prognosis cytogenetic profile, especially in M4, M5 and M6 subtypes and CD34+ blasts. On the other hand, low Bcl-2 protein levels are associated with a better prognosis for those AML patients with a poor risk karyotype [76, 80]. High levels of Bad and Bax mRNA are indicative of a failure to enter complete remission, while higher levels of Bad or Bad and Bax expression are highly predictive of poor outcomes despite response to chemotherapy. Additionally, any increases in Bax levels or the Bcl-2/Bax ratio following
chemotherapy are suggestive of poorer outcomes [77]. Interestingly, there is a synergistic association between an increased Bcl-2/Bax ratio and the growth-promoting MEK/MAPK pathway found in primary AML cells. This synergism is indicative of a poor AML prognosis [78]. Therefore, the Bcl-2 family of proteins appears to play an important role in the outcome of AML. Whether Noxa is specifically relevant in this regard, is still poorly studied. Yet, some interesting observations regarding a role for Noxa in the treatment of AML are further elucidated.

4.2.2. Treatment of Acute Myeloid Leukemia

As described above, Noxa plays a role in the induction of apoptosis in both p53-dependent and p53-independent pathways. Many chemotherapeutic agents used in treating AML trigger DNA damage and depend on p53 to induce apoptosis [147]. Recently, the ubiquitin-proteasome system (UPS) emerged as a potential target for treatment in AML. The UPS is involved in the degradation of many proteins, and in homeostasis of the cell, such as signal transduction, stress responses, DNA damage, and cell-cycle progression [148]. Ubiquitin ligation to target proteins marks them for degradation by the proteasome [149]. Cullin ring ligases (CRLs) are a subset of E3 ubiquitin ligases that, when activated, facilitate the ubiquitination of a subgroup of protein substrates that affect cell survival. When these proteins are turned over by proteasomal degradation, they create an environment conducive of proliferation and survival in cancer cells [149]. A process termed Neddylation, the covalent binding of the ubiquitin-like Nedd8 protein to target proteins, in turn, activates CRLs. Nedd8-activating enzyme (NAE) has been recently identified to control Neddylation and a novel small molecule inhibitor of NAE, pevonedistat or MLN4924, was developed [148, 150]. By inhibiting NAE, pevonedistat prevents the activation of CRLs and inhibits the degradation of their target proteins leading to cell death. Thus, the potential for use of pevonedistat in the treatment for AML is recognized especially since it is active in the absence of p53. In AML cell lines, pevonedistat leads to the accumulation of the substrate c-Myc by inactivating CRLs. c-Myc is found to transactivate the PMAIP1 gene encoding Noxa leading to its transcriptional upregulation. Thus, pevonedistat emerged as a tool to increase the expression of Noxa in AML. When used with the BH3 mimetic drugs ABT-199, an inhibitor of Bcl-2, and ABT-263, an inhibitor of Bcl-2, Bcl-xL, and Bcl-w, but not Mcl-1, pevonedistat exhibits significant synergy in promoting apoptosis of the AML cells indicating that it could be useful in situations where specific inhibition of Mcl-1, by Noxa, is needed [150]. Recently, a phase I study shows that pevonedistat is target-specific and shows modest clinical activity [151]. Thus, the use of pevonedistat not only gives new insight into the role Noxa plays in AML but also provides a new tool for the upregulation of Noxa when needed.

4.2.3. Pathogenesis of Chronic Lymphocytic Leukemia

Recent studies have shown that the Bcl-2 family is highly associated with B-cell chronic lymphocytic leukemia (CLL). More recently, studies have also shown that Noxa plays an integral part in the pathogenesis of CLL [79]. In 2007, it was postulated that Noxa suppression permits a persistent CLL reservoir in lymph nodes, and possibly bone marrow [79]. This study found that the Noxa/Mcl-1 ratio is low in lymph node CLL cells relative to the peripheral blood CLL cells.
This offers an explanation for the resistance of CLL to treatment where treatment regimens are usually successful in inducing peripheral lymphopenia but relapse invariably occurs due to the protection of bone marrow and lymph node CLL cells from treatment. Another study found Puma expression to correlate with several prognostic factors, with low expression associated with a poorer prognosis of CLL [82]. Based on these findings, Zhang, et al. (2013) hypothesized that low expression of Puma, Noxa and Bim is correlated with a high tumor burden, or lymphocyte proliferation, and does not necessarily correlate with tumorogenesis [81].

4.2.4. Treatment of Chronic Lymphocytic Leukemia

It has been previously established that Noxa has a high specificity for Mcl-1, and this specificity poses as a potential therapeutic target for CLL treatment [152]. In view of the central role played by the anti-apoptotic Bcl-2 family proteins in the pathogenesis of CLL, manipulating BH3-only proteins is rational when treating CLL. Inhibiting the anti-apoptotic Bcl-2 family proteins using BH3-only mimetic drugs or other drugs that manipulate BH3 proteins is proposed to increase the sensitivity of several drugs.

Fludarabine, a purine analog, works via the p53-dependent pathway to upregulate the downstream BH3-only proteins, especially Puma, in order to eliminate the CLL cells. However, Noxa appears to play a restricted role in this context [81, 82]. HDACi’s appear to upregulate Noxa in the context of CLL, exploiting Noxa’s role in CLL treatment [60]. Noxa’s role in CLL treatment, in this case, is mainly through inactivation of the anti-apoptotic Bcl-2 protein, Mcl-1, which is why the Noxa/Mcl-1 ratio might be an important prognostic determinant in HDACi-treated CLL patients [153]. Glucocorticoids are also apoptosis-inducers in CLL cells via caspases. It is unclear as to how Noxa plays a role in apoptosis of CLL cells after glucocorticoid (GC) treatment. However, Ploner, et al. (2009) found that GC-induced apoptosis involves repressed Noxa mRNA levels and its proteasomal degradation on a protein level in acute lymphoblastic leukemia (ALL) [154]. It has been found that increased Bim mRNA and protein levels inhibit Mcl-1 anti-apoptotic activity in GC treatment of ALL [155-157]. Based on this finding, one proposition as to why Noxa fails to downregulate Mcl-1 levels upon Bim induction but still contributes to apoptosis is that Noxa accelerates GC-induced apoptosis via a non-Mcl-1 degradation-dependent mechanism [81, 154]. It appears that the balance between Noxa and Mcl-1 is integral in determining the sensitivity of leukemia cells to GC-induced apoptosis [158]. While there are no studies on the role of GC-induced apoptosis through Noxa in CLL, Noxa’s ability to accelerate GC-induced apoptosis of ALL cells can have several implications in the treatment of CLL if Bim is also found to be upregulated in CLL patients [157].

4.3. Alveolar Rhabdomyosarcoma

4.3.1. Pathogenesis

Rhabdomyosarcomas are common soft tissue tumors that have a poor prognosis. There are two main histological subtypes, embryonal rhabdomyosarcoma (ERMS) and alveolar rhabdomyosarcoma (ARMS). ARMS is more aggressive and has a poorer prognosis [84]. One of the main mechanisms behind the development of rhabdomyosarcoma is the inactivation of p53 [85]. The lack of p53 in these tumors indicates that targeting Noxa may be more successful in treating them [83, 86].

4.3.2. Treatment

Two treatment options for alveolar rhabdomyosarcoma show upregulation of Noxa via a p53-independent manner. In one study, Noxa appears to play a role in glucose uptake, which makes it critical for apoptosis of ARMS cells when induced by the glycolytic inhibitor 2-deoxyglucose (2-DG). Noxa upregulation upon 2-DG use is associated with Mcl-1 downregulation, and consequently, cell death [86]. Another study demonstrates that Noxa is upregulated by the paired box 3-forkhead box protein O1 (PAX3-FOXO1) fusion transcription factor. ARMS cells expressing this transcription factor are more susceptible to γ-secretase inhibitor (GSI1, Z-LLNle-CHO), the proteasome inhibitor bortezomib, and BH3 mimic ABT-737 [87]. These findings have several implications for Noxa in treatment of ARMS.

4.4. Prostate Cancer

4.4.1. Pathogenesis

Many studies have found that high-grade prostate cancer and androgen-independent prostate cancer express increased levels of Bel-2, Bel-2, and Mcl-1. One study assesses whether Noxa and Puma are associated with prostate cancer recurrence. According to this study, there is a frequent increase in Noxa expression in prostate cancer, and additionally, Noxa expression is associated with clinical outcome [87]. Therefore, Noxa may be linked to prostate cancer progression, making it a potential marker of prostate cancer prognosis. Noxa, among many other existing clinical markers, may also aid in predicting the biochemical recurrence of prostate cancer [87].

4.4.2. Treatment

While several Bcl-2 family inhibitors are showing benefit in prostate cancer outcome, an interesting study demonstrates that the innate immune system may play a role in activating Noxa-dependent apoptosis. The retinoid acid-inducible gene 1 (RIG-1) is a pattern-recognition receptor involved in the innate immune response to several viruses that utilizes the mitochondrial antiviral signaling protein (MAVS) to launch the defensive response. This study found that the hemagglutinating virus of Japan envelope (HVJ-E) induces cell death in prostate cancer cell lines. Another study demonstrates that viral defective-interfering (DI) particles in the Cantell strain of HVJ contain many incomplete viral genomes after several replicative errors. The DI genomes with the highest binding affinity to RIG-1 are termed the “copy-back” type, known for replacing weaker replication promoters at the 3’ end with stronger anti-genomic promoters [159]. This “copy-back” type DI particle RNA genome activates pro-apoptotic genes such as Noxa more effectively than the Sendai/52 strain or Cantell strain [160]. The RIG-I/MAVS signaling pathway mediates this HVJ-E-induced cell death via Noxa and TNF-related apoptosis-inducing ligand (TRAIL) being upregulated just downstream of this pathway. TRAIL, however, is more involved in inducing apoptosis.
than Noxa, because it activates both the intrinsic and extrinsic pathway, while Noxa only activates the intrinsic pathway of apoptosis [161]. These studies illustrate the presence of more than one approach to engage Noxa in the treatment of prostate cancer.

4.5. Ovarian Cancer

4.5.1. Pathogenesis

Ovarian cancer is the most common gynecological malignancy-related cause of death. One major contributor to the progression and therapeutic resistance of ovarian cancer is the deregulation of apoptotic pathways, especially the p53 and/or p73-dependent pathways. Also, high levels of Bcl-2, Bcl-xL, and Mcl-1 as well as low levels of Bax and Bak correlate with greater resistance in ovarian cancer cells to chemotherapeutic agents [88].

4.5.2. Treatment

As mentioned above, Noxa is a transcription target of both p53- and p73-associated apoptotic pathways. Based on one study, ovarian cancer cells express Noxa in a p53-not p73-dependent manner after cisplatin use. Ovarian cell lines that lacked functional p53 are resistant to cisplatin but are rendered sensitive when transfected with Noxa [88]. The effectiveness of cisplatin depends on both Bax expression and SMAC release from mitochondria, events that are regulated by Noxa. This study suggests that upregulating Noxa is a useful approach for chemosensitizing cisplatin-resistant ovarian cancer cells lacking functional p53 [88].

4.6. Colorectal Cancer

4.6.1. Pathogenesis

In 2003, it was speculated that Noxa might not be of significance in colorectal cancer, because no mutations in the Noxa gene were found and Noxa mRNA expression was not altered [91]. Additionally, there was a lot of redundancy of other BH3-only members like Puma being involved in colorectal cancer [91]. More recently, however, Conti, et al. (2015) showed that Noxa expression is upregulated under certain circumstances. They demonstrated that KRAS G13D mutations upregulate basal Noxa levels dependent on ERK2, not ERK1, activation [89]. They also showed that Noxa expression sensitizes premalignant epithelial cells to apoptosis upon induction by treatment with various cytotoxic agents. However, Conti, et al. (2015) note that in colorectal cancer cells, Noxa levels are not upregulated by KRAS although their baseline levels are higher than those found in premalignant epithelial cells [89]. They discovered that this difference is due to aberrant induction of AKT, which counterbalances the KRAS effects on Noxa and apoptosis. Another study demonstrates that A-kinase anchor protein 4 (AKAP4) is expressed in colorectal cancer cells, but not in normal colon cells. This study shows that once AKAP4 is ablated, there is an increase in pro-apoptotic molecules such as Bad, Bid, Bak, Noxa, and Puma [90]. Therefore, it can be safely assumed that Noxa, as well as the other pro-apoptotic molecules, may play an indirect role in the pathogenesis of colorectal cancer upon AKAP4 expression.

4.6.2. Treatment

These findings led to a novel treatment approach that involves inhibiting AKT in order to restore the sensitizing activity of Noxa. Indeed, AKT inhibitors restore sensitivity to camptothecin (CPT)/SMAC mimetic drug when KRAS is mutated in colorectal cell lines. Noxa levels are not further increased upon AKT inhibition, thus implying that the AKT pathway is involved in acting against Noxa-dependent apoptosis in the presence of an oncogenic KRAS [89]. This discovery is highly significant, because it sheds light on the other signaling pathways involved in controlling or rather counteracting the Noxa pro-apoptotic pathway when KRAS is mutated such as the PI3/AKT pathway. Another study discovered that Noxa upregulation by CPT-11 or Trinitocetan, or even bortezomib, a proteasome inhibitor, sensitizes the colorectal cancer cells to the Bcl-2 family inhibitor, ABT-737, when Mcl-1 is expressed [113]. This finding is significant, because ABT-737, as previously discussed, does not antagonize Mcl-1. CPT-11-induced Noxa apparently displaces Bak from Mcl-1, thus facilitating this sensitization and improving the efficacy of ABT-737 [113]. These studies suggest that manipulating Noxa in colorectal cancer may be a useful approach to sensitize tumors to chemotherapy.

4.7. Malignant Melanoma

4.7.1. Pathogenesis

Melanoma, just like many other malignancies, is also associated with defects in cellular signaling and apoptotic activities [162]. For instance, melanoma can acquire metastatic and apoptosis-resistant properties because of downregulation of Apaf-1 expression due to hypermethylation and alterations in TNF, Fas, and TRAIL receptors in the death receptor apoptotic pathway [163]. The associations with signaling and apoptosis could be exploited for the treatment of this malignancy.

4.7.2. Treatment

In order to overcome the resistance to apoptosis and possible metastatic properties of melanomas, it is necessary to make use of apoptotic signaling. Noxa induction has proven to be a possible mechanism for the treatment of melanoma. Melanoma cells are found to express higher levels of Notch receptors compared to normal melanocytes [48]. Thus, inhibition of Notch receptors serves as a target for treating melanoma. Since Notch receptors are dependent on γ-secretase for activation, a γ-secretase tripeptide inhibitor (GSI), originally developed for the treatment of Alzheimer’s disease, can be used. Treatment of melanoma cell lines with GSI shows that cancer cell lines with low Apaf-1 expression levels are killed via a p53-independent upregulation of Noxa and Bim. This finding indicates that the p53-independent apoptosis pathway could be used in the treatment of melanoma [48]. Qin, et al. (2005) further investigated the use of proteasome inhibitors, such as bortezomib, in the treatment of melanoma, and discovered that Noxa significantly contributes to the death of these cancer cells [162]. One study shows that imiquimod also induces apoptosis of melanoma cells, with Noxa being the main mediator [164]. This study reports that Protein kinase RNA-like endoplasmic reticulum kinase-Inositol-requiring enzyme 1 alpha-activating transcription
factor 4 (PERK-IRE1α-ATF-4) and Apoptosis signal-regulating kinase 1-Juno amino-terminal kinase (ASK1-JNK) pathways induce Noxa by activating transcription factors, activating transcription factor 3 (ATF-3), activator protein 1 (AP-1) and p53 respectively [92, 94, 95]. Noxa then localizes to both the membrane and the endoplasmic reticulum (ER) and activates mitochondrial dysfunction and/or ER stress-dependent pathways [164]. Another drug, Mcl-1 inhibitor SC-2001, also upregulates Noxa when combined with ABT-737 for the treatment of melanoma [93]. These findings serve as potential advances involving Noxa in the treatment of life-threatening malignancies like melanoma.

4.8. Multiple Myeloma

4.8.1. Pathogenesis

Defects in apoptosis and cellular signaling also play a role in the pathogenesis of multiple myeloma [162]. Some studies have shown that TNF receptor-associated factor 3 (TRAF3) deletions or inactivating mutations constitutively activate the nuclear factor kappa-B2 (NF-κB2) pathway, prolonging B-cell survival [165-167].

4.8.2. Treatment

Multiple myeloma patients with TRAF3 mutations or deletions are also associated with resistance to certain chemotherapeutic drugs such as dexamethasone, but are more sensitive to bortezomib [166]. Bortezomib upregulates p21 and Noxa and downregulates Mcl-1 in TRAF3-deficient malignant B-cells [168]. A recent study demonstrates that reovirus receptor, junctional adhesion molecule-A (JAM-A), is overexpressed in advanced multiple myeloma primary cells [97]. It also elucidates that JAM-A expression is required for Reolysin, a reovirus formulation for cancer therapy, to induce apoptosis in both relapsed and refractory multiple myeloma models [97]. Reolysin then induces Noxa expression, especially in bortezomib-resistant multiple myeloma cell lines [96, 97].

CONCLUSION AND PERSPECTIVES

In summary, Noxa appears to communicate with several other Bcl-2 family proteins as well as act downstream both p53-dependent and p53-independent pathways to control cell proliferation. Although Noxa might have a restricted role in apoptosis, several studies established its significance in the pathogenesis of several cancers. Its role cannot be easily separated from other BH3-only proteins, because its versatile function in its interaction with different proteins in different pathways paves the way for chemotherapeutic advancements. Its role is largely dependent on the context in which it is expressed, whether it is the type of cancer, type of signal induced, or the counteracting pathways directly or indirectly affecting Noxa expression. Focus should be directed towards determining the role of Mcl-1 or A1 and how their expression plays a role in oncogenesis and chemotherapeutic resistance. Noxa-mimetics, those drugs that resemble Noxa more than the other BH3 proteins, such as BimS2A and SAHB, need to be further investigated in hopes of better cancer outcomes.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

This work was partially funded by grants to GD from the Lebanese National Counsel for Scientific Research and the Medical Practice Plan at the American University of Beirut.

REFERENCES

[17] Certo, M.; Del Giaco Moore, V.; Nishino, M.; Wei, G.; Korsmeyer, S.; Armstrong, S.A.; Letati, A. Mitochondria primed by death sig-


Morsali et al.
Noxa: Role in Cancer Pathogenesis and Treatment

Current Cancer Drug Targets, 2018, Vol. 18, No. 10 927


