Trends in Cell Biology

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Review

Molecular Comprehension of Mcl-1: From Gene Structure to Cancer Therapy

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Among cell death regulators, members of the Bcl-2 family are of interest because they are highly conserved across species and represent promising targets for anticancer therapy. This family and its associated proteins include more than 25 members, with either anti- or proapoptotic functions. Although the overall regulation of apoptosis by Bcl-2 family proteins is now well understood, targeted therapy requires careful consideration of individual members of the family and their crosstalk. One of the most studied representatives of the Bcl-2 family is antiapoptotic Mcl-1. After 25 years of investigations, a large amount of data regarding Mcl-1's regulation and functions has been compiled. In this review, we summarize current knowledge about Mcl-1, focusing on molecular aspects relevant to Mcl-1-targeted therapies.

Highlights

Recent advances in molecular oncology have led to the development of the BH3 mimetics – small-molecule drugs that neutralize antiapoptotic Bcl-2 family proteins and, thus, favor the mitochondrial pathway of apoptosis.

Mcl-1-selective BH3 mimetics are at relatively early stages of drug development, so their adverse effects, including those relating to the nonapoptotic functions of Mcl-1, are poorly understood.

Several BH3 mimetics preclude interactions between Mcl-1 and its proapoptotic partners while stabilizing Mcl-1. Understanding of the conformational changes that induce degradation of Mcl-1 would allow the rational design of alternative compounds that would cause a pronounced reduction of its cellular level.

In the context of oncology, it is worth examining alternative strategies to target Mcl-1, such as an alternative splicing switch toward a proapoptotic Mcl-1S isoform and indirect antagonism of Mcl-1.

Bcl-2 Family: Bullseye in Targeting Apoptosis

According to the Nomenclature Committee on Cell Death, there are more than ten types of 'programmed cell death' (PCD), each possessing its own biochemical and morphological features [1]. Apoptosis, the most investigated type of PCD, represents a genetically regulated process that results in the engulfment of a dying cell by adjacent ones. Consequently, apoptosis does not cause damage to neighboring cells, making it a more favorable process in cancer therapy than several other cell death modalities.

To commit suicide, cells require the activity of special proteases, **caspases** (see Glossary). Two sets of apoptotic caspases exist: initiator and executioner. Once activated, initiator caspases lead to the processing and activation of executioner caspases, which in turn cleave various proteins and cause biochemical and morphological changes specific for apoptotic cell death [2]. Apoptosis can be triggered extrinsically through the activation of death receptors or intrinsically in response to various stress stimuli (e.g., DNA damage). In the intrinsic apoptotic pathway, cytochrome *c* is released from the mitochondrial intermembrane space, which is considered a point of no return. This event results in the assembly of the apoptosome, an initiator caspase-9 activation complex, and consequent apoptosis [3]. As a crucial step, cytochrome *c* release is tightly regulated by numerous Bcl-2 family members [4]. Bcl-2 proteins play vital roles in cell death regulation, with some being antiapoptotic while others are proapoptotic (Box 1). Prosurvival members include Bcl-2, Bcl-xL, Bcl-w, Mcl-1, and A1, while proapoptotic members include Bim, Bad, Bid, Noxa, Puma, Bax, and Bak. Their vital role in regulating cell fate has made them attractive targets for cancer therapy. Most recently, venetoclax, an inhibitor of the antiapoptotic protein Bcl-2, has been approved for clinical use. Meanwhile, other members of the Bcl-2 family, including the antiapoptotic protein Mcl-1, have great potential as drug targets.

Myeloid cell leukemia-1 (*MCL1*) was the first reported gene found to be homologous to *BCL2* and has since been shown by numerous studies to have an antiapoptotic role in the regulation of cell survival [21–24]. However, the nature of Mcl-1 seems to be more complicated: its different splice variants play opposite roles in apoptosis, while its nonapoptotic functions are also of great signif-

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icance. Here we highlight the molecular composition of Mcl-1 and discuss current advances and possible pitfalls of targeting Mcl-1 for cancer treatment.

Isoforms and Structure of Mcl-1

Traditionally, Mcl-1L – the most abundant and largest product of the *MCL1* gene with a calculated molecular weight of 37.2 kDa – is referred to as Mcl-1. Similar to the other multidomain proteins of the Bcl-2 family, Mcl-1 contains four **Bcl-2 homology (BH) domains** [25]. Mcl-1 shares common structural features with other multidomain members of the Bcl-2 family, including a **BH3-binding groove** and transmembrane α-helix-9. However, Mcl-1 is substantially larger than its relatives due to the presence of a regulatory N-terminal region. This region contains a **mitochondrial targeting signal (MTS)** [26] and **PEST sequences** [21] (Figure 1), which are common in proteins with a short half-life. The half-life of Mcl-1 is estimated to be approximately 40 min when measured by **pulse–chase analysis** [27,28], although it is not constant and depends on cell type and intracellular conditions. Mcl-1 has a C-terminal transmembrane domain, which is essential for anchoring Mcl-1 to the outer mitochondrial membrane (OMM) as well as the nuclear and endoplasmic reticulum (ER) membranes. It is noteworthy that Mcl-1 also localizes to the nucleus [29,30] and mitochondrial matrix [26], where it exerts nonapoptotic functions.

Mcl-1S was first described as a product of cDNA amplified from human placenta and several human cell lines [7,31]. The lack of exon 2 in Mcl-1S mRNA results in a frame shift downstream of the BH3 coding domain. Therefore, Mcl-1S comprises 271 amino acids, among which 230 N-terminal amino acids are fully identical to those of Mcl-1. This N-terminal portion of Mcl-1S includes a large regulatory region, the BH4 and BH3 domains. However, BH1 and BH2 domains are absent in Mcl-1S. As these domains are necessary for the formation of the BH3-binding pocket, Mcl-1S lacks antiapoptotic activity. Conversely, Mcl-1S acts as a proapoptotic protein in a manner seemingly like that of BH3-only proteins (Box 2). Mcl-1S does not contain a transmembrane domain and localizes predominantly in the cytoplasm, although it has been found in the ER [32].

Mcl-1ES is the third human splice variant of *MCL1*. Its cDNA, which was identified as a minor RT-PCR product in some cancer and immortalized cell lines, lacks part of exon 1 due to splicing at a noncanonical pair (GC–AG) in this exon. Consequently, Mcl-1ES has a truncated N-terminal region devoid of the PEST motifs and putative BH4 domain, but retains BH3, BH1, and BH2 [8]. Mcl-1ES cDNA was isolated using Mcl-1-specific primers, although no endogenous Mcl-1ES protein has been detected. Moreover, Mcl-1ES protein functions were studied only under overexpression conditions [8,33]. Notably, there is no strong evidence supporting the existence of either the Mcl-1S or the Mcl-1ES isoform in rodents. Therefore, it remains unclear whether these isoforms are widely expressed among species.

Mcl-1 and Apoptosis

Mcl-1 has all the common characteristics of the antiapoptotic Bcl-2 family members. Simultaneously, Mcl-1 exhibits several unique features. First, Mcl-1 undergoes rapid degradation in response to various stress stimuli that may shift the threshold for induction of apoptosis [37]. Second, similar to other Bcl-2 family members, Mcl-1 displays its own binding-affinity profile [38]. Mcl-1 sequesters both activators (Bim and tBid, as well as the putative activators Puma and Noxa) [39] and pore-forming proteins (Bak and, to a lesser degree, Bax) [38,40]. Cell-free *in vitro* assays showed that Mcl-1 bound with the highest affinity to Bak, Bim, Bid, and Puma, whereas less stable complexes were formed between Mcl-1 and Bax or Noxa [39,40]. Of note, in most studies both truncated Mcl-1 and its binding partners were used to estimate Mcl-1-binding affinities, so the interpretation of interactions between full-length proteins in the cell is somewhat obscure [38].

Glossary

Bcl-2 homology (BH) domains: short amino acid regions of sequence homology in Bcl-2 family proteins.
BH3-binding groove: a hydrophobic cleft on the surface of antiapoptotic Bcl-2-family members that is formed by the BH1, BH2, and BH3 domains and accommodates the BH3 helix of proapoptotic Bcl-2 family proteins.
BH3 mimetics: small molecules that mimic the BH3 domains of proapoptotic Bcl-2 family proteins and block the BH3-binding grooves of antiapoptotic members of the family, thus antagonizing their prosurvival activity.
Caspases: a family of cysteine-aspartic proteases that serve as initiators or effectors in the progression of PCD or inflammation.
Mitochondrial outer membrane permeabilization (MOMP): a critical 'no-return' point during apoptosis characterized by the formation of protein-permeable pores on the OMM by Bax and/or Bak oligomers and the ensuing release of various proapoptotic factors (e.g., cytochrome *c*, SMAC/Diablo) from the mitochondrial intermembrane space.
Mitochondrial targeting signal (MTS): an amino-terminal sequence characterized by a positive net charge and implicated in trafficking proteins through mitochondrial membranes.
Peri-implantation lethality: a lethal outcome due to the failure of a blastocyst to attach to and/or implant in the lining of the uterus.
PEST sequence: a protein region that is enriched in proline (P), glutamic acid (E), serine (S), and threonine (T), and typically associated with short-lived proteins.
Pulse–chase analysis: a method for studying protein or mRNA dynamics in cells after a short period of labeling, or 'pulse', and subsequent replacement of the label with an unlabeled precursor, or 'chase'. Protein turnover rate is typically assessed by comparing the levels of target protein radioactivity after incubation of cells with [^{35}S]Cys/Met and the residual radioactivity at certain time points after the release from labeling.
Translocase of the outer/inner membrane (TIM/TOM) complex: a multiprotein machinery that transports proteins with a MTS from the cytosol into mitochondria.

Box 1. The Interplay of Bcl-2 Family Proteins Orchestrates Apoptosis

All Bcl-2 family proteins contain one or several regions of sequence homology termed BH-domains [5]. Bcl-2 family members are commonly classified into two subsets: proteins bearing only a BH3 domain (BH3-only proteins; e.g., Bim, Bad, Bid, Noxa, Puma) and multidomain proteins. Whereas BH3-only proteins have proapoptotic functions, multidomain proteins are further subdivided into prosurvival members (e.g., Bcl-2, Bcl-xL, Bcl-w, Mcl-1, A1) and proapoptotic effectors (e.g., Bax, Bak). Intriguingly, alternative splicing of some members of the Bcl-2 family can generate multiple isoforms with opposite functions (e.g., proapoptotic Bcl-xS and antiapoptotic Bcl-xL [6]). There are three known splice isoforms of Mcl-1 in humans: antiapoptotic Mcl-1L (long) and the proapoptotic isoforms Mcl-1S (short) [7] and Mcl-1ES (extra-short) [8].

Multidomain Bcl-2 family members adopt similar globular structures formed by nine α helices, where α helix-2 corresponds to the BH3 domain, α helix-9 represents a transmembrane domain, and α helices-2, -3, -4, and -5 constitute a hydrophobic groove [9]. This groove is critical for interactions with the BH3 domains of proapoptotic members of the Bcl-2 family and is thus called the BH3-binding groove (Figure IA,B). In contrast to antiapoptotic members of Bcl-2 family, Bax and Bak are structurally metastable and expose their BH3 domain on binding to other BH3 domains [9]. Most BH3-only proteins are intrinsically disordered and undergo a conformational change on binding to their partners (either proapoptotic or antiapoptotic) [9]. All of the interactions between BH3 domains and BH3-binding grooves underlie the regulation of apoptosis by Bcl-2 family proteins and determine the cell's fate in response to apoptotic stimuli.
The proapoptotic effectors Bax and Bak homodimerize and subsequently oligomerize to form protein-permeable pores on the OMM, resulting in MOMP and the ensuing release of various proapoptotic factors (e.g., cytochrome *c*, SMAC/Diablo) from the mitochondrial intermembrane space. In healthy cells, Bax is mainly cytosolic and Bak is localized to the OMM in a nonactivated state [10,11]. Interaction with BH3-only proteins promotes Bax translocation to the OMM and exposure of the BH3 domain of both Bax and Bak that is the crucial step in their activation [10,12]. Following activation, Bax and Bak form homodimers through BH3 domain–BH3-binding groove interaction [13].

Antiapoptotic Bcl-2 family members and BH3-only proteins regulate the activation and homodimerization of Bax and Bak. Notably, differences in the grooves of antiapoptotic proteins and in the BH3 domains of proapoptotic proteins account for the selectivity in their interactions. For instance, Bax is inhibited by all antiapoptotic proteins of the Bcl-2 family while Bak is sequestered predominantly by Bcl-xL and Mcl-1 [14] (although phosphorylated Bcl-2 exhibits high binding activity toward Bak [15]). Furthermore, while some BH3-only proteins (Bim, tBid – the active truncated form of Bid, Puma) neutralize all of the antiapoptotic Bcl-2 family members, others display pronounced selectivity for several binding partners (e.g., Bad for Bcl-2, Bcl-xL and Bcl-w, Noxa for Mcl-1 and A1) [16,17]. Finally, certain BH3-only proteins, particularly Bim and tBid, can both neutralize antiapoptotic members and directly activate proapoptotic effectors [16]. These proteins are, therefore, called 'activators'. By contrast, 'sensitizers' are capable only of neutralizing antiapoptotic members and cannot activate Bax and Bak (Figure IC). Initially, Bad, Puma, and Noxa were all considered to be sensitizers; however, now evidence exists that Noxa and Puma may also act as activators [18,19].

Taking these findings together, antiapoptotic members of the Bcl-2 family prevent apoptosis through two different modes of action [4,20]. The first mode involves the sequestration of BH3-only activators while the second involves the binding of active effectors. Both sensitizers and activators neutralize the antiapoptotic Bcl-2 family members, preventing the two modes of their prosurvival activity.

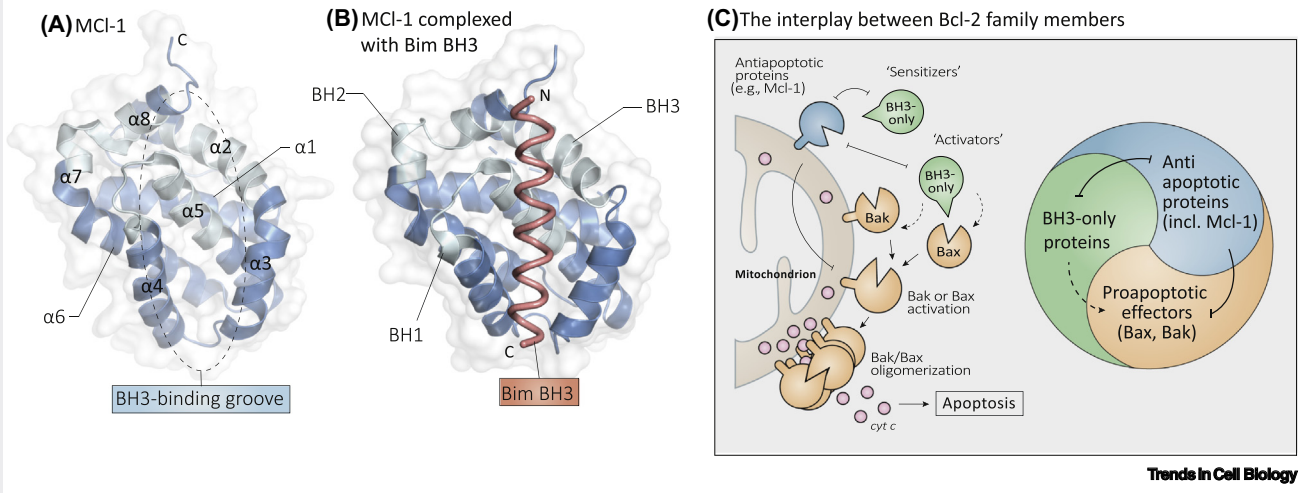

Figure I. Regulation of Apoptosis by the Bcl-2 Family of Proteins. The prosurvival properties of the antiapoptotic Bcl-2 proteins are structurally defined by their BH3-binding grooves, which sequester the exposed BH3 domains of proapoptotic Bcl-2 family members. The BH3-binding groove is formed mostly from helices α2–α5 and α8 and lined by residues from the BH1–BH3 domains (marked in light blue). The structures of free antiapoptotic proteins and their complexes with the BH3 domains of the proapoptotic proteins are exemplified by Mcl-1 (A) and the Mcl-1/Bim BH3 complex (B), respectively. Structures are based on the following Protein Data Bank (PDB) accessions: Mcl-1, NMR structure of human Mcl-1 (2MHS); Mcl-1 complexed with Bim BH3, Crystal structure of the Mcl-1:Bim BH3 complex (2NL9). (C) The family comprises BH3-only members, proapoptotic effectors, and antiapoptotic proteins. Some BH3-only proteins (activators) can both directly activate proapoptotic effectors and inhibit antiapoptotic members, while the others (sensitizers) balance a cell toward apoptosis only by inhibitive binding to the antiapoptotic Bcl-2 family members. By contrast, the antiapoptotic proteins neutralize proapoptotic effectors and BH3-only activators, supporting the survival of a cell.

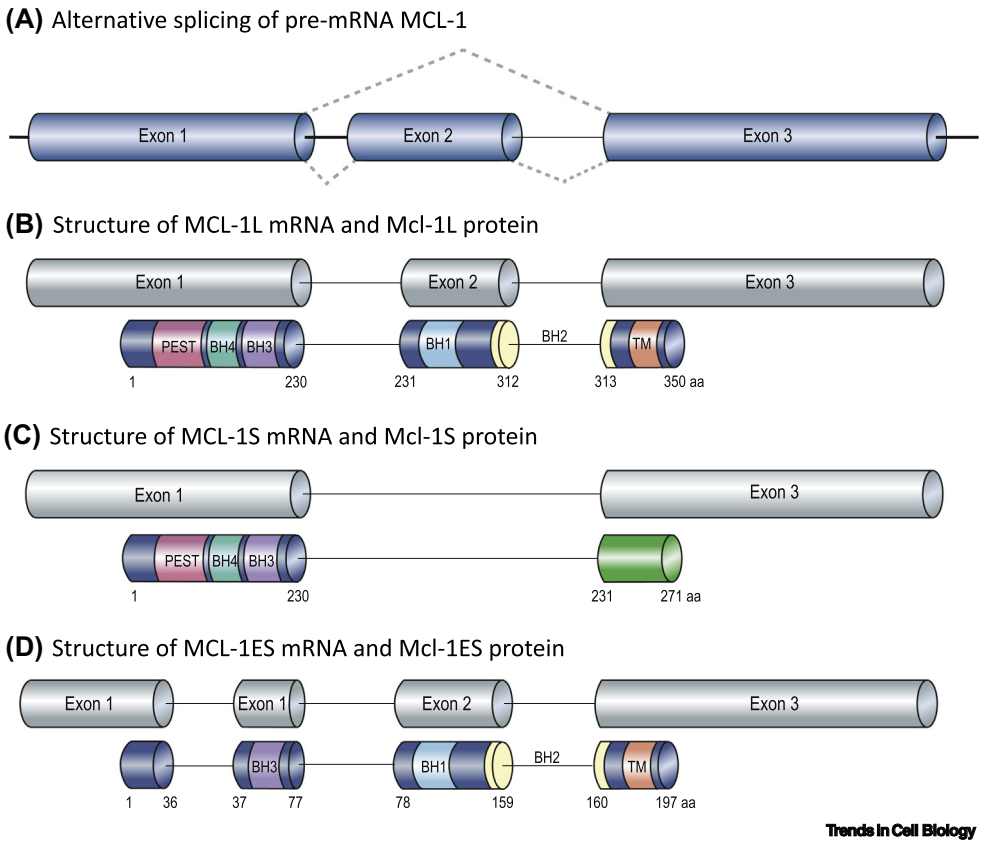

Figure 1. Alternative Splicing of *MCL1* Gives Rise to Three Structurally Different Isoforms. (A) MCL1 pre-mRNA contains three exons. (B) The inclusion of all of the exons in mRNA results in the translation of a full-size Mcl-1L protein, which is known to have antiapoptotic properties. Mcl-1L comprises a large N-terminal regulatory domain enriched in PEST sequences, a C-terminal transmembrane domain, and BH1–BH4 motifs that form the BH3-binding groove. (C) Skipping of exon 2 is conducive to the synthesis of the Mcl-1S isoform that lacks BH1 and BH2 domains, making Mcl-1S incapable of forming the BH3-binding groove. Since Mcl-1 still possesses a BH3 domain, it can act in a manner similar to proapoptotic BH3-only proteins. The C terminus of Mcl-1S (aa 231–271), a product of exon 3, is substantially different from that of Mcl-1L due to a reading frame shift. (D) mRNA of Mcl-1ES misses a portion of exon 1 that is responsible for encoding aa 37–189, which is normally present in the other isoforms. Therefore, the greater part of the regulatory N-terminal domain and also the BH4 motif are absent in the Mcl-1ES protein. It is unlikely that Mcl-1ES can fold the antiapoptotic BH3-binding groove. Mcl-1ES exhibits noncanonical proapoptotic activity. Notably, Mcl-1ES has been studied only on overexpression of the corresponding cDNA and remains undetected in unmodified cells. Abbreviations: aa, amino acid residue; BH, Bcl-2 homology; PEST, motif rich in proline, glutamic acid, serine, and threonine residues.

Comparing two pore-forming proteins in the cellular context, Bak is the preferable Mcl-1-binding partner while Mcl-1–Bax interactions are mainly undetectable [41,42]. Under basal conditions, BH3-only proteins appear to efficiently prevent Mcl-1–Bax interactions, whereas Mcl-1/Bak complexes remain untouched. At the same time, upregulation of BH3-only proteins, such as Bim, Noxa, and Puma may lead to disruption of Mcl-1/Bak complexes [14,43,44]. Notably, *BAX*$^{-/-}$ and *BAK*$^{-/-}$ cells demonstrate different susceptibilities to various apoptotic stimuli. For example, treatment with ABT-737 (the inhibitor of Bcl-2, Bcl-xL, and Bcl-w) kills *BAK*$^{-/-}$, but not *BAX*$^{-/-}$ HCT116 cells. Resistance to ABT-737-induced apoptosis in *BAX*$^{-/-}$ HCT116 cells is dictated by Mcl-1, which efficiently binds Bak [45].

Box 2. Short Variants of Mcl-1 and Apoptosis

To date, little is known about the roles of Mcl-1ES and Mcl-1S in the regulation of apoptosis. Overexpression of Mcl-1ES induced cytochrome *c* release in a Bax- and Bak-independent manner. Furthermore, for its proapoptotic activity, Mcl-1ES required Mcl-1, which facilitated mitochondrial Mcl-1ES localization and MOMP [33]. However, whether endogenous Mcl-1ES has any physiological significance is unclear.

Mcl-1S possesses proapoptotic activity, and among Bcl-2 family proteins only Mcl-1 was shown to interact with Mcl-1S. Consistently, Mcl-1 overexpression prevents Mcl-1S-induced apoptosis, whereas overexpression of Bcl-2 and Bcl-xL only partially rescues the cells [31]. Additionally, the stabilized BH3 α helices of Mcl-1S selectively interact with the hydrophobic groove of Mcl-1 but not that of other antiapoptotic proteins [34]. Taking these findings together, Mcl-1S apparently acts as a BH3-only protein, which specifically blocks the antiapoptotic activity of Mcl-1. An increased Mcl-1S:Mcl-1 ratio was found in preeclamptic placentae [35] and neurons exposed to ethanol [36], although the physiological significance of these phenomena was not studied. It remains unclear whether the Mcl-1/Mcl-1S splicing switch is implicated under some pathological and/or physiological conditions.

Among all BH3-only proteins, Noxa is a unique regulator of Mcl-1 for two reasons. First, due to structural peculiarities Noxa binds to Mcl-1 and A1 with much higher affinity than to Bcl-2 and Bcl-xL [46]. Second, in contrast to other BH3-only proteins, Noxa promotes proteasomal degradation of Mcl-1, thus switching off Mcl-1's antiapoptotic functions [47]. Mechanistically, Noxa controls the interactions between Mcl-1 and the ubiquitination and deubiquitination enzymes Mule and USP9X, respectively [48]. Noxa biases the Q221R222N223 (QRN) motif in the BH3 domain of Mcl-1 toward a helical conformation, which facilitates Mcl-1 ubiquitination by Mule, whereas Bim exerts the opposite destabilizing effect (Figure 2) [49]. This is consistent with the observation that the C-terminal sequence of Noxa, which occupies the BH3-binding groove of Mcl-1 in close proximity to the QRN motif, is essential for Mcl-1 degradation [47]. Additionally, Noxa promotes Mcl-1 site-specific phosphorylation by Chk2, resulting in subsequent ubiquitin-dependent degradation [50]. It is currently not fully understood which mechanisms of Noxa-mediated degradation of Mcl-1 prevail and whether a link between them exists.

Mcl-1 as a Target for Cancer Therapy

Mcl-1 is the most abundant product of the *MCL1* gene and appears to be essential for the survival of cells of various origins [51]. However, owing to Mcl-1's survival potential, tumor cells often exploit its upregulation. Mcl-1 is overexpressed across various types of B- and T-lineage non-Hodgkin lymphomas [52] and in a variety of solid malignancies, including hepatocellular carcinoma [53], esophageal squamous cell carcinoma [54], breast cancer [55], and others [56].

As Mcl-1 can neutralize a broad spectrum of proapoptotic proteins, it may functionally compensate for the lack of the other antiapoptotic proteins or related activity. Consistently, Mcl-1 can serve as a major resistance factor for compounds targeting both Bcl-2 and Bcl-xL (ABT-737, ABT-263/navitoclax) [41] or Bcl-2 only (ABT-199/venetoclax) [57]. These compounds are referred to as '**BH3 mimetics**' because they block the BH3-binding grooves of antiapoptotic proteins in a manner similar to that of BH3-only proteins. Since ABT-199 received approval for clinical use, therapeutic strategies to overcome its resistance have been under active development.

As with other antiapoptotic proteins of the Bcl-2 family, Mcl-1 could be neutralized by BH3 mimetics, which disrupt interactions between the target protein and its partners. However, unique molecular features of Mcl-1 leave room for maneuver in Mcl-1-targeted therapy.

BH3 Mimetics: Standard Approach or Targeted Degradation?

To affect Mcl-1-dependent tumors and evade drug resistance, a great effort was made to target Mcl-1 using small molecules [58]. This recently culminated in the discovery of the highly selective and potent BH3 mimetic S63845, which demonstrated tolerance and efficacy against several cancer types in mouse models [59]. Currently, a derivative of S63845, S64315/MIK665, is

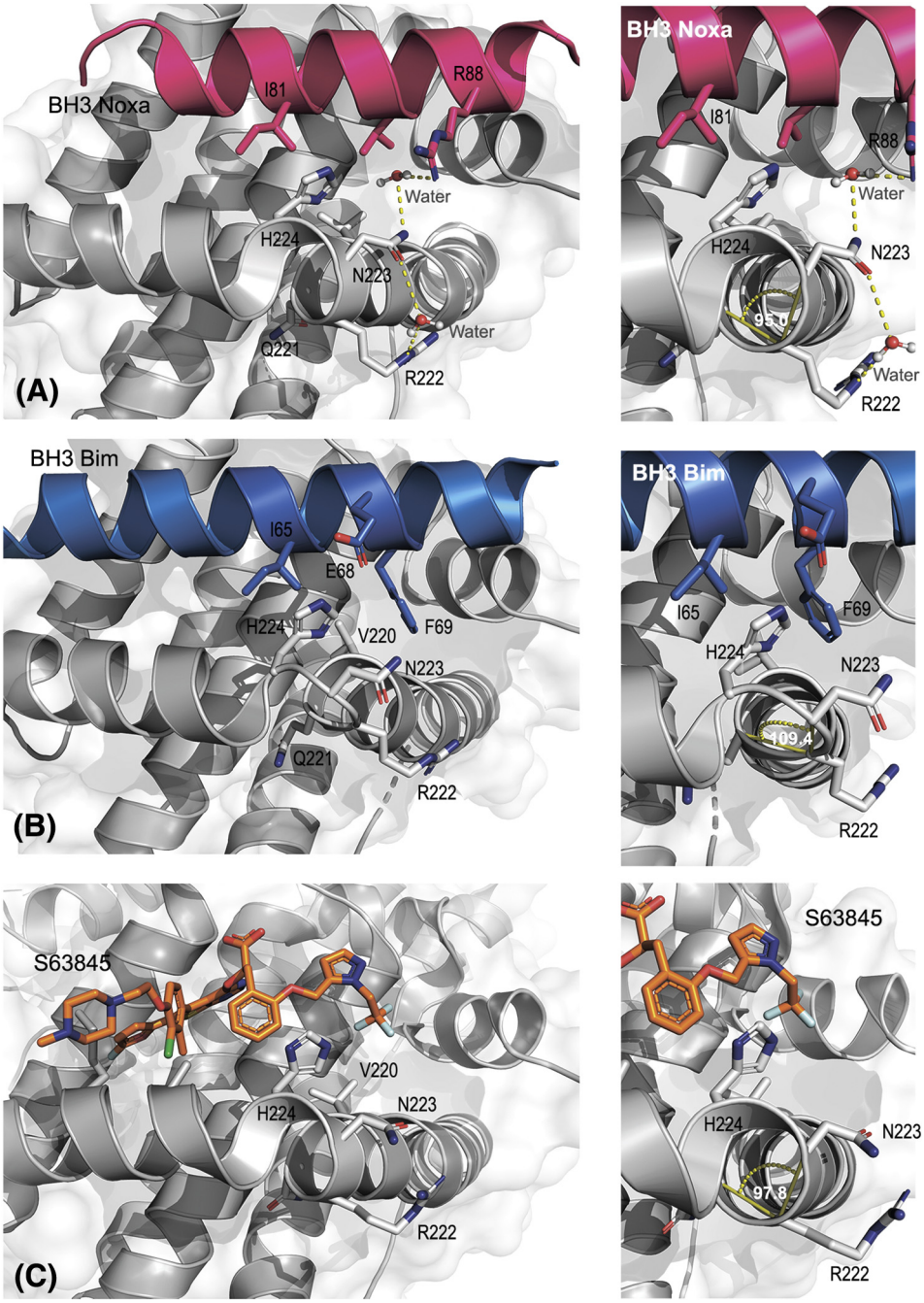

Figure 2. Molecular Mechanisms of Mcl-1 Degradation. Noxa (pink) was found to target Mcl-1 for proteasomal degradation, whereas Bim (blue) stabilizes Mcl-1. To explain why one BH3-only protein facilitates proteasomal degradation while another causes the opposite effect, Song *et al.* [49] assumed that the proteins have different conformational impacts on the Q221R222N223 (QRN) motif of Mcl-1. (A) Through binding, the Noxa BH3 domain sustains the α-helical structure of the QRN region that apparently makes the Mcl-1 conformation more likely to complex with the Mule ubiquitin ligase. The stabilization could be visualized as the interaction of R88 mNoxaB, an amino acid

(Figure legend continued at the bottom of the next page.)

undergoing Phase I clinical trials in patients with hematological malignancies (NCT02979366, NCT02992483). Meanwhile, there are reports of further promising signs of preclinical activity with S63845 [60]. The list of potential Mcl-1-targeting drugs is also represented by the recently described BH3 mimetics AMG176 [61] and AZD5991 [62], both of which are being evaluated in Phase I clinical trials (NCT02675452 and NCT03218683, respectively).

There were some concerns about possible on-target toxicities of S63845, as this compound has a higher affinity to human Mcl-1 than to its mouse counterpart [63]. This issue was recently addressed in experiments in which mouse Mcl-1 was substituted with its human homolog (huMcl-1 mice). Treatment of huMcl-1 mice with S63845 demonstrated its efficacy against lymphomas at tolerable doses. However, the maximum tolerated dose was lower in huMcl-1 mice than in the control group [64], suggesting that S63845 toxicity depends on its immediate target Mcl-1, although the precise mechanism is unknown. Arguably, toxic doses of S63845 disrupt high-affinity interactions between Mcl-1 and proapoptotic proteins, leading to apoptosis in normal cells and lesions in various tissues.

Meanwhile, some BH3-mimetic-like compounds might target Mcl-1 for proteasomal decay in a similar way to Noxa. Several Mcl-1 antagonists, including S63845, AMG176, and AZD5991, lead to Mcl-1 stabilization [49,59,61,62]. However, other inhibitors of Mcl-1, such as UMI-77 and maritoclax, promote degradation of this protein. Interestingly, both UMI-77 and maritoclax act in a Noxa-like manner on the QRN motif of Mcl-1 [49]. Whether the impact on the QRN motif has a decisive role in targeting Mcl-1 for degradation requires further verification. Regardless, Noxa mimetics that target Mcl-1 for proteasomal degradation could be used as a potential tool to neutralize Mcl-1 in cancer cells.

It is not yet clear which of these two groups of compounds (i.e., standard BH3 mimetics or Noxa mimetics) represents a more favorable tool to neutralize Mcl-1. The complete disruption of complexes between Mcl-1 and its proapoptotic partners seems to be sufficient to make the former compounds as effective as the latter ones. Therefore, the potency of Mcl-1 inhibitors to prevent the interactions between Mcl-1 and its binding partners should be thoroughly evaluated, including in the context of possible toxicity. Nevertheless, at least in some cases, cancer cells might have machinery with higher activity that is responsible for Noxa-mediated degradation of Mcl-1, which could be a prerequisite for the potential use of Noxa mimetics in the clinic.

Turning Mcl-1 into a Proapoptotic Protein

Mcl-1 splicing control could be used to overcome Mcl-1-mediated resistance to apoptosis [65]. No splicing factors have been described that regulate Mcl-1ES production. By contrast, a

residue corresponding to R39 of human Noxa, with the N223 and R222 (Mcl-1) residues by hydrogen bonds, with a calculated angle between the QRN alpha carbons of 95.0°. (B) The BH3 domain of Bim disrupts the α-helical conformation of QRN. By comparison, the QRN helix angle is 109.4° instead of 95.0°. As a result, the Bim BH3 domain stabilizes Mcl-1. (C) The Mcl-1 inhibitor S63845 does not substantially affect the conformational stability of QRN (the angle between the alpha carbons of these residues is only slightly different from that of Noxa), yet experimentally the compound substantially stabilizes Mcl-1. The nondegradative effect might be attributed to the lack of stabilizing interactions between S63845 and the QRN motif. Based on the described model of Mcl-1 degradation, Song *et al.* [49] also suggested a compound that stabilizes the QRN motif in a Noxa-like manner; interestingly, the chemical indeed leads to Mcl-1 degradation. However, more research needs to be conducted to solidify that structural hypothesis. Several side chains of amino acids and the BH3 mimetic S63845 are shown in stick representations where blue, red, cyan, green, and yellow correspond to nitrogen, oxygen, fluorine, chlorine, and sulfur atoms, respectively. Carbons in the side chains of amino acids and in S63845 are shown in gray and orange, respectively. Structures are based on the following Protein Data Bank (PDB) accessions: Mcl-1 and BH3 Noxa, Crystal structure of the Mcl-1:mNoxaB BH3 complex (2NLA); Mcl-1 and Bim BH3, Crystal structure of the Mcl-1:Bim BH3 complex (2NL9); Mcl-1 and S63845, Crystal structure of the MBP-MCL1 complex with highly selective and potent inhibitor of MCL1 (5LOF).

genome-wide small interfering (si)RNA screen disclosed dozens of factors that are associated with the splicing switch between Mcl-1 and Mcl-1S isoforms. Interestingly, some of these factors simultaneously regulate Bcl-x splicing [66]. Silencing of several splicing factors, including SF3B1 and SRSF1 (also known as ASF/SF2), switches alternative splicing toward a proapoptotic Mcl-1S isoform. Hence, SF3B1 and SRSF1 favor Mcl-1 formation. The RBM4 and SRSF2 splicing factors, conversely, act as pro-Mcl-1S factors by promoting the skipping of exon 2 in Mcl-1 pre-mRNA [67,68].

Thus, inhibitors of SF3B sensitize cancer cell lines of different origins to BH3-mimetic-induced apoptosis through a decrease in Mcl-1L isoform formation [65,69,70]. Other useful tools are antisense morpholino oligonucleotides for exon skipping, which could shift splicing from Mcl-1L to Mcl-1S, consequently leading to apoptosis [71].

Indirect Targeting of Mcl-1

Numerous regulatory mechanisms tightly control Mcl-1 functionality by changing its level in response to different stimuli. Several ubiquitin ligases (Mule, $SCF^{\beta\text{-}TrCP}$, SCF^{FBW7}, TRIM17, SCF^{FBXO4}, Parkin [72–74]) and deubiquitinases (USP9X, USP13, USP24, Ku70 [72,75–77]) orchestrate ubiquitination and the subsequent proteasomal degradation of Mcl-1. Consistently, treatment with the deubiquitinase inhibitor WP1130, which also targets the deubiquitinase USP9X, induced apoptosis in antiestrogen-resistant breast cancer cells in an Mcl-1-dependent manner [78]. The exhaustive list of Mcl-1 regulators could underlie precision-medicine approaches.

As Mcl-1 has a short half-life, different approaches were proposed to target Mcl-1 via the inhibition of protein synthesis. For example, transcriptional suppression by flavopiridol leads to a rapid drop in Mcl-1 levels, resulting in induction of apoptosis or sensitization to ABT-199 [79,80]. Likewise, other approaches, including inhibition of mTORC1 by calorie restriction or metformin treatment, decrease Mcl-1 level by translational suppression [81,82]. Inhibition of transcription or translation results in the selective depletion of Mcl-1 and other short-lived proteins [83,84]. Therefore, targeting of protein synthesis relies on the fast depletion of labile oncoproteins, and thus may have therapeutic potential. However, the practical application of such approaches remains questionable. For instance, in Phase I of two clinical trials, flavopiridol in combination with genotoxic agents failed to achieve a marked decrease in Mcl-1 levels in patients [85,86]. It is necessary to evaluate the clinical effectiveness of different approaches for indirect targeting of Mcl-1, as well as biochemical markers indicating their potential benefits.

Nonapoptotic Functions of Mcl-1

Besides its antiapoptotic utility, Mcl-1 displays a list of nonapoptotic functions in autophagy, mitochondrial homeostasis, nuclear events, and the regulation of protein kinase signaling. The latter aspect was recently reviewed [87] and thus is not discussed here. Interestingly, *MCL1* knockout causes **peri-implantation lethality** in mice in the absence of increased apoptosis in blastocysts [88]. One possible explanation for this phenomenon is the impact of Mcl-1 on STAT3 signaling and mesenchymal-to-epithelial transition during implantation [89]. Hence, the nonapoptotic functions of Mcl-1 are of great significance and should be taken into consideration as they could be affected by approaches targeting Mcl-1 (Figure 3).

Mcl-1 and Autophagy

Mcl-1 has been implicated in the regulation of autophagy, the process of degradation and recycling of intracellular content, which results in cell survival or death [90]. There is convincing evidence that Mcl-1, like other Bcl-2 family members (Bcl-2 and Bcl-xL), binds to a key autophagic protein, Beclin-1 [90–92]. This interaction, mediated by the BH3-binding groove of Mcl-1 and the Beclin-1 BH3 motif, precludes stimulatory phosphorylation of Beclin-1, thus blocking membrane

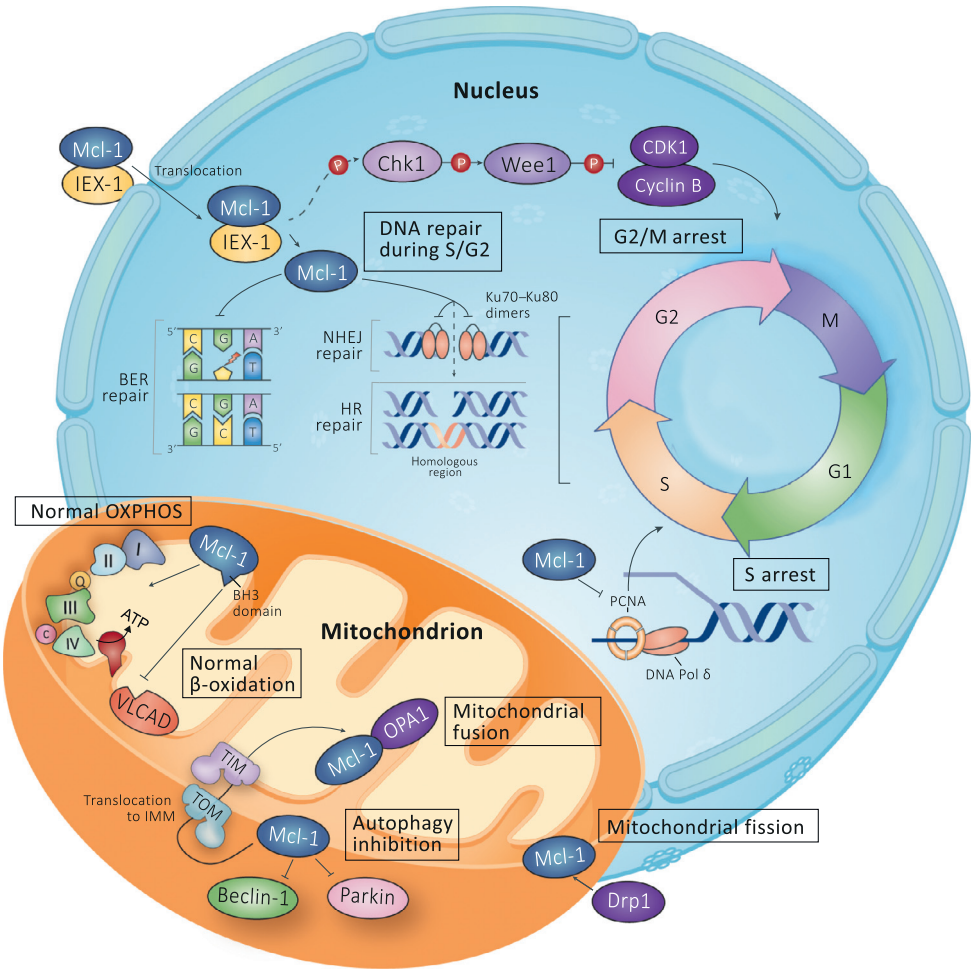

Figure 3. Nonapoptotic Functions of Mcl-1. Activated on DNA damage, IEX-1 promotes the translocation of Mcl-1 to the nucleus. Either together with IEX-1 or alone, Mcl-1 induces the phosphorylation of Chk1 and thereby mediates mitotic arrest for DNA repair. Mcl-1 can also enhance the accuracy of DNA repair during S/G2 phase by inhibitive binding to Ku70–Ku80 dimers and thus shifting from nonhomologous DNA end joining (NHEJ) to homologous recombination (HR). By contrast, Mcl-1 can suppress base excision repair (BER) under certain conditions. In S phase, Mcl-1 can inhibit a cofactor for DNA polymerase delta (DNA Pol δ), proliferating cell nuclear antigen (PCNA), and therefore impede further progression of the cell cycle. On the outer mitochondrial membrane (OMM), Mcl-1 interacts with Beclin-1 and Parkin thus suppressing the autophagy and mitophagy pathways. Mcl-1 also recruits a profission protein, Drp1, facilitating mitochondrial fragmentation. By contrast, Mcl-1 imported through the translocase of the inner membrane (TIM)/translocase of the outer membrane (TOM) complex to the inner mitochondrial membrane (IMM) increases the stability of the profusion protein OPA1. In the noncanonical conformation of a surface-exposed BH3 domain, Mcl-1 binds to very-long-chain acyl-CoA dehydrogenase (VLCAD), preventing excessive activity under stress. Mitochondrial matrix Mcl-1 also supports normal oxidative phosphorylation (OXPHOS), although the mechanism underlying the phenomenon is not yet established. Abbreviation: CDK1, cyclin-dependent kinase-1.

trafficking for autophagosome formation [92,93]. Moreover, Mcl-1 impairs the stability of Beclin-1 by competitively binding to the same region of the USP9X deubiquitinase, which can stabilize both proteins [94].

The downregulation of Mcl-1 can cause a robust autophagic response [90,92]. However, depending on the context, Mcl-1 loss can also lead to impaired autophagy [95]. Prodeath autophagy, shown on a decrease of Mcl-1, might be attributed to mitochondrial dysfunction (see below), which is accompanied by decreased mitochondrial membrane potential, ATP depletion, and the production of reactive oxygen species (ROS) [95].

Depending on the relative dominance of autophagic or proapoptotic factors, Mcl-1 can negatively regulate autophagy or apoptosis, respectively. In the former case, deletion of *MCL1* in postmitotic neurons, which express elevated levels of autophagic Atg7 and Atg5–Atg12 conjugates, fosters autophagy [92]. Additionally, Mcl-1 is phosphorylated, ubiquitinated, and subsequently degraded in dorsal root ganglion explant neurons, promoting axonal degeneration, caused by Beclin-1 activation and the expression of Atg5 and Atg7 [96]. In the latter case, neural progenitors that lack Mcl-1 are more susceptible to apoptosis (but not to autophagy) due to greater expression of proapoptotic machinery. Such patterns result in different stress responses in mature neurons and neural progenitors, owing to dissimilar profiles of autophagic and proapoptotic factors. Taking these findings together, Mcl-1 prevents both autophagy and apoptosis according to cellular conditions [92].

Mcl-1 and Mitochondrial Homeostasis

Mcl-1 localizes to both the OMM (hereafter referred to as Mcl-1^{OMM}) and the mitochondrial matrix (Mcl-1^{Matrix}) [97]. The translocation of Mcl-1 to the mitochondrial matrix is implemented via the **translocase of the outer/inner membrane (TIM/TOM) complex** and implies proteolytic cleavage, generating an N-terminally truncated form [26]. Mcl-1^{Matrix} maintains a supramolecular organization of the electron transport chain complexes and ATP synthase. Mitochondria lacking Mcl-1 disassemble and subsequently exhibit respiratory chain inefficiency, enhanced ROS production, and defective cristae structure [26]. Furthermore, Mcl-1^{Matrix} interacts with and elevates the stability of the mitochondrial fusion protein OPA1 that has been implicated in maintaining oxidative phosphorylation in cells [98]. By contrast, Mcl-1^{OMM} induces the recruitment of profission Drp1 to the organelle, promoting mitochondrial fragmentation when Mcl-1 is overexpressed [32,98]. Both OPA1 and Drp1 interact with Mcl-1 through its BH3-binding groove [98]. It was also demonstrated that Mcl-1^{OMM}, in common with other Bcl-2 family proteins, binds to Parkin and suppresses mitophagy in a Beclin-1-independent manner [99].

In murine experimental models, Mcl-1 ablation in the heart leads to cardiomyopathy, which manifests with mitochondrial abnormalities and poor ATP production [95,100]. A further explanation for this effect could also be that, since Mcl-1^{Matrix} appears to bind very-long-chain acyl-CoA dehydrogenase (VLCAD) in a noncanonical conformation (in which the Mcl-1 BH3 helix is exposed), such an interaction prevents hyperactivity of the fatty acid β-oxidation pathway under physiological stress, maintaining optimized substrate consumption and normalized acetyl-CoA levels [101]. Notably, the interactions of Mcl-1 with both OPA1 and Drp1 are disrupted by S63845, whereas the inhibitor does not influence long-chain fatty acid oxidation [98,101]. Overall, two forms of Mcl-1 – Mcl-1^{Matrix} and Mcl-1^{OMM} – influence major mitochondrial functions and network dynamics by binding to mitochondrial proteins.

Mcl-1 and the Nucleus

In the nucleus, Mcl-1 interacts with several partners, including cell cycle regulators [29,30,77]. In particular, Mcl-1 can directly bind proliferating cell nuclear antigen (PCNA) through the S phase [29]. Furthermore, Mcl-1 stimulates Chk1 phosphorylation both on DNA damage and in the absence of genotoxic stress, thus maintaining the G2/M checkpoint response [30,102,103]. These data support the involvement of Mcl-1 in cell cycle control.

Being dynamically regulated by cellular conditions, Mcl-1 can rapidly accumulate in the nucleus in response to double-strand breaks [77,102,103]. This nuclear translocation is mediated by the early-response gene product IEX-1, which relies on ATM activity (the latter corresponds to the degree of double-strand DNA breaks) [102,104]. Cooperatively, Mcl-1 and IEX-1 modulate the phosphorylation of Chk1 and thus mediate G2/M arrest for DNA repair and cell survival [102]. Recently, it was reported that following genotoxic stress Mcl-1 interacts with the Ku70/Ku80 heterodimeric complex during S/G2 phase, which results in switching from nonhomologous DNA end joining (NHEJ) to homologous recombination (HR) [105].

Based on these studies, Mcl-1 performs nuclear functions both under normal cellular conditions (cell cycle control) and after genotoxic stress. These roles might be essential for cell survival, since cells lacking Mcl-1 exhibit cell cycle aberrations and extended DNA abnormalities on DNA damage [102,103]. At the same time, Mcl-1 was proposed to suppress base excision repair (BER) in liver cell lines in response to bile salt glycochenodeoxycholate exposure [106], which points to a possible role of the nonapoptotic functions of Mcl-1 in the pathogenesis of hepatocellular carcinoma.

In general, the nonapoptotic functions of Mcl-1 are poorly investigated, especially their physiological significance. For instance, the roles of Mcl-1 in cell cycle regulation and DNA repair have been studied only in cultured cells. Nevertheless, the relevance of some nonapoptotic functions of Mcl-1 was confirmed *in vivo*. One particularly striking example is the role of Mcl-1 in the maintenance of mitochondrial homeostasis in cardiomyocytes. Hence, possible cardiotoxicity is one of the main caveats of the use of Mcl-1 antagonists. Intriguingly, BH3 mimetics, which only block the BH3-binding groove in the absence of Mcl-1 degradation, might have a decisive advantage, since, intuitively, they should not affect those functions that are independent of the BH3-binding groove. In this context, the molecular mechanisms of the nonapoptotic functions of Mcl-1 and the effects of BH3 mimetics (e.g., S63845, AMG176, AZD5991) on these functions should be carefully investigated.

Concluding Remarks

Currently, there is a large amount of data concerning the functions of Mcl-1. However, some of these functions, particularly those that are nonapoptotic, remain poorly understood. Numerous reports indicate that Mcl-1 regulates autophagy, mitochondrial homeostasis, the cell cycle, and protein kinase signaling. Further studies of such functions are required, especially in the context of possible side effects of Mcl-1 inhibition or ablation (see Outstanding Questions). Regarding its antiapoptotic functions, Mcl-1 undoubtedly plays an important role in the survival of both normal and cancer cells. A detailed study of the control of **mitochondrial outer membrane permeabilization (MOMP)** by Bcl-2 family members resulted in the development of BH3 mimetics, which demonstrate promising anticancer properties. Researchers eagerly await the results of clinical trials of Mcl-1-specific BH3 mimetics.

Meanwhile, it is important to consider alternative ways of targeting Mcl-1, such as utilizing Noxa mimetics to promote proteasomal degradation of Mcl-1. The indirect targeting of Mcl-1 by affecting its upstream regulators represents another strategy to neutralize the antiapoptotic activity of Mcl-1. In general, comprehension of the molecular biology of Mcl-1 brings us closer to exploiting this protein as a therapeutic target.

Outstanding Questions

What are the precise roles of short variants of Mcl-1 in the cell? What is the significance of the Mcl-1L/Mcl-1S splicing switch under physiological and/or pathological conditions? Is the Mcl-1 alternative splicing switch toward its proapoptotic isoform an efficient strategy to antagonize Mcl-1 in cancer cells?

Why does Noxa promote ubiquitination of Mcl-1 while Bim exerts the opposite, stabilizing effect? Does the QRN hypothesis explain all Mcl-1 interactions associated with its degradation or accumulation? Could this hypothesis be used for the structural design of Noxa mimetics, compounds that promote Mcl-1 degradation?

What are the mechanisms of the toxicities of the current Mcl-1-selective BH3 mimetics? Which nonapoptotic functions of Mcl-1 are independent of the BH3-binding groove and which will be interfered with by BH3 mimetics?

Could the applicable indirect approaches that lower Mcl-1 levels (e.g., mTORC1 inhibition by metformin treatment, inhibition of Mcl-1 deubiquitinases) be beneficial in the clinic? How would they compare with direct approaches in efficacy or safety?

Acknowledgments

This work was supported by a grant from the Russian Science Foundation (17-75-20102). The work in the authors' laboratories is supported by grants from the Stockholm Cancer Society (181301) and Swedish Cancer Society (160733), the Swedish Childhood Cancer Foundation (PR-2018-0058), and the Russian Foundation for Basic Research (19-015-00332, 18-29-09005).

References

1. Galluzzi, L. *et al.* (2018) Molecular mechanisms of cell death: recommendations of the Nomenclature Committee on Cell Death 2018. *Cell Death Differ.* 25, 486–541
2. Zamaraev, A.V. *et al.* (2017) Post-translational modification of caspases: the other side of apoptosis regulation. *Trends Cell Biol.* 27, 322–339
3. Acehan, D. *et al.* (2002) Three-dimensional structure of the apoptosome: implications for assembly, procaspase-9 binding, and activation. *Mol. Cell* 9, 423–432
4. Czabotar, P.E. *et al.* (2013) Control of apoptosis by the BCL-2 protein family: implications for physiology and therapy. *Nat. Rev. Mol. Cell Biol.* 15, 49–63
5. Yin, X.-M. *et al.* (1994) BH1 and BH2 domains of Bcl-2 are required for inhibition of apoptosis and heterodimerization with Bax. *Nature* 369, 321–323
6. Chang, B.S. *et al.* (1999) The BH3 domain of Bcl-x_S is required for inhibition of the antiapoptotic function of Bcl-x_L. *Mol. Cell. Biol.* 19, 6673–6681
7. Bingle, C.D. *et al.* (2000) Exon skipping in Mcl-1 results in a Bcl-2 homology domain 3 only gene product that promotes cell death. *J. Biol. Chem.* 275, 22136–22146
8. Kim, J.-H. *et al.* (2009) MCL-1ES, a novel variant of MCL-1, associates with MCL-1L and induces mitochondrial cell death. *FEBS Lett.* 583, 2758–2764
9. Moldoveanu, T. *et al.* (2014) Many players in BCL-2 family affairs. *Trends Biochem. Sci.* 39, 101–111
10. Wolter, K.G. *et al.* (1997) Movement of Bax from the cytosol to mitochondria during apoptosis. *J. Cell Biol.* 139, 1281–1292
11. Griffiths, G.J. *et al.* (1999) Cell damage-induced conformational changes of the pro-apoptotic protein Bak *in vivo* precede the onset of apoptosis. *J. Cell Biol.* 144, 903–914
12. Wei, M.C. *et al.* (2000) tBID, a membrane-targeted death ligand, oligomerizes BAK to release cytochrome *c*. *Genes Dev.* 14, 2060–2071
13. Dewson, G. *et al.* (2008) To trigger apoptosis, Bak exposes its BH3 domain and homodimerizes via BH3:groove interactions. *Mol. Cell* 30, 369–380
14. Willis, S.N. *et al.* (2005) Proapoptotic Bak is sequestered by Mcl-1 and Bcl-xL, but not Bcl-2, until displaced by BH3-only proteins. *Genes Dev.* 19, 1294–1305
15. Dai, H. *et al.* (2013) Contribution of Bcl-2 phosphorylation to Bak binding and drug resistance. *Cancer Res.* 73, 6998–7008
16. Kuwana, T. *et al.* (2005) BH3 domains of BH3-only proteins differentially regulate Bax-mediated mitochondrial membrane permeabilization both directly and indirectly. *Mol. Cell* 17, 525–535
17. Chen, L. *et al.* (2005) Differential targeting of prosurvival Bcl-2 proteins by their BH3-only ligands allows complementary apoptotic function. *Mol. Cell* 17, 393–403
18. Dai, H. *et al.* (2014) Evaluation of the BH3-only protein Puma as a direct Bak activator. *J. Biol. Chem.* 289, 89–99
19. Chen, H.-C. *et al.* (2015) An interconnected hierarchical model of cell death regulation by the BCL-2 family. *Nat. Cell Biol.* 17, 1270–1281
20. Llambi, F. *et al.* (2011) A unified model of mammalian BCL-2 protein family interactions at the mitochondria. *Mol. Cell* 44, 517–531
21. Kozopas, K.M. *et al.* (1993) *MCL1*, a gene expressed in programmed myeloid cell differentiation, has sequence similarity to BCL2. *Proc. Natl. Acad. Sci. U. S. A.* 90, 3516–3520
22. Lømo, J. *et al.* (1996) Expression of the Bcl-2 homologue Mcl-1 correlates with survival of peripheral blood B lymphocytes. *Cancer Res.* 56, 40–43
23. Zhou, P. *et al.* (1998) Mcl-1 in transgenic mice promotes survival in a spectrum of hematopoietic cell types and immortalization in the myeloid lineage. *Blood* 92, 3226–3239
24. Opferman, J.T. *et al.* (2003) Development and maintenance of B and T lymphocytes requires antiapoptotic MCL-1. *Nature* 426, 671–676
25. Kvansakul, M. *et al.* (2008) Vaccinia virus anti-apoptotic F1L is a novel Bcl-2-like domain-swapped dimer that binds a highly selective subset of BH3-containing death ligands. *Cell Death Differ.* 15, 1564–1571
26. Perciavalle, R.M. *et al.* (2012) Anti-apoptotic MCL-1 localizes to the mitochondrial matrix and couples mitochondrial fusion to respiration. *Nat. Cell Biol.* 14, 575–583
27. Chao, J.R. *et al.* (1998) *mcl-1* is an immediate-early gene activated by the granulocyte-macrophage colony-stimulating factor (GM-CSF) signaling pathway and is one component of the GM-CSF viability response. *Mol. Cell. Biol.* 18, 4883–4898
28. Liu, H. *et al.* (2005) Stabilization and enhancement of the antiapoptotic activity of mcl-1 by TCTP. *Mol. Cell. Biol.* 25, 3117–3126
29. Fujise, K. *et al.* (2000) Regulation of apoptosis and cell cycle progression by MCL1. *J. Biol. Chem.* 275, 39458–39465
30. Jamil, S. *et al.* (2008) An essential role for MCL-1 in ATR-mediated CHK1 phosphorylation. *Mol. Biol. Cell* 19, 3212–3220
31. Bae, J. *et al.* (2000) MCL-1S, a splicing variant of the antiapoptotic BCL-2 family member MCL-1, encodes a proapoptotic protein possessing only the BH3 domain. *J. Biol. Chem.* 275, 25255–25261
32. Morciano, G. *et al.* (2016) Mcl-1 involvement in mitochondrial dynamics is associated with apoptotic cell death. *Mol. Biol. Cell* 27, 20–34
33. Kim, J.-H. and Bae, J. (2013) MCL-1ES induces MCL-1L-dependent BAX- and BAK-independent mitochondrial apoptosis. *PLoS One* 8, e79626
34. Stewart, M.L. *et al.* (2010) The MCL-1 BH3 helix is an exclusive MCL-1 inhibitor and apoptosis sensitizer. *Nat. Chem. Biol.* 6, 595–601
35. Soleymanlou, N. *et al.* (2007) Hypoxic switch in mitochondrial myeloid cell leukemia factor-1/Mtd apoptotic rheostat contributes to human trophoblast cell death in preeclampsia. *Am. J. Pathol.* 171, 496–506
36. Sariyer, R. *et al.* (2017) Alcohol-mediated missplicing of Mcl-1 pre-mRNA is involved in neurotoxicity. *Alcohol. Clin. Exp. Res.* 41, 1715–1724
37. Senichkin, V.V. *et al.* (2018) Modulation of Mcl-1 transcription by serum deprivation sensitizes cancer cells to cisplatin. *Biochim. Biophys. Acta* 1862, 557–566
38. Kale, J. *et al.* (2018) BCL-2 family proteins: changing partners in the dance towards death. *Cell Death Differ.* 25, 65–80
39. Certo, M. *et al.* (2006) Mitochondria primed by death signals determine cellular addiction to antiapoptotic BCL-2 family members. *Cancer Cell* 9, 351–365
40. Ku, B. *et al.* (2011) Evidence that inhibition of BAX activation by BCL-2 involves its tight and preferential interaction with the BH3 domain of BAX. *Cell Res.* 21, 627–641
41. Konopleva, M. *et al.* (2006) Mechanisms of apoptosis sensitivity and resistance to the BH3 mimetic ABT-737 in acute myeloid leukemia. *Cancer Cell* 10, 375–388
42. Zhai, D. *et al.* (2008) Differential regulation of Bax and Bak by anti-apoptotic Bcl-2 family proteins Bcl-B and Mcl-1. *J. Biol. Chem.* 283, 9580–9586
43. Gillissen, B. *et al.* (2007) Mcl-1 determines the Bax dependency of Nbk/Bik-induced apoptosis. *J. Cell Biol.* 179, 701–715
44. Gomez-Bougie, P. *et al.* (2016) Repression of Mcl-1 and disruption of the Mcl-1/Bak interaction in myeloma cells couple ER stress to mitochondrial apoptosis. *Cancer Lett.* 383, 204–211
45. Wang, C. and Youle, R.J. (2012) Predominant requirement of Bax for apoptosis in HCT116 cells is determined by Mcl-1's inhibitory effect on Bak. *Oncogene* 31, 3177–3189
46. Smith, A.J. *et al.* (2011) Noxa/Bcl-2 protein interactions contribute to bortezomib resistance in human lymphoid cells. *J. Biol. Chem.* 286, 17682–17692
47. Czabotar, P.E. *et al.* (2007) Structural insights into the degradation of Mcl-1 induced by BH3 domains. *Proc. Natl. Acad. Sci. U. S. A.* 104, 6217–6222
48. Gomez-Bougie, P. *et al.* (2011) Noxa controls Mule-dependent Mcl-1 ubiquitination through the regulation of the Mcl-1/USP9X interaction. *Biochem. Biophys. Res. Commun.* 413, 460–464

49. Song, T. *et al.* (2016) Deactivation of Mcl-1 by dual-function small-molecule inhibitors targeting the Bcl-2 homology 3 domain and facilitating Mcl-1 ubiquitination. *Angew. Chem. Int. Ed. Engl.* 55, 14250–14256
50. Nakajima, W. *et al.* (2016) DNA damaging agent-induced apoptosis is regulated by MCL-1 phosphorylation and degradation mediated by the Noxa/MCL-1/CDK2 complex. *Oncotarget* 7, 36353–36365
51. Uhlen, M. *et al.* (2015) Tissue-based map of the human proteome. *Science* 347, 1260419
52. Fernández-Marrero, Y. *et al.* (2016) Survival control of malignant lymphocytes by anti-apoptotic MCL-1. *Leukemia* 30, 2152–2159
53. Sieghart, W. *et al.* (2006) Mcl-1 overexpression in hepatocellular carcinoma: a potential target for antisense therapy. *J. Hepatol.* 44, 151–157
54. Lin, J. *et al.* (2017) Mcl-1 inhibitor suppresses tumor growth of esophageal squamous cell carcinoma in a mouse model. *Oncotarget* 8, 114457–114462
55. Campbell, K.J. *et al.* (2018) MCL-1 is a prognostic indicator and drug target in breast cancer. *Cell Death Dis.* 9, 19
56. Uhlen, M. *et al.* (2017) A pathology atlas of the human cancer transcriptome. *Science* 357, eaan2507
57. Niu, X. *et al.* (2016) Binding of released Bim to Mcl-1 is a mechanism of intrinsic resistance to ABT-199 which can be overcome by combination with daunorubicin or cytarabine in AML cells. *Clin. Cancer Res.* 22, 4440–4451
58. Chen, L. and Fletcher, S. (2017) Mcl-1 inhibitors: a patent review. *Expert Opin. Ther. Pat.* 27, 163–178
59. Kotschy, A. *et al.* (2016) The MCL1 inhibitor S63845 is tolerable and effective in diverse cancer models. *Nature* 538, 477–482
60. Merino, D. *et al.* (2017) Synergistic action of the MCL-1 inhibitor S63845 with current therapies in preclinical models of triple-negative and HER2-amplified breast cancer. *Sci. Transl. Med.* 9, eaam7049
61. Caenepeel, S. *et al.* (2018) AMG 176, a selective MCL1 inhibitor, is effective in hematological cancer models alone and in combination with established therapies. *Cancer Discov.* 8, CD-18-0387
62. Tron, A.E. *et al.* (2018) Discovery of Mcl-1-specific inhibitor AZD5991 and preclinical activity in multiple myeloma and acute myeloid leukemia. *Nat. Commun.* 9, 5341
63. Letai, A. (2016) S63845, an MCL-1 selective BH3 mimetic: another arrow in our quiver. *Cancer Cell* 30, 834–835
64. Brennan, M.S. *et al.* (2018) Humanized *Mcl-1* mice enable accurate preclinical evaluation of MCL-1 inhibitors destined for clinical use. *Blood* 132, 1573–1583
65. Aird, D. *et al.* (2019) Sensitivity to splicing modulation of BCL2 family genes defines cancer therapeutic strategies for splicing modulators. *Nat. Commun.* 10, 137
66. Moore, M.J. *et al.* (2010) An alternative splicing network links cell-cycle control to apoptosis. *Cell* 142, 625–636
67. Lin, J.-C. *et al.* (2014) Elevated SRPK1 lessens apoptosis in breast cancer cells through RBM4-regulated splicing events. *RNA* 20, 1621–1631
68. Kędzierska, H. *et al.* (2016) Decreased expression of SRSF2 splicing factor inhibits apoptotic pathways in renal cancer. *Int. J. Mol. Sci.* 17, E1598
69. Laetsch, T.W. *et al.* (2014) Multiple components of the spliceosome regulate Mcl1 activity in neuroblastoma. *Cell Death Dis.* 5, e1072
70. Gao, Y. *et al.* (2014) Regulation of HPV16 E6 and MCL1 by SF3B1 inhibitor in head and neck cancer cells. *Sci. Rep.* 4, 6098
71. Shieh, J.-J. *et al.* (2009) Modification of alternative splicing of Mcl-1 pre-mRNA using antisense morpholino oligonucleotides induces apoptosis in basal cell carcinoma cells. *J. Invest. Dermatol.* 129, 2497–2506
72. Mojsa, B. *et al.* (2014) Mcl-1 ubiquitination: unique regulation of an essential survival protein. *Cells* 3, 418–437
73. Feng, C. *et al.* (2017) FBXO4 inhibits lung cancer cell survival by targeting Mcl-1 for degradation. *Cancer Gene Ther.* 24, 342–347
74. Carroll, R.G. *et al.* (2014) Parkin sensitizes toward apoptosis induced by mitochondrial depolarization through promoting degradation of Mcl-1. *Cell Rep.* 9, 1538–1553
75. Diao, J. *et al.* (2015) ATG14 promotes membrane tethering and fusion of autophagosomes to endolysosomes. *Nature* 520, 563–566
76. Peterson, L.F. *et al.* (2015) Targeting deubiquitinase activity with a novel small-molecule inhibitor as therapy for B-cell malignancies. *Blood* 125, 3588–3597
77. Wang, B. *et al.* (2014) Role of Ku70 in deubiquitination of Mcl-1 and suppression of apoptosis. *Cell Death Differ.* 21, 1160–1169
78. Thrane, S. *et al.* (2015) A kinase inhibitor screen identifies Mcl-1 and Aurora kinase A as novel treatment targets in antiestrogen-resistant breast cancer cells. *Oncogene* 34, 4199–4210
79. Gojo, I. *et al.* (2002) The cyclin-dependent kinase inhibitor flavopiridol induces apoptosis in multiple myeloma cells through transcriptional repression and down-regulation of Mcl-1. *Clin. Cancer Res.* 8, 3527–3538
80. Bogenberger, J. *et al.* (2017) Combined venetoclax and alvocidib in acute myeloid leukemia. *Oncotarget* 8, 107206–107222
81. Bhat, M. *et al.* (2017) Metformin requires 4E-BPs to induce apoptosis and repress translation of Mcl-1 in hepatocellular carcinoma cells. *Oncotarget* 8, 50542–50556
82. Meynet, O. *et al.* (2013) Caloric restriction modulates Mcl-1 expression and sensitizes lymphomas to BH3 mimetic in mice. *Blood* 122, 2402–2411
83. Lam, L.T. *et al.* (2001) Genomic-scale measurement of mRNA turnover and the mechanisms of action of the anti-cancer drug flavopiridol. *Genome Biol.* 2, RESEARCH0041
84. Manier, S. *et al.* (2017) Inhibiting the oncogenic translation program is an effective therapeutic strategy in multiple myeloma. *Sci. Transl. Med.* 9, eaal2668
85. Bible, K.C. *et al.* (2005) Phase 1 trial of flavopiridol combined with cisplatin or carboplatin in patients with advanced malignancies with the assessment of pharmacokinetic and pharmacodynamic end points. *Clin. Cancer Res.* 11, 5935–5941
86. Karp, J.E. *et al.* (2011) Phase 1 and pharmacokinetic study of bolus-infusion flavopiridol followed by cytosine arabinoside and mitoxantrone for acute leukemias. *Blood* 117, 3302–3310
87. Young, A.I. *et al.* (2017) Myeloid cell leukemia 1 (MCL-1), an unexpected modulator of protein kinase signaling during invasion. *Cell Adhes. Migr.* 12, 513–523
88. Rinkenberger, J.L. *et al.* (2000) Mcl-1 deficiency results in peri-implantation embryonic lethality. *Genes Dev.* 14, 23–27
89. Renjini, A.P. *et al.* (2014) STAT3 and MCL-1 associate to cause a mesenchymal epithelial transition. *J. Cell Sci.* 127, 1738–1750
90. Elgendy, M. *et al.* (2011) Oncogenic Ras-induced expression of Noxa and Beclin-1 promotes autophagic cell death and limits clonogenic survival. *Mol. Cell* 42, 23–35
91. Maiuri, M.C. *et al.* (2007) Functional and physical interaction between Bcl-X_L and a BH3-like domain in Beclin-1. *EMBO J.* 26, 2527–2539
92. Germain, M. *et al.* (2011) MCL-1 is a stress sensor that regulates autophagy in a developmentally regulated manner. *EMBO J.* 30, 395–407
93. Green, D.R. and Levine, B. (2014) To be or not to be? How selective autophagy and cell death govern cell fate. *Cell* 157, 65–75
94. Elgendy, M. *et al.* (2014) Beclin 1 restrains tumorigenesis through Mcl-1 destabilization in an autophagy-independent reciprocal manner. *Nat. Commun.* 5, 5637
95. Thomas, R.L. *et al.* (2013) Loss of MCL-1 leads to impaired autophagy and rapid development of heart failure. *Genes Dev.* 27, 1365–1377
96. Wakatsuki, S. *et al.* (2017) GSK3B-mediated phosphorylation of MCL1 regulates axonal autophagy to promote Wallerian degeneration. *J. Cell Biol.* 216, 477–493
97. Perciavalle, R.M. and Opferman, J.T. (2013) Delving deeper: MCL-1's contributions to normal and cancer biology. *Trends Cell Biol.* 23, 22–29
98. Rasmussen, M.L. *et al.* (2018) A non-apoptotic function of MCL-1 in promoting pluripotency and modulating mitochondrial dynamics in stem cells. *Stem Cell Rep.* 10, 684–692
99. Hollville, E. *et al.* (2014) Bcl-2 family proteins participate in mitochondrial quality control by regulating Parkin/PINK1-dependent mitophagy. *Mol. Cell* 55, 451–466

100. Wang, X. *et al.* (2013) Deletion of MCL-1 causes lethal cardiac failure and mitochondrial dysfunction. *Genes Dev.* 27, 1351–1364
101. Escudero, S. *et al.* (2018) Dynamic regulation of long-chain fatty acid oxidation by a noncanonical interaction between the MCL-1 BH3 helix and VLCAD. *Mol. Cell* 69, 729–743.e7
102. Pawlikowska, P. *et al.* (2010) ATM-dependent expression of IEX-1 controls nuclear accumulation of Mcl-1 and the DNA damage response. *Cell Death Differ.* 17, 1739–1750
103. Mattoo, A.R. *et al.* (2017) MCL-1 depletion impairs DNA double-strand break repair and reinitiation of stalled DNA replication forks. *Mol. Cell. Biol.* 37, e00535-16
104. Yoon, S. *et al.* (2009) IEX-1-induced cell death requires BIM and is modulated by MCL-1. *Biochem. Biophys. Res. Commun.* 382, 400–404
105. Chen, G. *et al.* (2017) Targeting Mcl-1 enhances DNA replication stress sensitivity to cancer therapy. *J. Clin. Invest.* 128, 500–516
106. Yu, H. *et al.* (2017) Mcl-1 suppresses abasic site repair following bile acid–induced hepatic cellular DNA damage. *Tumor Biol.* 39, 101042831771210