POSTTRANSLATIONAL MODIFICATIONS

Comment on "SUMO deconjugation is required for arsenic-triggered ubiquitylation of PML"

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Fasci *et al*. proposed that a SENP1-mediated switch from SUMO2 to SUMO1 conjugation on Lys^{65} in promyelocytic leukemia protein (PML) is required for arsenic-induced PML degradation, the basis for the antileukemic activity of arsenic. We found that PML or PML/RARA (retinoic acid receptor α) mutants that cannot be SUMO-conjugated on this specific site nevertheless underwent immediate arsenic-triggered SUMO modification. Moreover, these mutants were efficiently degraded in cells and even in vivo, demonstrating that SUMOylation of Lys^{65} was dispensable for arsenic response. The existence and putative role of a SUMO switch on PML should thus be reassessed.

Therapy-enhanced promyelocytic leukemia protein (PML)/retinoic acid receptor α (RARA) degradation is the driving force underlying acute promyelocytic leukemia (APL) cure (*1*, *2*). Arsenic triggers PML degradation through its hyperSUMOylation (*3*–*5*). Mechanistically, PML polymerization in nuclear bodies upon oxidative stress allows its subsequent SUMOylation by PML-bound UBC9 (*6*). PML SUMOylation is followed by its polyubiquitylation, mediated by the SUMO-dependent ligase RNF4, and PML proteasome-dependent degradation (*7*, *8*). Fasci *et al.* proposed that an arsenic-induced switch in the SUMOylation of Lys^{65} in PML from SUMO2 to SUMO1 initiates SUMO2 conjugation of Lys^{160} and drives arsenic-induced PML degradation (*9*). This statement was based on experiments using SUMO2 Q90P, which cannot be efficiently deconjugated, as well as SENP1 down-regulation.

In the experimental systems used by the authors, arsenic did not trigger PML degradation. The authors instead assessed PML SUMOylation and/or ubiquitylation in cell lines overexpressing tagged PML and/or SUMOs, allowing robust biochemistry and quantification. However, by changing the equilibrium between the multiple actors involved in the SUMO-initiated degradation cascade, this approach may introduce various biases. In this respect, previous studies using overexpressed tagged PML have failed to detect arsenic-triggered PML degradation (*5*). Moreover, in several of the authors' experiments involving stable expression of Flag-PML or Flag-PML-GFP (green fluorescent protein), arsenic paradoxically enhanced PML abundance, so the observed increases in SUMO or ubiquitin conjugates largely followed PML abundance [Figs. 5A and 6, C and D, in (*9*)]. Moreover, APL response is based on arsenic-induced PML/RARA degradation, and polyubiquitylation, as studied here, does not always trigger protein catabolism. Using primary cells ex vivo or in vivo, we found that PML or PML/RARA were hyperSUMOylated and subsequently fully degraded, sometimes as early as 1 hour after arsenic exposure (Fig. 1, A to C), pointing to the importance of the cellular system used to explore SUMO-initiated ubiquitin-dependent degradation. In our experience, the ideal conditions for observing the complex cascade of arsenic-triggered events that culminate in PML or PML/RARA proteolysis are when they are at low abundance and SUMOs are at endogenous amounts, preferably in primary cells (*3*, *4*, *7*).

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Comparing individual mutations of the three SUMO target sites on PML led the authors to propose that "Lys^{65} on the RING domain of PML was the switching lysine, the residue that needed to be modified by SUMO1 to drive SUMO2 chain synthesis." Under our experimental conditions, in contrast to the Lys^{160} consensus site whose SUMOylation is absolutely required for PML or PML/RARA degradation, mutation of Lys^{65} did not preclude arsenic-triggered PML degradation in stable Chinese hamster ovary (CHO) cell lines nor PML/RARA degradation in APL cells ex vivo [Fig. 1, D and E, and (*7*)]. SUMO conjugation of Lys^{65} was also dispensable for arsenic-induced PML/RARA hyperSUMOylation in vivo (Fig. 2A). These results indicate that PML Lys^{65} is dispensable for arsenic-triggered hyperSUMOylation and degradation (*7*).

The authors proposed that SENP1-mediated deconjugation of SUMO2-bound Lys^{65} was required for arsenic-induced PML SUMOylation or ubiquitylation on Lys^{65} and Lys^{160}. Mass spectrometry studies have failed to demonstrate substantial conjugation of Lys^{65} in PML by SUMO2, whereas SUMO2 conjugation of other sites (Lys^{490} and Lys^{160}) are readily detected under either basal or stress conditions [fig. S7 in (*10*) and references therein]. Fasci *et al.* mentioned that overexpressed tagged or mutant SUMO1 or SUMO2 could have protein targets other than PML. In particular, overexpression of these tagged SUMO paralogs or knockdown of SENP1 could change the SUMO conjugation of UBC9 itself, and this may preclude the processive activity of the E2 enzyme (*11*–*13*). Similarly, RNF4-mediated PML polyubiquitylation may be favored when RNF4 is conjugated by SUMO1. Like the authors, we found that knockdown of endogenous SUMO1 blunted arsenic-induced degradation of PML. However, it also blocked that of PML K65/490R [Fig. 2B and (*7*)]. In addition, SUMO1 silencing did not block arsenic-induced PML hyperSUMOylation [Fig. 2B, left, and (*7*)]. In the absence of arsenic, SUMO1 silencing selectively destabilized the PML K65/490R protein (Fig. 2B, right), suggesting that SUMO1 conjugation on Lys^{160} may limit basal SUMO2 chain formation and degradation. PML undergoes basal SUMOylation on three distinct sites and dimerizes upon arsenic exposure (*4*, *6*, *14*, *15*). Tandem SUMO-interacting motifs can interact with multiSUMOylated proteins, so RNF4 may interact with multiSUMOylated PML even without SUMO2 chain formation (*16*, *17*).

Collectively, our observations on the role of Lys^{65} in arsenic response do not support the model in which Lys^{65} must undergo arsenic-induced deSUMOylation from a basal SUMO2-conjugated state toward SUMO1 to drive SUMO2 polyconjugation on Lys^{160}, ultimately driving PML degradation. Although we do not exclude the role of SENPs in arsenic response, further work is required to understand how arsenic modulates

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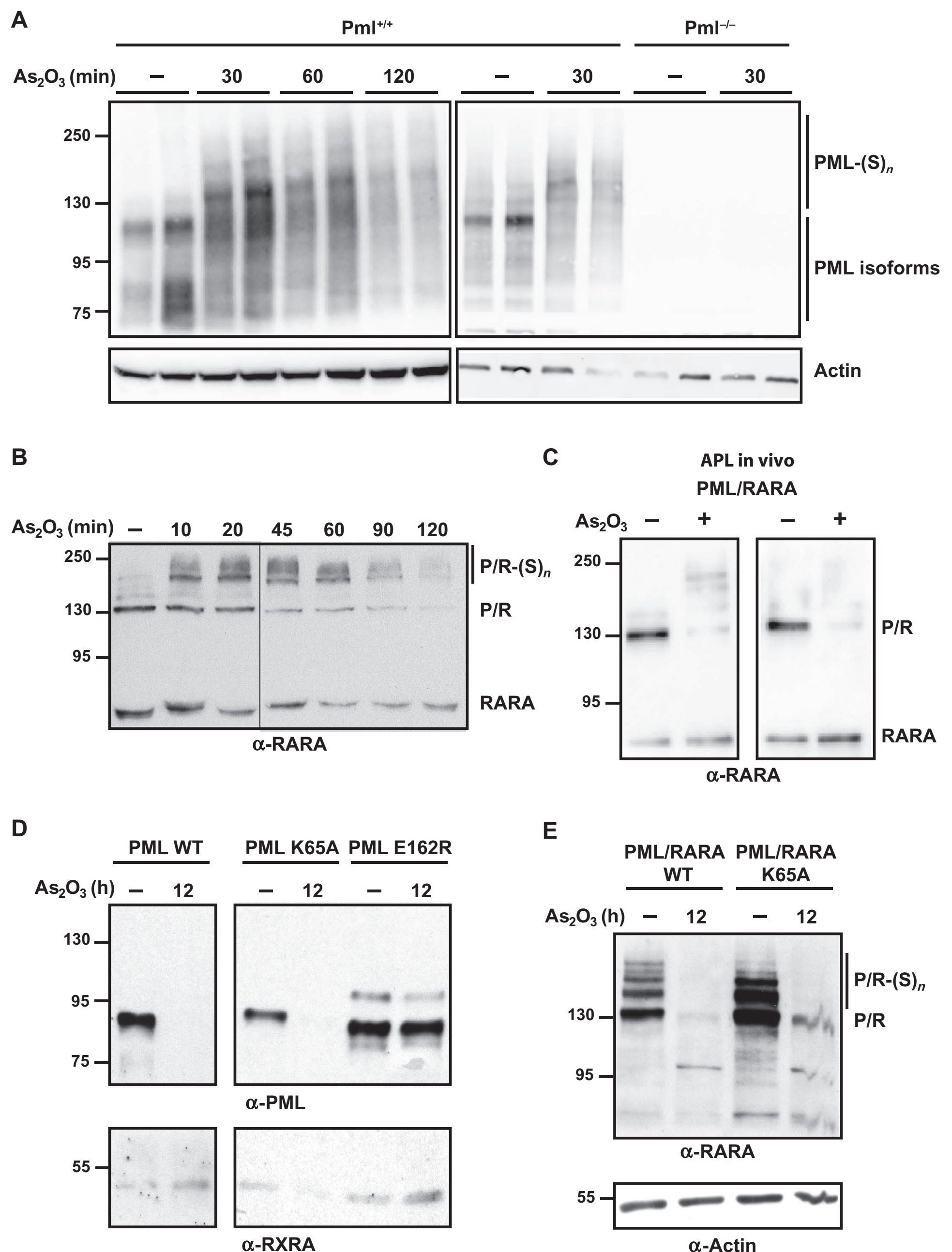

Fig. 1. Arsenic induces an efficient Lys^{65}-independent degradation of PML or PML/RARA. (**A**) Arsenic trioxide was prepared as previously described (*1*) and intraperitoneally injected into mice (129sv background) at 5 μg/g for the indicated times. Total cell extracts were obtained from mouse livers ground in liquid nitrogen using Tissue Lyser II (Thermo Fisher, Life Technologies) and immediately lysed in Laemmli buffer. Immunoblots using mouse monoclonal anti-mouse PML antibody, clone 36.1-104 (Merck Millipore, MAB3738), and rabbit anti-actin (Sigma, A2066) antibodies, and horseradish peroxidase–conjugated secondary antibodies (1:20,000; The Jackson Laboratory) were performed as described previously (*4*, *7*). Note the rapid SUMOylation and degradation of endogenous PML isoform in hepatocytes after in vivo treatment, as previously described (*4*). *Pml*$^{-/-}$ mice (129sv background) demonstrate the specificity of PML detection (right). Two representative experiments, each using two mice, of $n = 4$ independent experiments are depicted. (**B**) Hematopoietic progenitors were purified and transduced using pMSCV (mouse stem cell virus)–IRES-EGFP (MIE) retroviral vectors expressing wild-type (WT) PML/RARA (P/R) as in (*1*). Time course of PML/RARA SUMOylation and degradation in transformed primary hematopoietic progenitors treated with 10^{-7} M arsenic trioxide (Fluka) are shown. Cells were directly lysed in Laemmli buffer, and proteins detected by immunoblotting with rabbit polyclonal anti-RARA antibody. $n = 2$ independent experiments. (**C**) APL leukemic mice were treated with arsenic for 1 hour. The mice were obtained from serial transplantation of PML/RARA transgenic leukemic bone marrow cells into FVB (Friend leukemia virus B) strain, as previously described (*1*). PML/RARA SUMOylation and degradation as detected by rabbit polyclonal anti-RARA antibody from two independent experiments are shown. (**D**) Degradation of PML (left) or its SUMOylation mutants (right) stably expressed in CHO cells (*4*, *7*) (detected by chicken anti-human PML antibody) after treatment with 10^{-6} M arsenic trioxide and direct lysis in Laemmli buffer. Retinoid X receptor α (RXRA) was detected with rabbit polyclonal anti-RXRA antibody (Santa Cruz Biotechnology). (**E**) Degradation of PML/RARA or its K65AR mutant (detected by rabbit polyclonal anti-RARA) in primary hematopoietic progenitors treated with 10^{-7} M arsenic after transduction. Note the different SUMO conjugates in untreated cells. All experiments in cell lines in (D) and (E) were repeated at least three times.

SUMOylation of PML and PML/RARA to initiate degradation of the fusion protein and cure APL.

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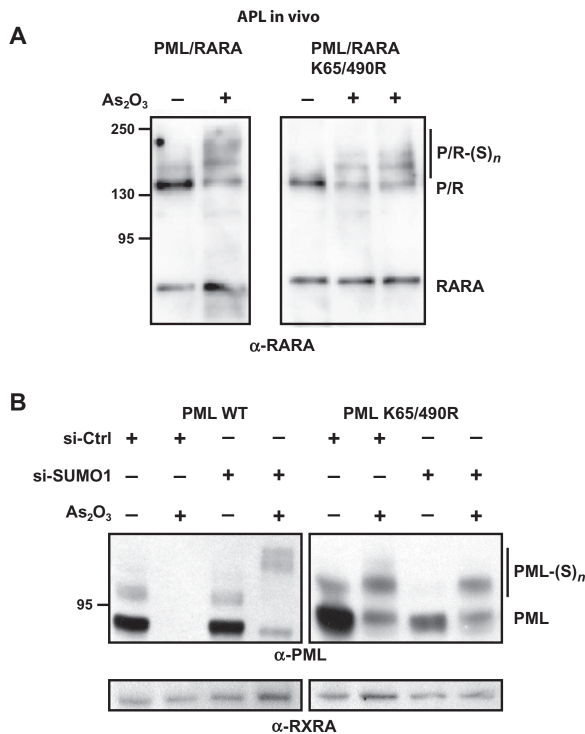

Fig. 2. Lys[65] is dispensable for PML and PML/RARA SUMOylation. (**A**) Western blot analyses of PML/RARA hyperSUMOylation (detected with rabbit polyclonal anti-RARA antibody) 1 hour after arsenic trioxide injections in PML/RARA or PML/RARA K65/490R-driven APL mice. Two arsenic-treated mice are shown for the PML/RARA K65/490R mutant. Data are representative of two independent experiments. (**B**) Arsenic treatment and SUMO1 knockdown [sequence and use as described in (*7*)] in CHO cells stably expressing PML or PML K65/490R. Immunoblotting was performed with chicken anti-human PML antibody and rabbit polyclonal anti-RXRA antibody. Results in (B) were independently obtained at least three times.

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