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Review

Mcl-1 is a potential therapeutic target in multiple types of cancer

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Abstract. Resistance to apoptosis is a common challenge in human malignancies contributing to both progress of cancer and resistance to conventional therapeutics. Abnormalities in a variety of cell intrinsic and extrinsic molecular mechanisms cooperatively promote tumor formation. Therapeutic approaches that specifically target components of these molecular mechanisms are getting widespread attention. Mcl-1 is a highly expressed pro-survival protein in human malignancies and its cellular expression is tightly regulated *via* multiple mechanisms. Mcl-1 differs from other members of the Bcl-2 family in having a very short half-life. So inhibition of its expression and/or neutralization of its anti-apoptotic function will rapidly make Mcl-1-dependent cells more susceptible to apoptosis and provide an opportunity to combat several types of cancers. This review summarizes the current knowledge on the regulation of Mcl-1 expression and discusses the alternative approaches targeting Mcl-1 in human cancer cells whose survivals mainly depend on Mcl-1.

Keywords. Apoptosis, cancer, Bcl-2 family, Mcl-1, therapeutic.

Introduction

Apoptosis is a morphologically distinct form of cell death in multicellular organisms and involves a series of biochemical events leading to a variety of morphological changes including blebbing, change in membrane asymmetry, cell shrinkage, nuclear fragmentation and chromatin condensation [1]. Its regulation and execution require contributions and interactions of several groups of proteins and other molecules. Two major pathways that regulate apoptosis have been defined in a number of different cell types. The first is the death receptor pathway (the extrinsic apoptosis pathway) initiated mainly by TNFRs and Fas that can activate a caspase cascade *via* activation of caspase-8 as an initiator caspase. The second is termed the intrinsic apoptosis pathway and involves mitochondria and Bcl-2 family members. It results in the activation of a caspase cascade *via* activation of caspase-9 as an initiator caspase [2].

Bcl-2 family proteins are the main regulators of apoptotic processes and include anti-apoptotic and pro-apoptotic members. The balance between the relative levels of these antagonistic proteins is critical for cell fate [3]. Any mechanism breaking down this balance and failures in normal apoptosis pathways may contribute to several diseases including carcinogenesis. Commitment of cells to apoptosis is controlled largely by protein-protein interactions between members of the Bcl-2 protein family that can be divided into three subgroups based on their structural and functional properties. The anti-apoptotic subgroup includes Bcl-2, Bcl-x_L, Mcl-1 (myeloid cell leukemia-1), Bcl-w, and A1. Whereas Bcl-2, Bcl-x_L and Bcl-w contain four BH (Bcl-2 homology) domains (BH1, BH2, BH3, BH4), Mcl-1 and A1 distinguish

themselves from the other pro-survival Bcl-2 family proteins because they lack a well-defined BH4 domain. The first of two pro-apoptotic groups includes Bax and Bak with multiple BH domains (BH1, BH2, BH3). Members of the second pro-apoptotic group including Bad, Bid, Bim, Bik, NOXA, and PUMA contain only BH3 domains (and are therefore called "BH3-only proteins") [4].

Structural studies revealed that BH1, BH2, and BH3 domains of the anti-apoptotic Bcl-2 family proteins form a hydrophobic groove on their surface. This structural property is important since the hydrophobic groove of an anti-apoptotic member can bind to an α-helical BH3 domain of a pro-apoptotic protein and neutralize its pro-apoptotic function [5]. Furthermore, anti-apoptotic Bcl-2 family members prevent effector pro-apoptotic proteins Bax and Bak from being activated. When apoptotic signals are received, BH3-only proteins competitively bind to the hydrophobic groove of the anti-apoptotic proteins and displace Bax and Bak [6]. Bax and Bak can then form oligomers, permeabilize outer mitochondrial membrane and trigger the release of the apoptotic mitochondrial proteins including cytochrome c [7]. Cytochrome c which associates with Apaf-1 activates a caspase cascade leading to cleavage of specific cellular proteins and thereby execution of cell death [7].

Mcl-1 is an anti-apoptotic member of the Bcl-2 family and was originally cloned as an early induction gene during differentiation of the myeloid cell line, ML-1 [8]. Mcl-1 had sequence and functional similarity to Bcl-2, which is the founding member of the Bcl-2 protein family. Although Mcl-1 and Bcl-2 share the ability to promote cell survival, there is accumulating evidence showing that the expressions of these proteins are independently regulated and the tissue distributions of Mcl-1 and Bcl-2 show significant differences [9, 10]. Furthermore, differential regulation of Mcl-1 and Bcl-2 depending on the developmental stages implies that these two anti-apoptotic proteins may function independently [11]. All these reports suggest that Mcl-1 may have a distinct role in controlling apoptotic pathways.

Structural and functional properties of Mcl-1

The human *Mcl-1* gene is located on chromosome 1q21 and comprises three exons. Alternative splicing gives rise to two distinct Mcl-1 mRNAs either containing or lacking exon 2 and encoding the Mcl-1_L and Mcl-1_S isoforms, respectively [12]. Excluding exon 2 in Mcl-1_S causes exon 3 sequences to be translated in a different reading frame and C-terminal transmembrane (TM) domain, which is a part of Mcl-1_L, is not included in Mcl-1_S (Mcl-$1_{S/\Delta TM}$). Whereas wtMcl-1_L comprises 350 amino acid residues and has BH1, BH2, BH3 and C-terminal TM domains, Mcl-1_S contains 271 amino acid residues and only a BH3 domain. The C-terminal TM domain serves to localize Mcl-1_L mainly to the outer mitochondrial membrane [13]. Surprisingly, although Mcl-1_L and Mcl-1_S are expressed from the same gene *via* alternative splicing, they have opposing functions, Mcl-1_L being anti-apoptotic and Mcl-1_S pro-apoptotic [12]. Two PEST sequences located N-terminal part of the both Mcl-1 proteins are characteristic sequences that are found in proteins with rapid turn over. They are thought to be as the main reasons of the short half-life of Mcl-1 protein. Whereas two residues in PEST domains (Asp^{127} and Asp^{157}) have been reported to be critical for caspase cleavage of Mcl-1 [14], several other residues (Ser^{64}, Thr^{92}, Ser^{155}, Ser^{159}, Thr^{163}) have been shown as the potential sites for phosphorylation [15–17].

Mcl-1 (Mcl-1_L will be simply called as Mcl-1 hereafter) is primarily localized to the outer mitochondrial membrane and promotes cell survival by suppressing cytochrome c release from mitochondria *via* heterodimerization with and neutralization of effector pro-apoptotic Bcl-2 family members including Bak [18]. Mcl-1 also selectively interacts with BH3-only proteins, Bim, tBid, Bik, PUMA and NOXA [18–22]. As the most plausible mechanism of anti-apoptotic action of Mcl-1, it has been suggested that Mcl-1 may function as an anti-apoptotic factor by sequestering Bak on the outer mitochondrial membrane, preventing Bak oligomerization and cytochrome c release from mitochondria [18, 22]. However, when apoptotic signals are received, Bik, NOXA and tBid can selectively disrupt Mcl-1-Bak interaction to displace Bak from Mcl-1, leading to Bak oligomerization and cytochrome c release (displacement model) [18, 20, 22] (Fig. 1). As an alternative mechanism, activator BH3-only proteins (Bim, PUMA, and tBid) bind and activate Bax and/or Bak directly if they are not bound and neutralized by Bcl-2-like proteins including Mcl-1 [6, 19, 20, 23]. However, NOXA (perhaps also Bik) can competitively bind to Mcl-1 and prevent it from sequestering activator BH3-only proteins [23] (Fig. 1). When we consider our previous finding that Mcl-1 is mainly localized to outer mitochondrial membrane *via* its C-terminal TM domain [13], it seems reasonable that Mcl-1 may be primarily acting as a factor sequestering Bak at the outer mitochondrial membrane in an inactive state. Besides these two models explaining the pro-survival function of Mcl-1, further experimental analysis is required to determine whether Mcl-1 plays any other direct and/or indirect role in performing its anti-apoptotic function.

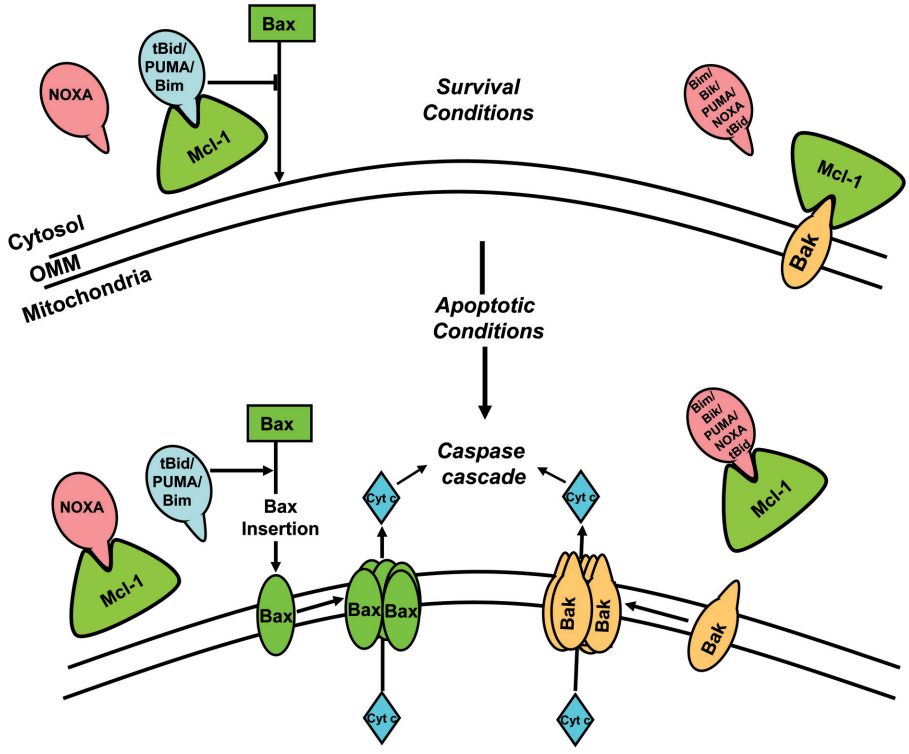

Figure 1. Mcl-1 plays two main roles in the cellular apoptosis machinery. In survival conditions, Mcl-1 may function as an anti-apoptotic factor by sequestering Bak on the outer mitochondrial membrane (OMM). However, when apoptotic signals are received, specific BH3-only proteins can displace Mcl-1 from Bak leading to Bak oligomerization and cytochrome c release from mitochondria. Mcl-1 can also display its pro-survival functions by heterodimerizing with activator BH3-only proteins including tBid, PUMA and Bim. In apoptotic conditions, NOXA displaces Mcl-1 from these activator binding partners. Then, Bim, PUMA and tBid can interact with Bax causing its insertion into outer mitochondrial membrane, oligomerization and cytochrome c release.

Regulation of the Mcl-1 expression

Mcl-1 has a short half-life and is a highly regulated protein. Whereas its expression is induced by survival and differentiation signals, it is also rapidly down-regulated during apoptosis in many cell systems. The rapid regulation of the Mcl-1 expression suggests that it plays a critical role in apoptosis in response to rapidly changing conditions. Regulation of Mcl-1 expression can occur at multiple levels as summarized in Figure 2.

Transcriptional regulation of Mcl-1 expression

Several constitutively activated and/or extracellular signal-activated transcription factors can regulate Mcl-1 transcription. The signal transducers and activators of transcription (STATs) represent a family of transcription factors and regulate cell growth, survival and differentiation in many types of cells. Mcl-1 promoter has been shown to be a target of STAT proteins. Whereas Mcl-1 transcription is up-regulated through a STAT3 pathway upon IL-6 treatment in cholangiocarcinoma cells [24], STAT5 activation contributes to BCR/ABL-dependent expression of Mcl-1 in leukemic cells [25]. IL-3 activates Mcl-1 transcription either by the PI3-K/Akt-dependent pathway through a transcription factor complex containing CREB [26] or by activation of the PU.1 transcription factor through a p38MAPK-dependent pathway [27]. Up-regulation of Mcl-1 is critical for protection of melanoma cells against endoplasmic reticulum stress-induced apoptosis and is due to increased transcription that involves IRE1α and activating transcription factor 6 (ATF6) [28]. Mcl-1 transcription can also be up-regulated by hypoxia-inducible factor-1 (HIF-1) that protects cells against apoptosis under hypoxia [29]. *Mcl-1* gene promoter has also been identified as a target for the ternary complex factor (TCF)-serum response factor (SRF) complex and TCF-SRF-regulated Mcl-1 expression protected cells from apoptosis [30].

Post-transcriptional regulation of Mcl-1 expression

Alternative splicing gives rise to two distinct Mcl-1 mRNA encoding Mcl-1 and Mcl-1_S isoforms with opposing functions [12]. It has been recently reported that alternative splicing of Mcl-1 can be regulated in a cell-specific manner. Macrophages up-regulate anti-apoptotic Mcl-1 expression during bacterial infections, and their commitment to apoptosis for the resolution of infection is dynamically regulated by switching Mcl-1 expression from anti-apoptotic Mcl-1 to pro-apoptotic Mcl-1_S [31].

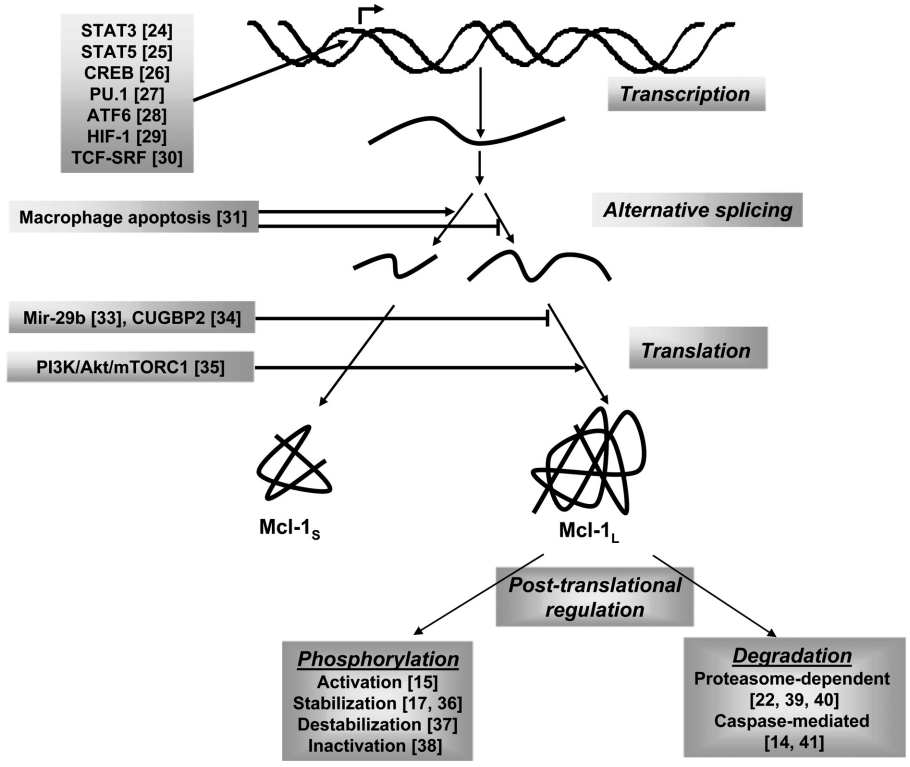

Figure 2. Multiple modes of regulation of the Mcl-1 expression. Mcl-1 is a short-lived and highly regulated protein. Whilst its expression is induced by survival and differentiation signals, it is also rapidly down-regulated during apoptosis in many cell systems. Regulation of Mcl-1 expression can occur at multiple levels. Several transcription factors contribute constitutive and regulated transcription of Mcl-1. Alternative splicing of Mcl-1 pre-mRNA produces two proteins with antagonistic functions and splicing mechanisms can be regulated to shift the balance in favor of one of these proteins according to signals received by the cell. Translational control is also widely used to regulate the Mcl-1 expression. MicroRNAs and RNA binding proteins have been shown to inhibit the translation of Mcl-1 mRNA as discussed in the text. Multiple modes of the post-translational regulation of Mcl-1 protein have been described. Mcl-1 possesses several phosphorylation sites, and it is likely that differential phosphorylation of Mcl-1 results in different fate of this protein. Proteasome and/or caspase-mediated degradation of Mcl-1 are two mechanisms to rapidly diminish cellular levels of this protein. (Numbers in brackets show the references cited and details of regulation are given in the text).

Translational regulation of Mcl-1 expression

We and others have shown that Mcl-1 mRNA and protein both have very short half-lives and their cellular levels depend on balance between *de novo* synthesis and degradation [13, 32]. Mcl-1 was shown to be regulated at the translational level by microRNAs through a mir-29b binding in the 3'-UTR of Mcl-1 mRNA [33]. Mir-29b directly inhibits expression of Mcl-1 by binding to its target sequence. Furthermore, mir-29b was found to be overexpressed in non-malignant compared to malignant cholangiocytes, implying a critical role of Mcl-1 protein up-regulation in malignant cells [33]. CUGBP2, an RNA binding protein, can also bind to Mcl-1 mRNA 3'-UTR and inhibits its translation, driving the cells in HCGU2 cell line stably expressing CUGBP2 in the HCT-116 colon cancer cells to apoptosis [34]. mTORC1, the mammalian target of rapamycin complex 1, is a serine/threonine protein kinase and a downstream target of PI3K/Akt. It has been reported that Mcl-1 is a translationally up-regulated genetic determinant of mTORC1-dependent survival [35].

Post-translational regulation of Mcl-1 expression

Multiple modes of post-translational regulation of Mcl-1 have been defined. Mcl-1 possesses many phosphorylation sites, and it is likely that differential phosphorylation of Mcl-1 results in different fates of this protein. Whereas ERK-mediated phosphorylation of Mcl-1 at Thr^{92} and Thr^{163} prolongs the Mcl-1 half-life [17, 36], Ser^{159} phosphorylation by GSK-3β reportedly enhances Mcl-1 ubiquitylation and degradation [37]. On the other hand, a distinct pathway involving phosphorylation at Ser^{64} enhances its anti-apoptotic function [15]. Furthermore, Mcl-1 is phosphorylated at Ser^{121}/Thr^{163} and inactivated by JNK in response to oxidative stress [38].

Caspase-mediated and proteasome-dependent degradations are two main routes responsible for the rapid

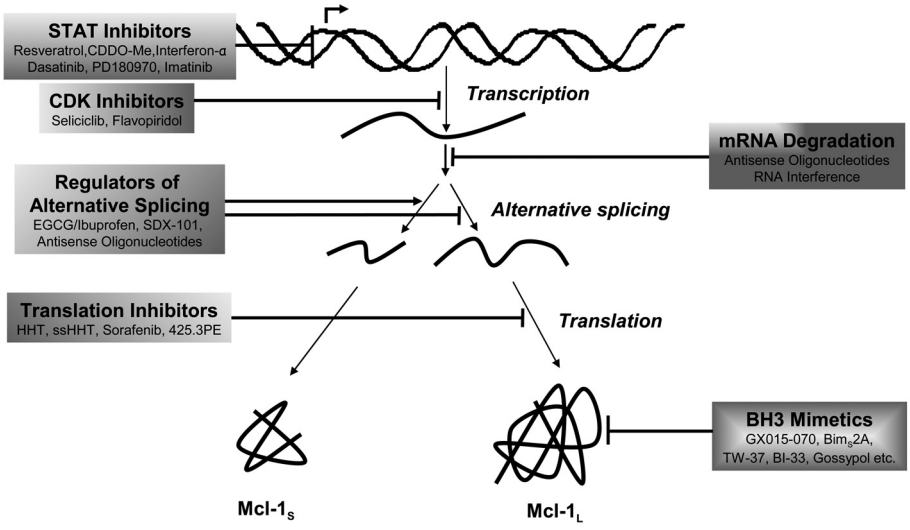

Figure 3. Multiple types of interventions can down-regulate Mcl-1L expression or, alternatively, small-molecule inhibitors can bind to surface hydrophobic groove of Mcl-1 and neutralizes its pro-survival functions. Mcl-1 is overexpressed in a variety of human hematopoietic, lymphoid cancers and solid tumors and also appears to be a key factor in the resistance of some cancer types to conventional cancer therapies. Mcl-1 down-regulation and/or neutralization of its pro-survival functions with BH3 mimetics are often sufficient to promote apoptosis in cancer cells, suggesting that Mcl-1 can be a potential therapeutic target in the treatment of several human malignancies. The figure shows the potential types of interventions either to down-regulate Mcl-1 expression or to neutralize its anti-apoptotic function.

turnover of Mcl-1. The degradation of Mcl-1 can be blocked by proteasome inhibitors, suggesting a role for the ubiquitin proteasome pathway in apoptosis [39]. It has been demonstrated that Mcl-1 is ubiquinated at five lysines by Mule (Mcl-1 ubiquitin ligase E3) [40]. NOXA is the best-characterized BH3-only protein, and can displace Bak from Mcl-1 to initiate apoptosis. Binding of NOXA to Mcl-1 also induced Mcl-1 degradation *via* a proteasome-dependent mechanism [22]. The role of Mcl-1 phosphorylations on its proteasome-dependent degradation has also been implicated since Mcl-1 hyperphosphorylation accelerated both Mcl-1 turnover and apoptosis [37, 39]. On the other hand, specific cleavage of anti-apoptotic Mcl-1 at Asp^{127} and Asp^{157} by caspase-3 during the apoptotic process produced a C-terminal domain with a death-promoting activity [14, 41].

Mcl-1 as a potential therapeutic target in human malignancies

Although Mcl-1 is one of the essential anti-apoptotic factor in the development and differentiation of normal cells, deregulation of signaling pathways regulating Mcl-1 expression often results in its overexpression, which contributes to several human diseases including malignancies. Mcl-1 overexpression has been shown in a variety of human hematopoietic, lymphoid cancers and solid tumors [25, 42–44], and also appears to be a key factor in the resistance of some cancer types to conventional cancer therapies [45–47]. Mcl-1 down-regulation is often sufficient to promote apoptosis in cancer cells, suggesting that Mcl-1 can be a potential therapeutic target in the treatment of several human malignancies [48–50].

Interventions to neutralize the pro-survival function of Mcl-1 in malignant cells

Mechanisms that abrogate the anti-apoptotic function of Mcl-1 can be divided into two categories: (1) Mcl-1 levels can be diminished in target cells by inhibiting its expression and/or inducing its rapid degradation; (2) pro-survival function of Mcl-1 can also be abolished by disrupting Mcl-1/Bak interaction *via* supplying exogenous binding partners, such as BH3 mimetics that interfere with the direct interaction between Mcl-1 and Bak (Fig. 3).

Many types of therapeutic interventions have been tested to neutralize the pro-apoptotic function of Mcl-1 in overexpressing cell lines and their effects on Mcl-1 are summarized in Figure 3. They can be categorized as either nonspecific or specific interventions. Nonspecific treatments mainly include chemotherapeutic agents directed towards more general molecular mechanisms with effects on a wide range of intracellular regulators. On the other hand, specific interventions include antisense oligonucleotides (ASOs), RNA interference (RNAi) and BH3 mimetics, which are able to specifically down-regulate the expression of deregulated proteins of cancer cells. Gene-specific interventions, which first became available in 1990 s,

have had a significant influence on apoptosis induction in some types of cancer cell lines, and are currently a very popular research area. However, advanced drug delivery systems have to be developed so that the treatment can be directed towards the localization of the malignant disease to reduce the risk of serious side effects [51].

Many synthetic chemicals and natural products have been investigated for their anticancer activities in a wide variety of cancer cell lines and down-regulation of the Mcl-1 expression has been reported as one of the mechanisms for the activities of these agents [17, 52–54]. However, the most important characteristic of these compounds with anticancer activities is that they lack specificity and they often affect multiple targets *via* distinct signaling pathways.

STAT proteins are often constitutively activated in many human cancer cells and tumor tissues. They have been shown to induce expression of genes involved in cell proliferation and survival [55]. Mcl-1 is transcriptionally regulated by STATs and this represents a promising target for cancer therapy. In recent years, many synthetic and natural compounds including Resveratrol, CDDO-Me, interferon-α, Dasatinib, PD180970 and Imatinib (STI-571) have been demonstrated to have inhibitory effects on STAT3 and STAT5, leading to Mcl-1 down-regulation and apoptosis induction [53, 56–60].

The fact that both Mcl-1 mRNA and protein have relatively short half-lives can be exploited in cancer therapy since inhibition of transcription and/or translation can rapidly diminish Mcl-1 levels in the cells whose survivals mainly depend on Mcl-1 expression. Treatments with cyclin-dependent kinase (CDK) inhibitors including Seliciclib and flavopiridol resulted in down-regulation of Mcl-1 mRNA *via* inhibition RNA polymerase II-dependent transcription [61, 62]. Inhibition of translation has been an alternative mechanism to down-regulate anti-apoptotic proteins in cancer cells. Homoharringtonine (HHT, a natural compound), its semi-synthetic derivative (ssHHT), Sorafenib (BAY 43–9006, a kinase inhibitor), and 425.3PE (an unmodified *Pseudomonas* exotoxin covalently linked to the 425.3 antibody) have all been shown to inhibit translation and rapidly reduced the levels of short-lived Mcl-1 protein, albeit with different mechanisms [48, 52, 54, 63]. Furthermore, it has also been demonstrated that phosphorylation of Mcl-1 by ERK leads to its stabilization *via* Pin 1, and inhibition of ERK by Sorafenib reverses this stabilization [17].

Although a clear mechanism has yet to be defined, histone deacetylase (HDAC) inhibitors, alone or in combination with other therapeutics, induced apoptosis *via* down-regulating the anti-apoptotic proteins including Mcl-1 [64, 65].

Treatments regulating alternative splicing of Mcl-1 can have a therapeutic potential if the splicing mechanisms can be directed towards the production of pro-apoptotic Mcl-1_S. Several therapeutic agents have already been shown to up-regulate Mcl-1_S splicing variant. For example, epigallocatechin-3-gallate (EGCG) and ibuprofen synergistically suppressed proliferation and induced apoptosis of prostate cancer cell lines *via* altering the ratio of the splice variants of Bcl-x and Mcl-1, down-regulating the mRNA levels of anti-apoptotic Bcl-x_L and Mcl-1 with a concomitant increase in the mRNA levels of pro-apoptotic Bcl-x_S and Mcl-1_S [66]. Similarly, SDX-101 (the R-enantiomer of etodolac) induced up-regulation of Mcl-1_S and enhanced the activity of dexamethasone in multiple myeloma [67]. Antisense technology has been a useful tool to manipulate alternative splicing mechanisms and ASOs were efficiently used to shift the splicing pattern of Bcl-x pre-mRNA from anti-apoptotic Bcl-x_L to Bcl-x_S [68–70]. Similarly, specificity of ASOs can also be exploited to direct alternative splicing of Mcl-1.

Some of the anticancer agents targeting Mcl-1 have been commercialized and used in the treatment of several types of cancer. For example, imatinib (STI571) is currently on the market as an anticancer drug and used in the treatment of chronic myeloid leukemia (CML). BCR/ABL is an oncoprotein that is expressed in leukemic cells as a result of the reciprocal translocation t(9;22) and displays constitutive tyrosine kinase activity. It has been shown that primary CML cells express Mcl-1 in a constitutive manner and that BCR/ABL promotes the expression of MCL-1 through activation of the RAS/RAF/MAP kinase pathway [25]. Several kinase inhibitors including imatinib and U0126 were used to down-regulate Mcl-1 expression in human cancer cell lines [25, 71, 72].

Recently, gene-specific interventions have drawn more attention and they have been successfully applied to neutralize anti-apoptotic proteins in a wide variety of cancer cell lines. Antisense technologies and RNAi are two gene-specific interventions based on inhibition of expression of specific genes [73, 74]. ASOs block translation of target mRNAs in a sequence-specific manner either by sterically blocking translation, or by degradation of the bound mRNA *via* RNase H. On the other hand, RNAi is a mechanism in which genes are specifically silenced at the level of mRNA degradation. Both technologies, alone or in combination with other conventional therapeutics, have been used efficiently to down-regulate Mcl-1 in several cancer cell lines and sensitized them by

specifically inhibiting Mcl-1 expression [25, 44, 49, 50 75, 76].

Agents that mimic BH3 domains of the pro-apoptotic Bcl-2 family members can neutralize anti-apoptotic proteins by binding to their surface hydrophobic groove. Whereas BH3 domains of the pro-apoptotic proteins contain conserved residues and generally function in a similar manner, it has been reported that only some pro-apoptotic Bcl-2 family members bind strongly to Mcl-1 [22, 23]. A number of small-molecule inhibitors of anti-apoptotic Bcl-2 family members have been recently developed and shown to inhibit multiple anti-apoptotic Bcl-2 family members [77, 78]. Although these small-molecule inhibitors can be peptide or non-peptide, one major advantage of non-peptide small-molecule BH3 mimetics over peptide-based inhibitors is their superior cell permeability. Differential binding specificities of BH3 domains suggest that it might be possible to design BH3 mimetics to specifically target an anti-apoptotic protein that are overexpressed in a particular type of cancer [21]. For example, ABT-737, a potent and specific BH3 mimetic, has high affinity for Bcl-x_L and Bcl-2 but it binds poorly to Mcl-1 [79]. So novel BH3 mimetics that can also neutralize Mcl-1 are needed since Mcl-1 overexpression plays a major role in resistance to ABT-737 [80–82]. TW-37, MIPRALDEN, BI-33, Gossypol, Apogossypolone and pyrogallol-based molecules have been shown to inhibit multiple anti-apoptotic Bcl-2 family members including Mcl-1 [47, 83–88]. Furthermore, several small-molecule inhibitors that have high affinities for Mcl-1 have been recently developed and used selectively to neutralize Mcl-1. A novel BH3 mimetic, obatoclax (GX015–070), has been shown to interfere with the direct interaction between Mcl-1 and Bak and overcome Mcl-1-mediated resistance to apoptosis [45, 89]. On the other hand, a novel BH3-like peptidic ligand derived from Bim, Bim_S2A, which is highly selective for Mcl-1, can efficiently antagonize Mcl-1 by tightly engaging its binding groove [90].

Mcl-1 and other survival factors can also be targeted simultaneously to induce enhanced apoptosis. SiRNA (Mcl-1)/Rituximab, siRNA(Mcl-1)/ABT-737, *N*-(4-hydroxyphenyl)retinamide/ABT-737, Sorafenib/Rapamycin, Rapamycin/UCN-1 and Mcl-1 down-regulation/NOXA up-regulation combinations have been used to obtain a synergistic apoptotic response and efficiently overcome resistance in several cancer types [46, 81, 82, 91–93].

Conclusions and future perspectives

Survival of a malignant cell often depends on the presence of multiple anti-apoptotic proteins [94, 95]. Furthermore, types and levels of survival factors may vary from one type of cancer cell to another [10, 42, 94, 96]. For example, whereas Bcl-2 and Bcl-x_L are the main anti-apoptotic proteins in small-cell lung cancer cell lines [94], Mcl-1 is the essential anti-apoptotic Bcl-2 family member in human myeloma cells [42].

Simultaneous targeting of multiple survival factors generally induces much stronger apoptotic response [52, 80, 82, 93]. However, singular targeting of Mcl-1 using antisense technologies, RNAi or BH3 mimetics may be sufficient to trigger apoptosis in specific types of cancer cell lines [42, 44, 90]. The expression levels of Mcl-1 vary substantially among normal and malignant cells [32, 96, 97]. Overexpression of Mcl-1 is often a critical determinant of extended survivals of cancer cells, and specific targeting of Mcl-1 can potentially overcome the resistance to apoptosis in malignant cells [44, 98].

In sum, Mcl-1 is a critical survival factor for both normal and malignant tissues and its cellular expression is tightly regulated *via* multiple mechanisms. Deregulation of signaling pathways often results in Mcl-1 overexpression contributing to development of malignancies and formation of resistance to conventional therapeutics. Over the past decade, there has been significant progress towards understanding the function and regulation of Mcl-1. Appreciable advancement has also been made towards finding relevant interventions to down-regulate Mcl-1 expression and/or to neutralize its pro-survival functions.

Almost all the experimental evidence summarized in this review has been obtained using cancer cell culture systems and they need to be tested in more complex living organisms including humans. In addition to this, improvements in drug delivery systems have to be made so that the treatment can be directed towards the localization of the malignant disease to reduce the risk of serious side effects. Production of better drug delivery systems, development of novel interventions specifically targeting Mcl-1, and testing efficiencies of all kinds of specific interventions in clinical trials of cancer treatment will certainly be priorities in the near future.

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