

Death by arsenic: Implications of PML sumoylation

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PML is a multifunctional protein that plays an important role in programmed cell death, albeit by mechanisms that remain unclear. In this issue of *Cancer Cell*, Hayakawa and Privalsky associate a MAP kinase pathway that mediates As₂O₃-induced PML phosphorylation with sumoylation and increased apoptotic activity of PML. Thus, specific MAP kinases may potentiate apoptosis in response to As₂O₃, a compound that has dramatic activity against acute promyelocytic leukemia (APL) cells. This novel mechanism may have important implications for use of As₂O₃ as a chemotherapeutic agent, especially in malignancies less sensitive to As₂O₃ than APL.

The tumor suppressor PML was originally identified as the gene fused to RAR α by the t(15;17) chromosomal translocation unique to acute promyelocytic leukemia (APL). Although still incompletely understood, there is good evidence that the PML/RAR α fusion protein exerts its oncogenic effects by interfering with the function of both RAR α and PML. The role of PML in normal cells has been carefully examined in mice with genetic manipulations of PML. Mice with a genetic deletion in PML are viable and develop normally, although they are prone to mycotic infections (Wang et al., 1998a). However, PML^{-/-} mice are more susceptible to carcinogen-induced tumor formation than their wild-type counterparts. Evidence that PML plays an important role in apoptosis was provided by experiments showing that mouse embryo fibroblasts from PML^{-/-} mice are significantly protected against several apoptotic inducers, including γ irradiation, Fas, TNF, IFN, and ceramide (Wang et al., 1998c). This is consistent with results from PML/RAR α transgenic animal models of APL, where hematopoietic cells expressing PML/RAR α have altered PML function and are less sensitive to the effects of the same apoptosis inducers (i.e., TNF and IFNs) on colony formation (Wang et al., 1998c). These mice develop a preleukemic state that progresses to APL; however, this happens with a low frequency and over several months (Grisolano et al., 1997; Wang et al., 1998a). When they are crossed with PML^{-/-} mice, further reducing PML function, the incidence of leukemia is increased and hastened, suggesting that PML has proapoptotic functions in malignant or premalignant cells (Rego et al., 2001).

Wild-type PML localizes to the nuclear bodies (NBs), also known as PML oncogenic domains (PODs), and is required for their proper formation. Deletion of PML, as well as expression of PML/RAR α , causes disruption of the NBs (Melnick and Licht, 2000; Wang et al., 1998a). Within the NBs, PML has been shown to interact with p53, pRb, DAXX, and CBP. Two other proteins that localize to PML NBs are the ubiquitin-conjugating enzyme Ubc9 and SUMO-1, which coordinate to modify the PML protein. Ubc9 binds PML and mediates the conjugation of SUMO-1 to PML. There is significant debate about how sumoylation of PML affects its function. Expression in PML^{-/-} cells of a PML mutated in its three sumoylatable lysine residues is unable to rescue NB formation (Zhong et al., 2000). However, if the lysine residue within the RING domain of PML that binds Ubc9 and SUMO-1 is mutated, there is no dysfunction of NB formation (Boddy et al., 1997). Sumoylation may also regulate the

binding of PML to other NB constituents and, as a result, regulate the movement of these proteins in and out of the NB. The identification of desumoylases indicates that sumoylation is a dynamic process with potential points of regulation within cells. New data from the Hayakawa article suggest that the sumoylation of PML may be further regulated by its phosphorylation state, and that this regulation is linked to the ability of PML to induce apoptosis.

The authors show that As₂O₃ activates ERK, defining PML as a novel target for As₂O₃-induced, ERK-mediated phosphorylation. They confirm that PML is sumoylated in response to As₂O₃ treatment and show that a pharmacologic ERK inhibitor can block this sumoylation. They further link these events to apoptosis. Cells transfected with PML, Ubc9, and SUMO-1 were significantly more sensitive to As₂O₃-induced apoptosis than those transfected with PML alone. In addition, the oncogenic PML/RAR α protein is a target for As₂O₃-induced phosphorylation. However, Hayakawa and Privalsky did not find sumoylation of PML/RAR α when expressed with Ubc9 and Sumo, consistent with previous reports that PML/RAR α is less sensitive than PML to sumoylation (Kamitani et al., 1998a, 1998b). They further correlated this reduced sumoylation with the failure of transiently transfected PML/RAR α to enhance arsenic-induced apoptosis. These results are consistent with our previous results that stable expression of PML/RAR α in U937 cells has a minimal effect on arsenic sensitivity (Davison et al., 2003). The finding that a chemical inhibitor nevertheless decreases the sensitivity of APL cells to arsenic may point to involvement of additional MAPK pathways in cell death by arsenic, as suggested by others (Davison et al., 2003; Kyriakis and Avruch, 1996; Verma et al., 2002).

There are many examples of how one posttranslational modification can affect another. Here, phosphorylation in both the N and C termini of PML potentiates sumoylation. Phosphorylation has also been shown to enhance sumoylation of heat shock factor 1 and the transcription factor, Smad4 (Hietakangas et al., 2003; Ohshima and Shimotohno, 2003). However, the sumoylation of several other transcription factors, c-jun, p53, and Elk-1, is abrogated by phosphorylation (Muller et al., 2000; Yang et al., 2003). In the case of c-jun, this is further complicated by the fact that ubiquitination is decreased as well as sumoylation. This is in contrast to I κ B, the inhibitor of NF- κ B signaling, where phosphorylation decreases sumoylation and increases ubiquitination, thereby marking the protein for degra-

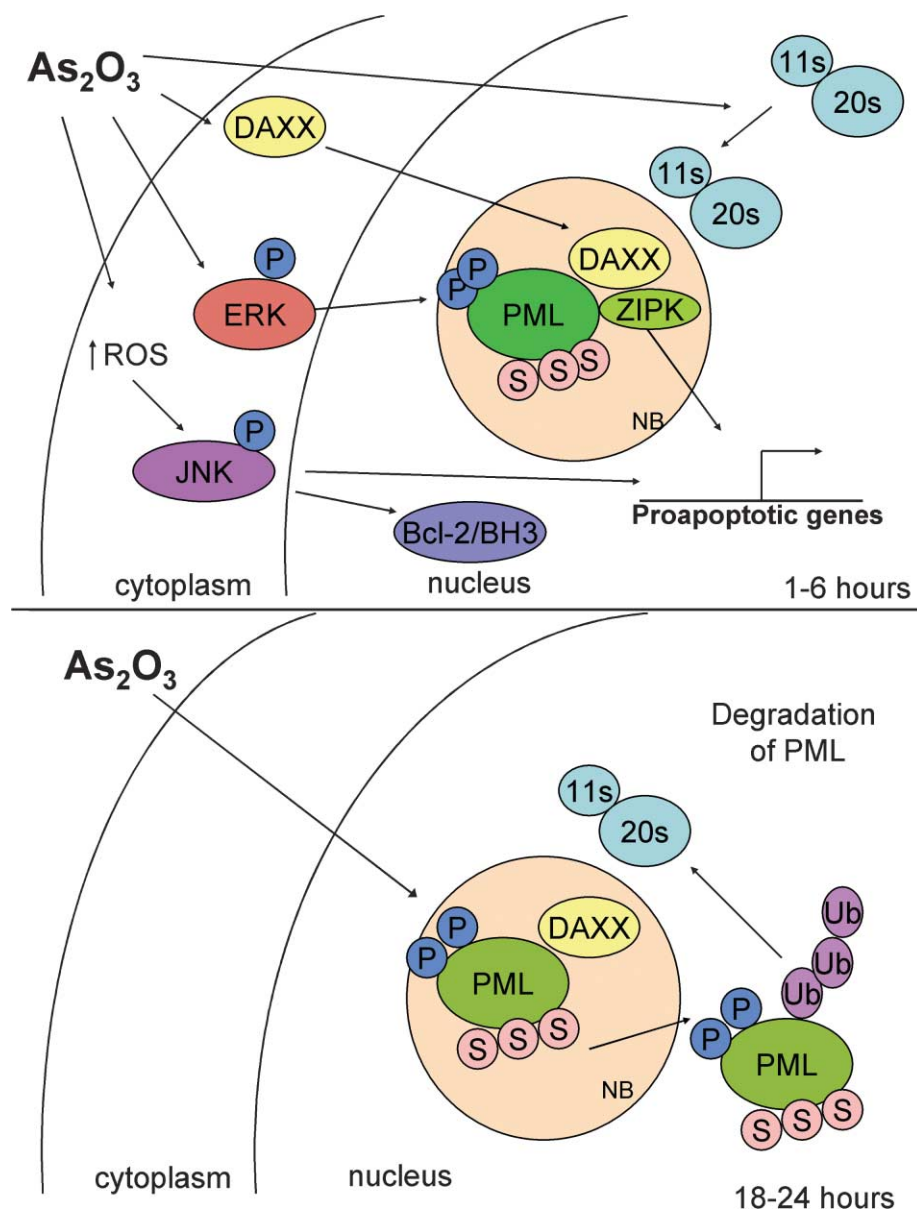


Figure 1. A model of PML-dependent and -independent As_2O_3 -induced apoptosis

The upper panel represents 1–6 hr after As_2O_3 treatment. ERK and JNK are phosphorylated (P) and thereby activated. JNK may activate proapoptotic genes via AP-1 binding or may modulate bcl-2/BH3 proteins. ERK phosphorylates PML, which is subsequently sumoylated (S). DAXX is translocated from the cell membrane to the nuclear bodies (NB), where it forms a complex with PML and ZIPK. The result of these binding is to turn on transcription of proapoptotic genes. Proteasomal components (11s and 20s) relocate near or at NBs. The bottom panel represents later time points of at least 18–24 hr after As_2O_3 exposure. Sumoylated PML is potentially ubiquitinated (Ub) and degraded by the proteasome.

As_2O_3 -induced apoptosis (Kawai et al., 2003). In two models suggested by Bernardi and Pandolfi, PML interacts with DAXX to either enhance transcription of proapoptotic genes or interfere with its function as a transcriptional repressor of antiapoptotic genes (Bernardi and Pandolfi, 2003). Perhaps PML, which is both phosphorylated and SUMO-1-modified, interacts more effectively with DAXX to enhance cell death. Recently, As_2O_3 has been shown to increase the association of another kinase, ZIPK, with the NBs, which in turn may recruit DAXX (Kawai et al., 2003). Perhaps As_2O_3 -induced sumoylation of PML increases the recruitment of ZIPK and DAXX to NBs.

Some key questions remain unanswered. First, there is the paradox of how As_2O_3 can enhance sumoylation of PML and apoptosis and yet cause PML degradation. As_2O_3 also degrades PML/RAR α , which led to the hypothesis that As_2O_3 differentiates APL cells by degrading the dominant-negative effect of PML/RAR α on RA and PML signaling. One possibility

is that PML sumoylation activates a death pathway and then signals a degradation of the protein. The authors show that As_2O_3 sumoylates PML very soon after treatment (i.e., partially by 1 hr and maximally after 6 hr). In our hands, PML/RAR α , at least, is not degraded after 6 hr, but only after 18–24 hr (our unpublished data). As_2O_3 -induced sumoylation of PML causes proteasome components to colocalize near the NBs and recruits DAXX and other proapoptotic machinery to the NBs, while the non-sumoylatable PML mutant does not (Lallemand-Breitenbach et al., 2001). SUMO-modification of a protein may not directly mark proteins for degradation, but could possibly change their subcellular localization or facilitate ubiquitination (Melchior, 2000). As_2O_3 may bring the proteasome to meet the sumoylated PML, resulting in degradation (Lallemand-Breitenbach et al., 2001). Of interest, degradation of PML/RAR α by RA is associated with the expression of UBE1L, a ubiquitin-activating E1-like enzyme, although the relevance of this to As_2O_3 -induced PML/RAR α degradation is unresolved

dation (Desterro et al., 1998). The regulation of protein function by posttranslational modifications is likely to be protein-specific and merits further investigation. It is unclear what relevance the position of phosphorylation and sumoylation sites may have on each other. Of note, the N-terminal phosphorylation site is adjacent to the RING box of PML where Ubc9 and SUMO bind. Are there physical interactions between the RING box and the C-terminal portion of PML? Clarification of this awaits the three-dimensional structure of PML, but to date, only the structure of the RING domain has been resolved.

ERK phosphorylation of PML is clearly linked to its sumoylation, but how do these protein modifications lead to increased apoptosis? DAXX is a protein that bridges the signaling between death receptors at the cytoplasmic membrane and the nucleus. PML and DAXX interact directly within the NBs and cooperate to increase FAS-mediated apoptosis (Torii et al., 1999). In addition, small-interfering RNA to DAXX decreases

(Kitareewan et al., 2002).

Second, As₂O₃ alters many cellular signaling molecules and pathways. There is considerable evidence that additional signaling mechanisms, at least some of which are PML-independent, must be involved in As₂O₃-induced apoptosis. Although resistant to many apoptotic stimuli, fibroblasts with a genetic deletion in PML are as sensitive as wild-type fibroblasts to the cytotoxic effects of As₂O₃ and other arsenic-containing compounds (Wang et al., 1998b). Hayakawa and Privalsky find that addition of PML and the constitutively active MEK1 did not cause apoptosis and conclude that other factors are required for cell death. The induction of reactive oxygen species by As₂O₃ is important for its ability to induce death (Davison et al., 2003). Perhaps this change in redox puts the cell in a state favoring apoptosis that is enhanced by ERK signaling and subsequent sumoylation of PML. This hypothesis is supported by recent data showing TPA-enhanced, As₂O₃-induced apoptosis is associated with enhanced ERK activation and decreased glutathione levels (Fernandez et al., 2004). Another kinase, JNK, is activated by As₂O₃ and is a mediator of As₂O₃-induced apoptosis in APL cells (Davison et al., 2004). Whether this is mediated by activation of AP-1 transcription of proapoptotic genes or through effects on bcl-2/BH3 domain proteins remains unclear. It would be interesting to see if PML is also a target for JNK phosphorylation and if the ability of As₂O₃ to sumoylate PML correlates with increased activation of JNK. Importantly, many of the effects of As₂O₃ may be cell-type specific. In both neurons and leukemic cells, p38 Map kinase is activated in response to arsenic treatment. In neurons, a p38 inhibitor blocked arsenic-induced apoptosis; however, in leukemic cell lines, the same inhibitor did not block apoptosis, but rather slightly enhanced it (Namgung and Xia, 2000; Verma et al., 2002). Thus, it will be interesting to determine the generality of the ERK-PML pathway in mediating apoptosis.

Despite lingering questions concerning mechanisms, this PML-ERK connection may potentially be exploited to enhance As₂O₃ clinically. Although As₂O₃ is used to treat APL and shows some promise as a treatment for patients with multiple myeloma or myelodysplastic syndrome, it has less antitumor activity against other tumor types. Therefore, many groups are investigating the possibility of combining As₂O₃ with other drugs, including both conventional cytotoxic agents and more novel, targeted agents. The new data of Hayakawa and Privalsky suggest that agents that activate or amplify ERK signaling may enhance the sensitivity of tumors to As₂O₃, perhaps especially those that express significant levels of PML.

A proposed model for As₂O₃-induced apoptosis is shown in Figure 1, which includes both PML-dependent and -independent pathways. This model is by no means exhaustive, and further investigation is required to link all the elements. As illustrated in PML^{-/-} fibroblasts, PML expression enhances many apoptosis inducers, but surprisingly not As₂O₃. Together, these data serve to emphasize the unique aspects of As₂O₃ as an apoptosis inducer that have yet to be explored.

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