

# USP11: A key regulator of cIAP2 stability and sensitivity to SMAC mimetics

Eun-Woo Lee and Jaewhan Song\*

Department of Biochemistry; College of Life Science and Biotechnology; Yonsei University; Seoul, Korea

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The critical function of cellular inhibitor of apoptosis proteins (cIAPs) in the protection of cancer cells from numerous apoptotic stimuli prompted the development of second mitochondria-derived activator of caspases (SMAC) mimetics. We recently addressed a novel survival pathway in which cIAP2 is induced by tumor necrosis factor- $\alpha$  and is stabilized by its specific deubiquitylase, USP11, rendering cells resistant to SMAC mimetics.

Inhibitor of apoptosis proteins (IAPs) constitute a family of proteins that strongly suppress various types of cell death, such as apoptosis and necroptosis.<sup>1,2</sup> Although the direct inhibition of caspases is a well-characterized function of IAPs, ubiquitylation of other target proteins is an emerging mediator of IAP-induced suppression of cell death. For example, cIAPs mediate the ubiquitylation of receptor-interacting protein kinase (RIPK1), thereby inducing nuclear factor kappa-B (NF- $\kappa$ B) activation and preventing cell death following tumor necrosis factor- $\alpha$  (TNF $\alpha$ ) stimulation.<sup>3</sup> The central roles of IAPs in RIPK1 regulation are crucial not only for TNF $\alpha$  signaling but also for ripoptosome signaling upon activation by various stimuli such as genotoxic stress and Toll-like receptor (TLR) activation.<sup>4</sup> The crucial role of IAPs in the inhibition of cancer cell death prompted the development of second mitochondria-derived activator of caspases (SMAC) mimetics, which are being clinically tested in cancer patients worldwide.<sup>5</sup> However, the efficacy of SMAC mimetics is occasionally hampered by the resistance of some cancer cell types to these agents. The lack of a mechanistic understanding of this resistance is a major obstacle to the effective application of these drugs.

One of the missing links in understanding cIAPs is the functional or regulatory distinction between baculoviral IAP repeat containing 2 (BIRC2, best known as cIAP1) and BIRC3 (best known as cIAP2), which are known to display high homology and functional redundancy.<sup>2</sup> Because cIAP1 is easily detectable and constitutively expressed in cells, most studies on cIAPs have focused on cIAP1. Here, we provide new insight into the specific regulation of cIAP2. The cIAP2 protein is barely detectable in most cancer cells but strongly accumulates in response to various stimuli, including TNF $\alpha$  and lipopolysaccharide (LPS).<sup>6-8</sup> The detection of cIAP2 in tumor xenografts suggests that cIAP2 may be susceptible to activation by cytokines in the tumor microenvironment (data not shown). Previous studies proposed that transcriptional activation of cIAP2 via the non-canonical NF- $\kappa$ B and PI3K pathways leads to resistance to SMAC mimetics.<sup>6,9</sup> In addition, we recently suggested that the post-translational stabilization of cIAP2 by ubiquitin-specific protease 11 (USP11) contributes to SMAC mimetic resistance.<sup>10</sup> Therefore, understanding the mechanism by which cIAP2 is regulated appears to be important for cancer treatment.

In our recent study, we first observed that cIAP1 expression levels are relatively high but are rapidly decreased by SMAC mimetics in most cancer cell types. However, cIAP2, which is expressed at low levels, is enhanced by TNF $\alpha$  stimulation and remains stably expressed despite SMAC mimetic treatment.<sup>10</sup> Accordingly, cIAP2 knockdown, but not cIAP1 knockdown, sensitized these cells to apoptotic cell death induced by TNF $\alpha$  and SMAC mimetics. These findings of a differential degradation rate between cIAP1 and cIAP2 led us to screen for related deubiquitylases and the identification of USP11 as a key regulator of cIAP2. Several *in vitro* experiments confirmed that USP11 acts as a *bona fide* deubiquitylase of cIAP2. Interestingly, USP11 depletion led to the downregulation of cIAP2 without affecting the expression of other IAPs, such as cIAP1, BIRC4 (best known as XIAP), BIRC5 (best known as ML-IAP), and BIRC7 (also known as Survivin), suggesting the specificity of USP11 for cIAP2. Ectopic expression of USP11 ameliorated SMAC mimetic-induced cIAP2 degradation, which may explain why some cell lines display resistance to SMAC mimetics. Supporting this hypothesis, we observed an inverse correlation between USP11 expression and SMAC mimetic

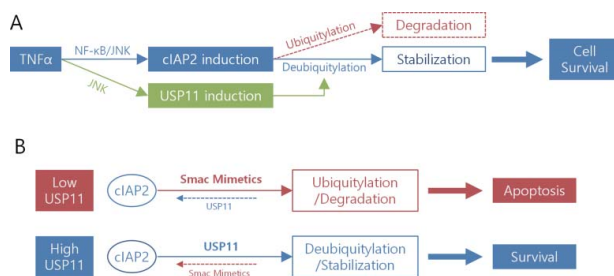
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\*Correspondence to: Jaewhan Song; Email: jso678@yonsei.ac.kr

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**Figure 1.** Effects of the TNF $\alpha$ /USP11/cIAP2 signaling axis on cellular sensitivity to SMAC mimetics. (A) TNF $\alpha$  activates the transcription of both cIAP2 and USP11 via the NF- $\kappa$ B and/or JNK pathways. USP11 induction by TNF $\alpha$  prevents cIAP2 degradation via direct deubiquitylation. High expression of USP11 and cIAP2 contributes to cell survival in the presence of various apoptotic stimuli. (B) SMAC mimetics rapidly induce cIAP2 autoubiquitylation and subsequent degradation, leading to the death of cells displaying low USP11 expression. In cells with high USP11 expression, USP11 abrogates the SMAC mimetic-induced ubiquitylation of cIAP2, resulting in cIAP2 stabilization. Therefore, USP11 prevents cell death induced by SMAC mimetics and other stimuli, such as TRAIL. cIAP2, cellular inhibitor of apoptosis protein-2; JNK, c-Jun N-terminal kinase; NF- $\kappa$ B, nuclear factor kappa-B; TNF $\alpha$ , tumor necrosis factor- $\alpha$ ; USP11, ubiquitin-specific protease 11.

sensitivity in some panels of colon cancer cells or melanoma cells. For example, cell lines expressing high levels of USP11 showed slow degradation of cIAP2 upon treatment with SMAC mimetics, whereas cell lines expressing low levels of USP11 exhibited rapid degradation of cIAP2 (Fig. 1B). In addition, *USP11*-depleted cells showed rapid degradation of cIAP2 following treatment with SMAC mimetics; these cells ultimately underwent rapid apoptosis compared to control cells. Our results suggest that the stabilization of cIAP2 by USP11 might be responsible for the viability of certain cancer cell types in the presence of SMAC mimetics.

One interesting observation of our studies was that USP11 was transcriptionally induced by TNF $\alpha$ , indicating that USP11 is an innate component of the TNF $\alpha$  signaling pathway. Using several

inhibitors, we found that c-Jun N-terminal kinase (JNK) is responsible for USP11 induction. We hypothesized that TNF $\alpha$  activates the survival pathway via not only the activation of cIAP2 transcription but also through USP11-dependent cIAP2 stabilization (Fig. 1A). In the absence of USP11 expression, TNF $\alpha$ -induced cIAP2 accumulation was suppressed despite its normal mRNA induction, resulting in the sensitization of cancer cells to SMAC mimetics. Why cIAP2, but not cIAP1, is specifically regulated at both the transcriptional and post-translational levels remains unclear. Mice specifically deficient in either cIAP1 or cIAP2 may reflect the physiological differences between these 2 homologous proteins. Because previous studies using *clap1* or *clap2* knockout mice have primarily focused on the phenotypes related to

inflammation and immunity,<sup>9</sup> the roles of cIAP1 and cIAP2 in tumorigenesis and cancer treatment should be investigated by crossing each *clap* knockout mouse with various murine tumor models.

Finally, we showed that USP11 down-regulation promotes tumor suppression following combination treatment with TNF $\alpha$ -related apoptosis-inducing ligand (TRAIL) and SMAC mimetics in mouse xenograft models. *Usp11* knockout mouse experiments would provide more elaborate and defined physiological roles of USP11, as well as cIAP2, in tumorigenesis. Because SMAC mimetics synergistically induce cell death with various types of anticancer drug, it would be interesting to develop an USP11 inhibitor for the treatment of cancer using SMAC mimetics. In conclusion, our findings demonstrate that a novel TNF $\alpha$ /USP11/cIAP2 axis constitutes a crucial and previously unrecognized cell survival pathway that mediates the cellular resistance to anticancer drugs, including SMAC mimetics.

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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