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The anti-apoptotic protein MCL1, a novel target of lung cancer therapy

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ABSTRACT

Evasion of apoptosis is one of the typical hallmarks of cancer and a major mechanism for cancer development, tumor growth, and acquisition of resistance to chemotherapy. The anti-apoptotic Bcl-2 protein family, particularly MCL1 and BCL-XL, play an important role in acquisition of apoptosis evasion. MCL1 is a highly unstable protein that is constantly degraded by the ubiquitin-proteasome system. An increase in MCL1 protein has been reported in many cancers, including lung cancer, through high mRNA expression or impairment of its degradation systems. To date, much evidence has shown that MCL1 is important for cancer cell survival and drug resistance in lung cancers. In this review, we discuss the role and mechanism of high MCL1 expression in lung cancer.

MCL1 was originally identified as an up-regulated gene in a human myeloid leukemia cell line, and its amino acid sequence has similarity to the anti-apoptotic protein BCL2[1]. BCL-2 family proteins are critical regulators of mitochondrial apoptosis, a major regulatory pathway of mammalian apoptosis and a typical target to induce cell death by anti-cancer drugs[2]. The BCL-2 protein family has conserved BCL-2 homology (BH) domains and are classified as pro- or anti-apoptotic proteins. The pro-apoptotic "multi-domain" members that contain several conserved BH domains, BAX and BAK, function as apoptosis executors in mitochondria, while the anti-apoptotic "multi-domain" members, such as BCL-2, BCL-X_L, and MCL1, inhibit BAX/BAK-mediated apoptosis. Among the BCL-2 homology domains, the BH3 domain directly associates with anti-apoptotic BCL-2 members, and BH3-only members of the BCL-2 protein family trigger mitochondrial apoptosis by activation of BAX/BAK and inhibition of the anti-apoptotic BCL-2 members in the response to numerous stimuli such as developmental signals, stress signals, the DNA damage response, and various anticancer drugs[3] (Figure 1). Because anti-apoptotic BCL-2 family proteins secure survival of many cancer cells, it is possible that suppression of their anti-apoptotic functions induce apoptosis of cancer cells. In this context, inhibitors of anti-apoptotic BCL-2 members have been extensively explored, and several candidate compounds are now being analyzed for their efficacy against various cancers including lung cancer[3-5] (see below).

Lung cancer is the leading cause of cancer mortality worldwide, and accumulating evidence has suggested that high expression of anti-apoptotic MCL1 protein by various mechanisms is important for oncogenesis, tumor development, and chemotherapeutic drug

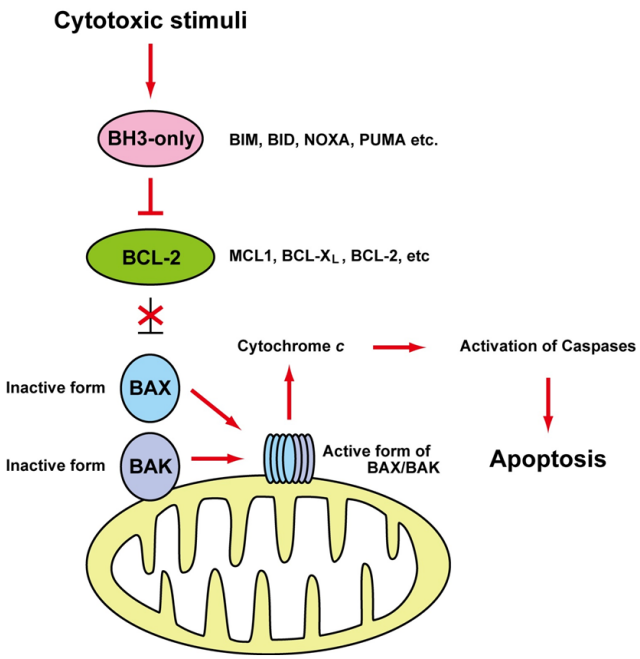

Figure 1. Mitochondrial apoptotic pathway. In response to cytotoxic stimuli, pro-apoptotic BH3-only proteins, such as BIM, BID, NOXA, and PUMA, inactivate anti-apoptotic multi-domain members, such as MCL1, BCL-XL, and BCL-2, through direct interaction with their BH3 domain. Inactivation of anti-apoptotic members changes the inactive form of BAX/BAK to the active form, resulting in oligomerization of BAX/BAK in the mitochondrial outer membrane. Oligomerized BAX/BAK form a pore to release mitochondrial protein cytochrome c into the cytoplasm. Released cytochrome c activates the Apaf-1 apoptosome, and subsequent activation of caspases results in induction of apoptosis[2,3].

resistance of lung cancer cells. Indeed, in a mouse lung adenocarcinoma model, in which the oncogenic transcription factor Myc was targeted to pulmonary alveolar cells, it has been shown that MCL1 overexpression augments tumor progression by circumventing Myc-induced apoptosis[6]. This mini-review introduces the role and mechanism of MCL1 overexpression in lung cancer cells and discusses the possibility of treatments targeting MCL1.

Gene amplification and transcriptional activation of MCL1

High resolution analyses of somatic copy number alterations revealed gene amplification of *BCLX* and *MCL1* in a substantial proportion of human cancers, especially lung and breast cancers[7]. Moreover, growth of MCL1 gene-amplified lung cancer cell lines is affected by inhibition of MCL1 expression using RNA interference[7,8], suggesting a crucial role of MCL1 in lung cancer. This notion is also supported by results showing that copy number variation of MCL1 predicts overall survival of patients with non-small cell lung cancer (NSCLC)[9].

MCL1 gene transcription is upregulated by cytokines, such as interleukin (IL)-3, IL-6, granulocyte-macrophage colony-stimulating factor, and growth factors such as epidermal growth factor and vascular endothelial growth factor[10,11]. Moreover, the MCL-1 promoter has been shown to be activated by the transcription factors STAT3, NF-κB, CREBP, PU.1, SP-1, ELK-1, ATF-6, and HIF-1[11]. In lung cancer, activation of STAT3 and NF-κB are frequently observed and their roles have been analyzed experimentally[12-16]. Moreover, STAT3 and ELK-1 are activated by EGFR (epidermal growth factor receptor)[13,14]. In addition, a recent report has shown that expression of a microRNA, which directly suppresses MCL1 mRNA, is suppressed in lung cancer[17], suggesting that MCL1 mRNA expression is activated by transcriptional and post-transcriptional regulation in lung cancer.

The MCL1 protein stabilization by inhibition of ubiquitin ligases

Accumulating evidence has shown that expression of MCL1 protein is tightly regulated by the ubiquitin-proteasome system[10,18]. In the course of these experiments, several ubiquitin ligases were identified. MULE (Mcl-1 ubiquitin ligase E3) is a HECT domain ubiquitin ligase and contains the BH3 domain that allows MULE to specifically interact with MCL1[19]. Although it has been shown that inhibition of MULE expression induces hepatocarcinogenesis through stabilization of MCL1[20], it has not been reported whether it is involved in the onset of lung cancer. In addition, β-TrCP (β-transducin repeat-containing protein) degrades MCL1 via its phosphorylation at serine 155, serine 159, and threonine 163 by GSK-3β (glycogen synthase kinase-3β)[21]. β-TrCP is an F-box protein and functions as a substrate recognition component of the SCF (SKP1-cullin 1-F-box protein) family of ubiquitin ligases[22]. The inactive phosphorylation of GSK-3β at serine 9 and EGFR expression are both negatively linked to survival of lung cancer patients[23]. However, the relationship between lung cancer and GSK3 is currently under investigation[24].

The F-box protein FBW7 is a well characterized tumor suppressor acting as an ubiquitin ligase that targets MCL1 and well characterized as a tumor suppressor gene[25-27]. The FBXW7 gene, which encodes FBW7, is frequently mutated in diverse cancer types including leukemia and breast, colon, liver, ovarian, and lung cancers[26-28]. FBW7 expression is transcriptionally regulated by the tumor suppressor p53[29], and loss-of-function of p53 reduces the expression of FBW7[30]. Therefore, the tumor-suppressive function of FBW7 may be considered only in the context of p53. Since, FBW7 degrades several proto-oncogenes that function in cell growth, such as c-MYC, cyclin E, Notch, and c-JUN[26,28], its tumor-suppressing function of FBW7 is not only limited to MCL1 degradation. In addition to these mechanisms, we recently found that chaperone-mediated autophagy[31], a specific protein degradation system, promotes survival of several lung cancer cell lines through the selective stabilization of MCL1 by degradation of the ubiquitin ligase

that targets MCL1[32]. Despite these findings, much is still unknown about the regulation of MCL1 protein stability in healthy and malignant cells.

MCL1 protein stabilization by overexpression of deubiquitinase

The deubiquitinase USP9X binds to MCL1 and removes polyubiquitin chains that mark MCL1 for proteasomal degradation[33]. Moreover, increased USP9X expression correlates with increased MCL1 protein in human cancers, and knockdown of USP9X enhances MCL1 turnover, suggesting that USP9X stabilizes MCL1 and promotes cancer cell survival. In lung cancer, it has been reported that USP9X expression in NSCLC tissue is significantly higher than in normal lung tissue, and that an elevated expression level of USP9X is associated with a poor prognosis[34]. A global map of p53 transcription factor-binding sites revealed that USP9X might be a p53 target gene[35]. Induction of USP9X by radiation renders cancer cells more therapy resistant as high MCL1 protein levels prevent apoptosis[36]. These findings suggest that USP9X performs its oncogenic activity through stabilization of MCL1.

The role of MCL1 and its targeted therapy in lung cancer

During the process of oncogenic transformation, cells show higher expression of pro-apoptotic proteins resulting from cell cycle checkpoint activation, DNA replication stress, and/or many other stresses[37]. However, cancer cells survive by adapting to the effects of increasing levels of MCL1 and other anti-apoptotic BCL-2 family proteins. Although some lung cancer cells do not depend on MCL1 for survival[38], the expression of MCL1 is elevated in most lung cancer cells by various mechanisms (Figure 2). Many reports have shown that suppression of MCL1 increases the sensitivity of lung cancer cells to anticancer drugs[10,18,27]. Considering these facts, BH3 mimetics that are selective inhibitors of MCL1 and other anti-apoptotic BCL-2 proteins may be potential therapeutic agents for lung cancer.

BH3 mimetics are small compounds that antagonize anti-apoptotic BCL-2 family proteins, leading to apoptosis induction in cancer cells[3,4,39]. Similar to the BH3 domain in BH3-only proteins, BH3 mimetics specifically interact with anti-apoptotic BCL-2 family proteins and disrupt their

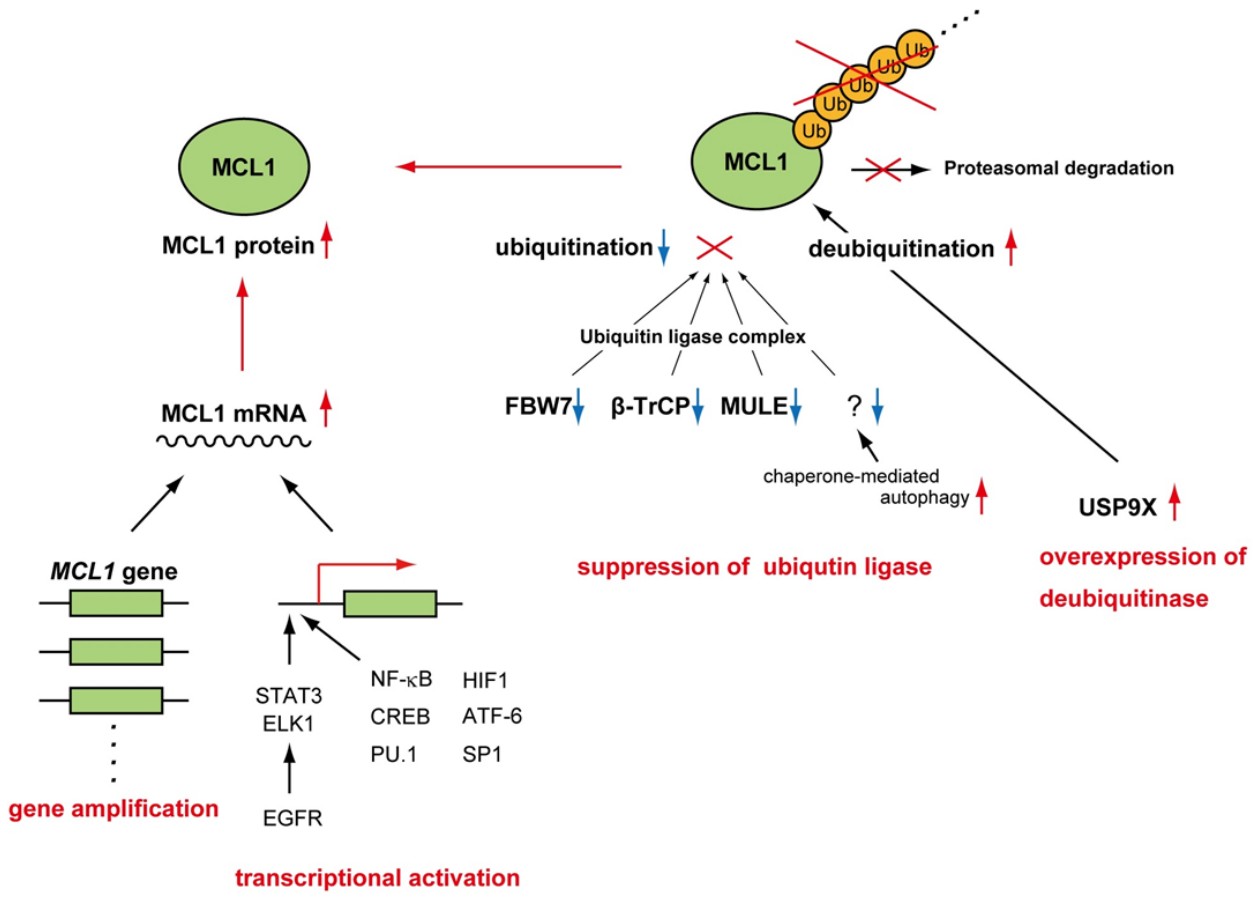

Figure 2. Schematic representation of the mechanism of high MCL1 expression in cancer cells. In cancer cells, MCL1 protein is highly expressed, mainly by three mechanisms: gene amplification, enhanced gene expression by transcription factors involved in cell proliferation, and protein stabilization by decreased expression of the ubiquitin-ligase complex or high expression of deubiquitinating enzymes. See main text for details.

ability to interact with pro-apoptotic BCL-2 proteins to induce BAX/BAK-dependent apoptosis[2,39]. Among such compounds, ABT-263 (navitoclax), a dual inhibitor of BCL-X_L and BCL-2, has been shown to be significantly effective in most chronic lymphocytic leukemia (CLL) patients in clinical trials, and ABT-199 (venetoclax), a selective BCL-2 inhibitor, is also effective in patients with relapsed or refractory CLL[2,40]. The three-dimensional structures of anti-apoptotic BCL-2 proteins, such as BCL-2 and BCL-X_L, share a common motif consisting of four amphipathic helices that form a hydrophobic groove serving as the binding site for pro-apoptotic BH3 domains. High- affinity binding of BH3 peptides to both BCL-2 and BCL-X_L is mediated primarily by interactions in two hydrophobic pockets, termed P2 and P4, and navitoclax specifically binds to these P2 and P4 pockets[39]. The efficacy of ABT-263 and its related compound, ABT-737, has been demonstrated in lung cancer[8,41-46]. Because of the key role of MCL1 in protecting malignant cells against anti-cancer treatments, combinatorial therapy with BH3-binding molecules such as navitoclax may enhance the therapeutic effects of radiotherapy and other treatments. Multiple approaches have been undertaken to directly target MCL1, and several MCL1-specific BH3 mimetics have been identified[39,47]. For example, S63845 binds with high affinity to human MCL1 without appreciable binding to BCL-2 or BCL-X_L[47]. Analyses of these compounds and further molecular development are expected to lead to effective treatments for lung cancer.

Conflict of Interest

The authors declare no conflict of interest.

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