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DUB-le Trouble for Cell Survival

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Expression of MCL-1 is frequently elevated in cancer and is implicated in the resistance to chemotherapy by the BCL-2 small molecule inhibitor ABT-737. A recent paper in *Nature* identified USP9X as an antagonist of MCL-1 ubiquitinylation and degradation. Often upregulated in tumor cells, USP9X activity influences the response to ABT-737.

The intricate decision processes that dictate cell life and death frequently converge on the BCL-2 family of proteins that control mitochondrial outer membrane integrity and the mitochondrial pathway of apoptosis (Letai, 2008). One family member, antiapoptotic myeloid cell leukemia sequence 1 (*MCL-1*), is an essential survival factor for stem and progenitor cells of multiple cellular lineages, and its overexpression is common in human cancers, including B cell and mantle cell lymphomas, acute lymphoblastic leukemia, chronic myelogenous leukemia, chronic lymphocytic leukemia, and multiple myeloma. High expression of MCL-1 correlates with chemotherapeutic resistance and disease progression, although, unlike BCL-2, chromosomal translocations have not been implicated in dysregulating MCL-1 levels. Instead, cellular signaling regulates MCL-1 function and expression at the posttranslational level and aberrations in signaling lead to elevation of MCL-1 in human cancer. Although posttranslational modifications are known in other BCL-2 family members, MCL-1 is unique in its short half-life, partly because of regulated ubiquitinylation and proteasomal degradation. Cancer cells often violate cellular checkpoints that should induce apoptosis, leading to the hypothesis that cancer cells are "addicted" to antiapoptotic BCL-2 family members that support their survival under adverse conditions. ABT-737, a small-molecule inhibitor of BCL-2, BCL-X_L, and BCL-w, promotes apoptosis in some cancer cells. However, high MCL-1 expression renders cancer cells resistant to ABT-737; thus, MCL-1 expression represents a critical resistance mechanism to ABT-737 efficacy.

Recently, a new player in the control of MCL-1 stability was identified: ubiquitin specific peptidase 9 X-linked (USP9X) (Schwickart et al., 2010). Deubiquitinases (DUBs) are proteins that act to remove conjugated ubiquitin, thereby antagonizing the effect of ubiquitin E3 ligases. RNAi-mediated silencing of USP9X resulted in loss of MCL-1 without affecting its mRNA expression (see Figure 1). Biochemically, USP9X binds to MCL-1 and directly removes degradative Lys-48-linked polyubiquitin chains on the protein. Intriguingly, high levels of MCL-1 correlated with elevated USP9X expression in follicular lymphoma, ductal adenocarcinoma, colon adenocarcinoma, and small-cell lung carcinoma samples. Furthermore, increased expression of USP9X mRNA significantly associated with poor prognosis in a retrospective study of multiple myeloma samples. For determining whether the interaction between USP9X and MCL-1 might affect ABT-737 sensitivity, USP9X was silenced by RNAi in a panel of ABT-737-resistant tumor cell lines. Loss of USP9X expression reduced MCL-1 levels in these cell lines and increased their sensitivity to ABT-737.

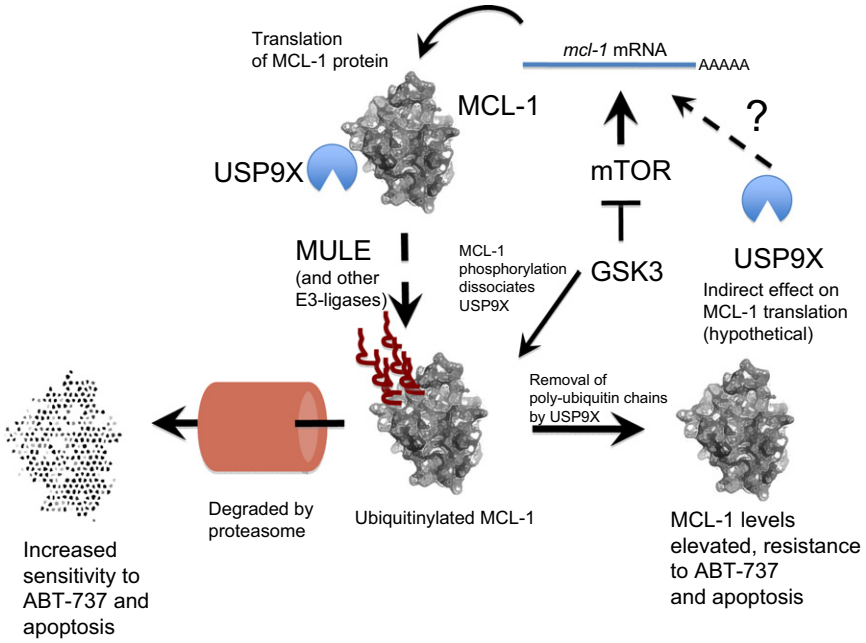

Figure 1. The Regulation of MCL-1 Protein Levels

MCL-1 associates with a DUB, USP9X, which counters the effects of MULE and other E3 ubiquitin ligases and reduces proteasome-dependent degradation of the antiapoptotic protein. GSK3 phosphorylates MCL-1, promotes its dissociation from USP9X, and thereby increases ubiquitinylation and decreases its half-life. MCL-1 translation is regulated by mTOR, which can also be inhibited by GSK3, and thus inhibition of GSK3 elevates MCL-1 levels in two ways (via increased translation and half-life). Knockdown of USPX9 dramatically promotes loss of MCL-1, leading to the speculation that USPX9 may not only decrease half-life but also production of MCL-1.

Phosphorylation in response to cellular signaling represents a critical control point for regulating MCL-1. Phosphorylation of Ser-159 on human MCL-1 by GSK3 promotes MCL-1 degradation by a proteasome-dependent mechanism (Ding et al., 2007; Maurer et al., 2006). Strikingly, mutation of these three residues to alanine (blocking phosphorylation) enhanced the interaction between USP9X and MCL-1 and increased MCL-1 protein levels, whereas mutation of these residues to aspartic acid (phosphomimic) decreased the interaction and decreased the steady-state level of MCL-1. Furthermore, cellular stress such as UV irradiation disrupted the interaction between MCL-1 and USP9X, enhancing MCL-1 turnover. Treatment of UV-irradiated cells with a GSK3 inhibitor maintained the interaction between MCL-1 and USP9X and stabilized MCL-1.

The control of MCL-1 protein levels is critical to our understanding of normal and cancer biology (Figure 1). Two E3 ligases have been implicated in promoting MCL-1 turnover. The first, MCL-1-ubiquitinating ligase E3 (MULE), a HECT-domain (homologous to the E6-AP carboxyl-terminus) E3 ligase, possesses a BH3 domain, similar to that of proapoptotic BAK, that allows it to target MCL-1. Interestingly, although RNAi-mediated silencing of MULE slows MCL-1 turnover, degradation nevertheless occurs, suggesting that additional pathways foster MCL-1 elimination (Zhong et al., 2005). The other E3 ligase, Skp1/Cul1/F-box protein β-transducin repeat-containing protein ($SCF^{\beta\text{-}TrCP}$), requires MCL-1 phosphorylation by GSK3 at Ser-159 for mediating recognition (Ding et al., 2007). In contrast to MULE, the interaction between β-TrCP and MCL-1 is facilitated by phosphorylation of the same serine and threonine residues that Schwickart and colleagues identified to be potentially recognized by USP9X. Thus, the possibility exists that β-TrCP and USP9X might compete for MCL-1 binding. Although both MULE and β-TrCP can target MCL-1 for degradation, neither is MCL-1 specific as they have been implicated in ubiquitinylating a myriad of substrates. The relative contributions of these or other unidentified MCL-1-specific E3 ligases in regulating MCL-1 remain unclear.

Although to date there are no reported genetic models for loss of MULE or β-TrCP, RNAi-mediated silencing of either does not completely block MCL-1 elimination (Zhong, et al., 2005; Ding, et al., 2007). Furthermore, although MCL-1 is clearly ubiquitinylated in cells, mutagenesis of the lysine residues required for ubiquitinylation slows, but does not block, MCL-1 elimination (Zhong et al., 2005). In contrast, blocking proteasome function appears to have a much stronger effect in blocking MCL-1 elimination, leading to the possibility that additional factors or pathways may exist to regulate MCL-1 stability (Maurer et al., 2006; Nijhawan et al., 2003). One possibility is that additional interacting proteins, regulated by ubiquitinylation, may affect the degradation of MCL-1. Interactions with BH3-only family members have been implicated in modulating MCL-1 expression; NOXA has been proposed to promote MCL-1 degradation and BIM has been implicated in stabilizing MCL-1 protein (Czabotar et al., 2007). The mechanism by which BH3-only molecules act to regulate MCL-1 expression is unclear, but may be at the level of competition with MULE (Zhong et al., 2005). Additionally, it is possible that MCL-1 may undergo proteasome-dependent, but ubiquitinylation-independent, degradation as has been identified for an expanding number of proteins (Hoyt and Coffino, 2004).

Although RNAi-mediated silencing of USP9X led to dramatic changes in steady-state MCL-1 protein levels, kinetic assays indicated that a loss of USP9X had only minor effects on the half-life of MCL-1 (i.e., ~17 versus 9 min). Given that MCL-1 mRNA levels were unaffected by gain or loss of USP9X, the effects of this DUB on MCL-1 are presumably dictated by the rates of translation and degradation. Little is known, however, about the control of MCL-1 translation. In an AKT-driven, Eμ-Myc lymphoma mouse model, translational regulation of MCL-1 by mTOR has been implicated in promoting lymphomagenesis (Mills et al., 2008). Given that GSK3 inhibits mTOR, it remains possible that pharmacologic blockade of GSK3 activity not only increases the stability of MCL-1 but also its translation.

This leads to an intriguing speculation. Might there be other targets of USP9X that affect the translation of MCL-1, thereby influencing accumulation of the protein not only in terms of its stability

but also in its rate of production? Precedent for such an effect comes from the control of TP53 levels by its E3 ligase, MDM2. In addition to controlling the stability of TP53, MDM2 targets a ribosomal protein, L26, which is important in regulating the rate of TP53 translation through interactions in the 3′ UTR of the mRNA (Ofir-Rosenfeld et al., 2008). Thus, regulation of MDM2 function has effects on TP53 protein levels that are independent of the interaction of MDM2 with TP53 protein itself. Might ubiquitination-de-ubiquitination events similarly affect the translation of MCL-1? If so, then changes in the expression of USP9X might produce important "off-target" effects on MCL-1 levels through multiple, integrated mechanisms. The combination of a slightly increased half-life and a decreased rate of translation will lead to a more complete loss of MCL-1 (as observed upon knock-down of USP9X) than would the direct effect on half-life alone. This might help to account for how small changes in the half-life of MCL-1 correlate with dramatic changes in MCL-1 levels, through the functions of this fascinating DUB.

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