Marked for death

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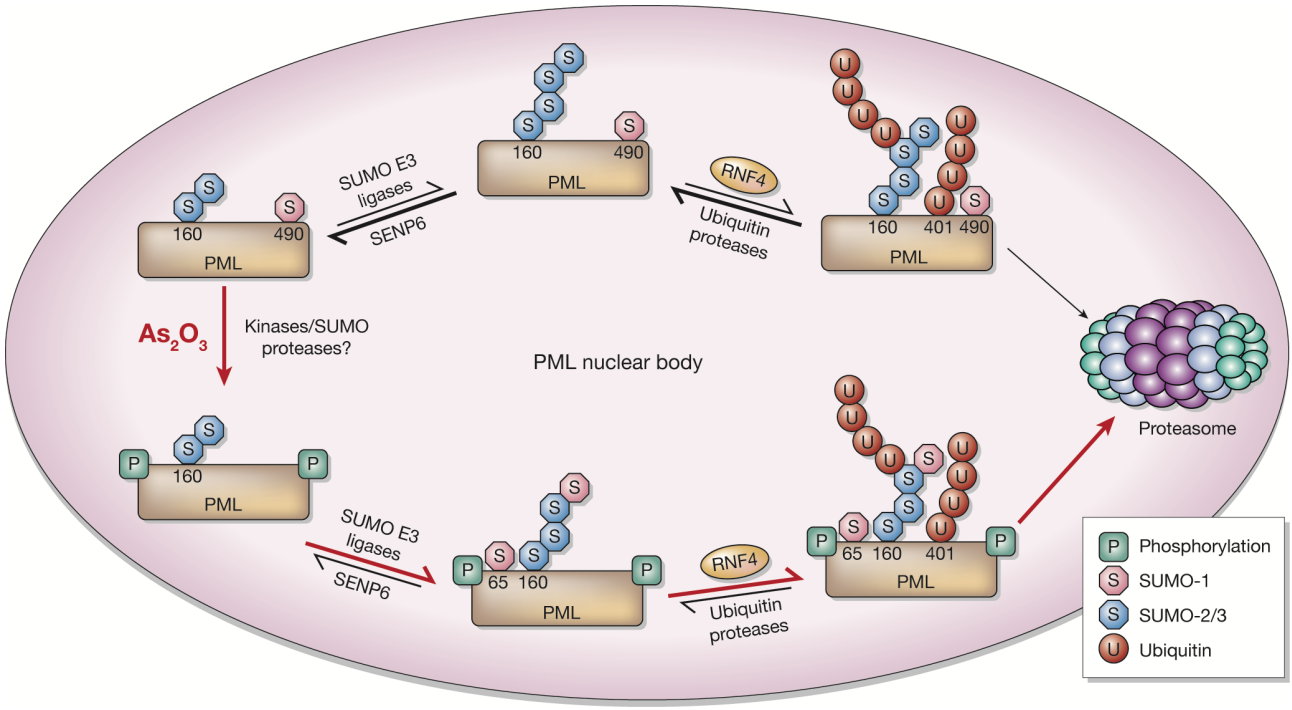
SUMOylation of PML–RARα oncoprotein has been linked to its arsenic-induced degradation and the therapeutic response in acute promyelocytic leukaemia. Two groups identify PML as an *in vivo* target of the RING finger ubiquitin E3 ligase RNF4, which specifically binds polySUMOylated PML and is essential for the arsenic-induced catabolism of both PML and PML–RARα.

Although better known as a poison and famously rumoured to have been used to kill Napoleon, arsenic is one of the oldest medicines known to man. First described nearly 2500 years ago by the ancient Greek physician Hippocrates, it has been used to treat a variety of ailments and diseases including syphilis and cancer (for a review see ref. 1). The use of arsenic declined during the twentieth century due to concerns about its toxicity and advances in modern medicine. However, in 1992 it was reported that Ai-ling 1, a Chinese traditional medicine containing high levels of this semi-metal induced dramatic remissions in patients suffering from acute promyelocytic leukaemia (APL). Arsenic trioxide (ATO, As_2O_3) was identified as the active component and the finding that it was therapeutically effective in APL but not in other subtypes of acute myeloid leukaemia suggested that it targeted the PML–RARα fusion oncoprotein, which exerts a dominant-negative effect on both retinoic acid and PML signalling during APL.

Although the specificity of ATO for PML–RARα-associated APL is clear, how it acts has remained contentious. ATO exerts a number of different effects that are to a degree concentration-dependent, inducing apoptosis at high concentrations and promoting differentiation at lower levels. Documented effects of ATO include interference with interactions between the SMRT co-repressor and nuclear receptors and induction of reactive oxygen species[1]. These activities could potentially contribute to the therapeutic effect of ATO in APL; however, it has been demonstrated that both PML–RARα and wild-type PML (but not wild-type RARα) are degraded in APL cells after ATO treatment. This supports the notion that the PML moiety is the primary target[2]. Subsequent studies in APL cells showed that ATO treatment leads to both PML and PML–RARα becoming highly modified by SUMO proteins, which are related structurally to ubiquitin, and that PML sumoylation can take place at Lys 65, 160 and 490 (ref. 3). A previous study suggested that only Lys 160 SUMOylation is important for ATO-induced degradation of PML and PML–RARα, as this modification also mediates recruitment of the proteasome to PML nuclear bodies[4]. In this issue of *Nature Cell Biology*, Lallemand-Breitenbach *et al*[5] and Tatham *et al*[6] demonstrate that the RING finger ubiquitin E3 ligase RNF4/SNURF is critical for the SUMOylation-dependent ubiquitination and subsequent catabolism of PML. For PML to be ubiquitinated by RNF4, it must be modified by a polySUMO chain on Lys 160, which is specifically targeted by RNF4 through four tandemly arranged SUMO interaction motifs (SIMs) (Fig. 1; ref. 6). ATO-induced differentiation was impaired when primary haematopoietic cells expressed a non-degradable PML–RARα Lys 160 mutant, but not wild-type PML–RARα. Impaired differentiation in response to ATO also occurred in PML–RARα-positive primary haematopoietic cells transduced with a dominant-negative mutant of RNF4 (ref. 5). Thus these results directly implicate RNF4-mediated PML–RARα degradation in the APL therapeutic response.

An interesting aspect of these studies is that for arsenic to degrade PML effectively, SUMO-1 and SUMO-2/3 are required. In this respect, it is noteworthy that a PML mutant lacking Lys 65 and Lys 490, but containing Lys 160 is still SUMO-1 conjugated after ATO treatment[5]. As SUMO-1 is unable to form polymers, in contrast to SUMO-2 or -3, it probably functions as a chain terminator. This notion is supported by the findings that the SUMO-1-modified PML K65/490R mutant has a molecular weight consistent with being polySUMOylated[5] and that SUMO-1-'capping' can indeed occur[7]. Given that a SUMO-1 cap is not required for RNF4 to interact with and ubiquitinate polySUMO-2-modified PML[6], attachment of SUMO-1 may serve some other purpose. One attractive possibility is that SUMO-1 termination of the Lys 160-attached chain

inhibits its depolymerization by the SUMO-specific protease SENP6/SUSP1 (Fig. 1). Recent research has suggested that SENP6 acts preferentially against polySUMO chains comprising three or more SUMO-2/3 moieties, and that SENP6 does not effectively deconjugate SUMO-1 (ref. 8). It remains to be seen whether a SUMO-1 cap can prevent the dismantling of a SUMO-2/3 chain, thus enhancing RNF4 binding and PML ubiquitination/degradation.



The upper pathway (A) represents the modification states that may exist between functional PML (top left) and its proteasomal degradation product. The PML 'pro- degradation' activities of as yet unspecified SUMO E3 ligases and RNF4 are opposed by the SUMO protease SENP6 and ubiquitin proteases, respectively. A balance is achieved in response to normal signalling that maintains the appropriate level of PML protein for a given cell type or developmental stage. RNAi-mediated knockdown of *RNF4* leads to an accumulation of PML nuclear bodies enriched for SUMO proteins, supporting the notion that it has an important role in this process. In the lower pathway (B), ATO treatment alters the pathway kinetics (highlighted in red) to favour degradation of PML. The initial sequence of events that occur in response to arsenic remain to be determined but probably involves phosphorylation of residues at the N- and C-terminal regions of PML and perhaps also the removal of SUMO-1 at Lys 490. SUMO-1 is conjugated to PML at Lys 65 and the extended SUMO-2/3 chain on Lys 160, where it functions as a chain terminator. Arsenic-induced SUMOylation of Lys 65 and Lys 160 is tightly coupled and SUMO-1 conjugation of Lys 65 may serve to recruit or stabilize the binding of a SUMO E3 ligase such as RanBP2, which contains a SIM that preferentially binds SUMO-1 (ref. 18). Furthermore, SUMO-1 capping of the SUMO-2/3 chain on Lys 160 could diminish the effectiveness of SENP6, for which SUMO-1 is a poor substrate, thus favouring binding of RNF4 and subsequent ubiquitination and degradation of PML.

Both articles have highlighted an important step linking PML SUMOylation and its degradation; however, the primary events through which arsenic directs this process remain to be uncovered. It is known that ATO activates several kinases and a requirement for MAP kinase phosphorylation of PML has been demonstrated in ATO-induced apoptosis[9]. Although the activities of MAP kinases in ATO-induced PML degradation were not specifically examined, the study found that PML phosphorylation in response to ATO enhanced its SUMOylation. This strongly suggests that ATO-induced PML phosphorylation is involved in the catabolic pathway, possibly as a trigger for an interaction with a known or as yet unidentified SUMO E3 ligase (Fig. 1). There may also be crosstalk between PML phosphorylation and SUMOylation, which mediates an interaction between PML and Pin1, a peptidyl-prolyl *cis-trans* isomerase that induces a conformational change in its substrate proteins. Recent research has shown that Pin1 activity is associated with PML degradation, although the potential role of Pin1 in mediating the effects of ATO has not yet been investigated. Interestingly, although Pin1 binding is dependent on phosphorylation of PML within its C-terminal region, PML SUMOylation blocks this interaction[10]. Given the link between PML SUMOylation and its degradation, this seems paradoxical but the study by Lallemand-Breitenbach *et al*. may provide an explanation[5]. They

show that wild-type PML is SUMO-1 modified on Lys 490 and mutation of this residue enhances degradation of PML in response to ATO. This suggests a stabilizing effect of PML Lys 490 SUMOylation, possibly due in part to inhibition of Pin1-mediated degradation. Furthermore, mutation of the 'pro-degradation' PML Lys 65 residue promotes Lys 490 SUMOylation, suggesting that SUMO modification of PML is subject to strict control by a variety of factors, whose activities can have antagonistic effects on its stability.

Questions arising from the results presented in the two papers are whether RNF4 recognizes other polySUMOylated proteins and also, whether there are other proteins that contain multiple SUMO-interaction motifs. RNF4 has been reported to associate with a number of transcription factors and steroid hormone receptors[11, 12, 13, 14, 15] but it is not known whether these interactions are mediated through the binding of polySUMO chains and/or related to the function of RNF4 as a ubiquitin E3 ligase. Although *Schizosaccharomyces pombe* contains two RNF4 homologues (Rfp1 and Rfp2), so far only RNF4 has been identified as possessing multiple SIMs in humans. However, a search of the Entrez database at the NCBI revealed the presence of a highly homologous 4× SIM-containing region in a predicted but as yet unverified isoform of a testis-specific RING protein encoded by the human *RNF36* gene (accession number EAW77280). Interestingly, both RNF36 (TRIM69) and PML (TRIM19) are members of the tripartite motif (TRIM) family of RING domain-containing proteins and, given that mouse RNF36 localizes to PML nuclear bodies, the function of its human counterpart perhaps merits a more detailed investigation[16].

In conclusion, results presented in the two manuscripts identify RNF4 as a polySUMO chain-binding protein and also PML as a *bona fide* RNF4 target; this is the first example of a protein degraded by this SUMO-dependent ubiquitination pathway. Moreover, the findings by Lallemand-Breitenbach *et al*[5] reinforce the scientific rationale for the use of low-dose ATO in differentiation therapy for PML–RARα-associated APL. However, given the role of PML as a growth/tumour suppressor[17], the finding that ATO also targets PML presents a 'catch 22' situation with regard to its use as a treatment for cancers in general. Nevertheless, the success of ATO in APL therapy has provoked renewed interest in its use and a large number of clinical trials investigating its potential in haematological malignances and solid tumours are currently underway or recently have been completed.[1] This, along with the development of other organic arsenic compounds could well see the emergence of new applications for this semi-metal in the wider range of cancer therapies.

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