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Therapy-induced PML/RARA Proteolysis and Acute Promyelocytic Leukemia Cure

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Abstract

Acute promyelocytic leukemia (APL) is characterized by a specific t(15;17) chromosomal translocation that yields the PML/RARA fusion gene. Clinically, besides chemotherapy, two drugs induce clinical remissions: retinoic acid (RA) and arsenic trioxide (As). Both agents directly target PML/RARA-mediated transcriptional repression and protein stability, inducing to various extent promyelocyte differentiation and clinical remission of APL patients. RA targets the RARA moiety of the fusion, whereas arsenic targets its PML part. PML/RARA expression in the mouse is sufficient to initiate APL. The RA-As association, which synergizes for PML/RARA degradation but not for differentiation, rapidly clears leukemia initiating cells (LIC), resulting in APL eradication in murine APL models, but also in several APL clinical trials. Cyclic AMP triggered PML/RARA phosphorylation also enhances RA-induced APL regression, PML/RARA degradation, and LIC clearance, raising new options for therapy-resistant patients. Although differentiation has a major role in debulking of the tumor, PML/RARA degradation seems to be the primary basis for APL eradication by the RA-As association. Oncoprotein degradation could be a general therapeutic strategy that may be extended beyond APL.


Background

Acute promyelocytic leukemia (APL) is a well-characterized subtype of acute myeloid leukemia (AML). APL, which accounts for 10 to 15% of AML, is morphologically characterized by a clear differentiation block of the granulocytic lineage at the promyelocytic stage. The genetic hallmark of APL is a balanced reciprocal translocation t(15;17)(q22;q11-12), leading to the fusion of the promyelocytic gene (PML) on chromosome 15 and the retinoic acid receptor alpha gene (RARA) on chromosome 17, and generating the PML/RARA fusion gene and protein (reviewed in ref. 1). Although, more than 97% of APL patients have the t(15;17) translocation, several rare variant translocations that always involve RARA are observed in the remaining APL patients. The most common is the t(11;17) translocation that fuses the promyelocytic leukemia zinc finger (PLZF) gene to RARA (2) in clinically retinoic acid (RA)-resistant APLs. The prognosis of APL with anthracyclin-based therapies used to be poor. Over the past 20 years, introduction of two novel agents, RA and arsenic, has radically changed the prognosis of this disease. Importantly, biological studies have unraveled a posteriori that these active drugs actually targeted the PML/RARA fusion. Actually, APL is one of the rare malignancies that may be cured by targeted agents, even without DNA-damaging chemotherapies (3–7). Hence, APL is not only a success story for clinical improvement of a fatal malignancy, but also for the development of rational combinations and translational research at large.

The partners: RARA and PML. RARA is a member of the RA nuclear receptor family that binds DNA as heterodimers with its cofactor, the retinoid X receptor (RXR; ref. 8). The RARA/RXR complex binds to specific RA response elements (RARE) composed of the canonical AGGTCA half site in a direct repeat orientation, with a spacing of five nucleotides. Binding of the RARA/RXR heterodimer onto RARE yields transcriptional repression in the absence of the RA ligand, but sharp activation in its presence. RARA seems to be particularly efficient to repress transcription, because of its strong interaction with co-repressors (9). Basal repression and hormone-induced activation by RARs play a critical role in several aspects of embryogenesis, and RARG has been implicated in hematopoietic stem cell self-renewal (10).

PML was discovered through the molecular cloning of PML/RARA. It belongs to the RBCC/TRIM family, many of which are ubiquitin ligases (11, 12). PML has drawn much attention to cell biologists because of its ability to nucleate PML nuclear bodies, discrete structures that recruit a large number of ubiquitylated proteins (13). Although their exact function is still controversial, these structures have been implicated in a large...
The oncogenic properties of PML/RARA have been associated with several features. PML/RARA is a DNA-binding transcription factor that may bind not only to consensus RARE, but also to novel binding sites, in which the two AGGTCA half-sites can be in any orientation or spacing (16–18). This characteristic is due to the existence in PML of a powerful dimerization domain, which allows formation of stable PML/RARA homodimers. Such homodimer formation also enhances corepressor recruitment, leading to transcriptional repression and silencing of target genes (19, 20). It is assumed that the differentiation block is the consequence of the transcriptional inhibition of key developmental regulators of myeloid differentiation (1), as in many other myeloid leukemia in which translocations turn master transcriptional activators into dominant repressor oncogenes (21). Recent studies have shown that the differentiation block and the transcriptional repression induced by PML/RARA involve not only RARA homodimerization (22), but also PML sumoylation, RXR binding, and recruitment of the polycomb complex (23–27). Other studies have shown that PML/RARA may be tethered onto DNA through specific interactions with other transcription factors, identifying yet another way for the fusion to alter gene expression (28). Apart from transcriptional regulation, PML/RARA also disrupts PML nuclear bodies (29–32), which regulate stem cell self-renewal (14, 15), raising the tantalizing prospect that other molecular mechanisms than transcriptional repression may be implicated in APL leukemogenesis.

**Therapeutic effects of retinoic acid in APL.** Before the 1980s, chemotherapy was the only available treatment for APL. Anthracyclines induce a complete remission (CR) rate of between 50 and 90%, but unfortunately this was followed by a very high rate of relapse (3). Then later, RA was discovered and has marked a major advance in APL treatment, as it dramatically increases the clinical remission (4). However, with RA as a single agent, the great majority of patients will also experience a relapse within a few months. The cloning of PML/RARA allowed the detection of minimal residual disease, which, despite clinically complete remissions, was never abolished by RA therapy (35). It was subsequently shown that the combination of RA with conventional chemotherapy greatly increases cure (4): RA combined with anthracycline-based chemotherapy can achieve CR in 90 to 95% of patients with APL and overall 5-year disease-free survival in up to 75% of patients (6). At the cellular level, RA induces terminal differentiation of APL cells along the granulocytic lineage. RA binds PML/RARA through the ligand-binding domain of RARA, triggering a conformational change that releases transcriptional corepressors.
and recruits transcriptional co-activators, allowing the RARA moiety of the fusion to activate transcription of its target genes and to differentiate cells expressing PML/RARA. It was first believed that the therapeutic effect of RA stems from its ability to reverse repression of critical target genes required for myeloid differentiation, thereby inducing granulocytic differentiation. RA-treatment of APL indeed constitutes the first, and only, example of differentiation therapy (36). RA binding also allows the direct recruitment of the proteasome to the ligand-activated transcriptional activation domain AF2 of RARA, leading to PML/RARA degradation (Fig. 1B; refs. 37, 38). A RA-induced PML/RARA degradation by a specific cleavage by caspases was also reported (39). Because RA directly targets the PML/RARA oncoprotein for activation and/or degradation, it also constitutes one of the first examples of oncogene-targeted treatments (4, 40, 41). However, the relative contributions of transcriptional activation, APL cell differentiation, and PML/RARA degradation to the therapeutic effect of RA have remained controversial.

**Therapeutic effects of arsenic trioxide in APL.** Arsenic trioxide (As) is one of the oldest drugs known to man (42). After its reintroduction in 1990s, it proved to be of great benefit to the treatment of APL patients. Several studies have shown that As has improved the clinical outcome of refractory or relapsed APL. It was shown to induce a CR in 85 to 90% of patients with both newly diagnosed and relapsed APL (43–46). Today, As is effective and approved for treatment of relapsed or refractory APL cases resistant to RA.

At the cellular level, arsenic induces apoptosis at high concentrations and promotes partial differentiation at lower levels (47, 48). PML/RARA protein is also targeted by As (48–51). However, in contrast to RA that targets the RARA moiety of the fusion, arsenic targets its PML moiety (40, 41). The molecular basis of this specific induction of proteolysis was only recently discovered. As treatment promotes small ubiquitin-related modifier (SUMO) conjugation of PML at amino acid K160 (52). Although in the basal state K160 sumoylation is required for the binding of the corepressor DAXX to PML/RARA (24, 53), poly-sumoylation of PML triggers its poly-ubiquitination by the SUMO-dependent ubiquitin ligase RNF-4 (Fig. 1B; ref. 54–56). Because of this As-induced PML/RARA degradation, As was identified as the second oncogene-targeted therapy in APL (40, 41).

**Mice get in the game.** The expression of PML/RARA suffices to induce APL in mice (57, 58). Several studies have investigated in mice the mechanisms through which PML/RARA triggers APL leukemogenesis. Interestingly, these have yielded conclusions somehow at variance to the studies done in cell-lines. In particular, whereas RARA dimerization seemed not only pivotal, but sufficient to trigger differentiation arrest in cell-lines (59, 60), it quite inefficiently promotes leukemia in vivo (22, 61). In contrast, the essential role of specific post-translational modifications, such as sumoylation of the PML moiety of PML/RARA or RXR binding, were primarily achieved in mice (23, 24). Mice also played a role to implicate immune response against PML/RARA in the eradication of the disease (62, 63), and in characterizing in situ the cellular effects of therapeutic agents. In particular, these studies have shown that RA and arsenic both promoted significant apoptosis in vivo (52, 58). These models have also shown that PLZF/RARA leukemias are completely resistant to As (64). Unexpectedly, they terminally differentiate in response to RA (65), but this does not suffice to clear the disease.

Finally, these mouse models have had a significant role in developing therapeutic combinations for APL and in elucidating their actual mechanisms of action. Because RA and As target the PML/RARA oncoprotein for degradation through different sites and via distinct pathways, it could be envisioned that they should be synergistic. Indeed, whereas studies in cell-lines were contradictory, those done in APL mouse models have all shown synergistic effects of both drugs in prolonging survival and even eradicating the leukemia (64, 66–68).

**How is RA-As synergy accomplished?** In mice, the combination of RA and As causes a rapid disappearance of APL cells and cures leukemia in several models. Yet, RA and As do not synergize to induce differentiation (47, 48, 50), but they do cooperate to induce PML/RARA degradation (Fig. 1B; refs. 40, 41, 65). Recent work from several groups has shown that the apparent contradiction between the observed RA-As antagonism for differentiation and dramatic synergy for APL cure could be clarified by a careful examination of the heterogeneity of APL cells. Cancer stem cells (CSC) or leukemia initiating cells (LIC) are a minority of tumor cells that are able to self-renew, but usually do not cycle (69). Because of their inefficient targeting by many treatments (notably chemotherapy), LIC are believed to drive many clinical relapses. Several studies have shown that these cells have a very peculiar biology and are regulated by a small number of highly conserved pathways. In addition to the differentiation block, PML/RARA confers immortalization and self-renewal properties to APL leukemic cells (Fig. 1A; refs. 70, 71), raising the possibility that these two events may be controlled by a different gene network. That not all APL cells are identical is well shown by both the requirement for a few hundred cells to efficiently propagate APL in mice, but also by the small fraction (1%) of clonogenic cells in ex vivo transduced primary hematopoietic progenitors (24, 71). Such asymmetry raised the possibility that differentiation block and progenitor immortalization may be uncoupled, in particular during therapy response.

Several recent studies have compared the fate of LIC upon ex vivo or in vivo treatment with RA or arsenic (65, 72–74). Unexpectedly, it was shown that whereas RA could clear some LIC, previously overlooked issues of doses were critical in this process. Indeed, in vivo, a very wide range of RA concentrations suffice to initiate similar levels of differentiation. In contrast, LIC clearance is strictly observed with high concentrations and is sharply dose-dependant (65). Full blast differentiation is indeed observed with much lower RA concentration than those required for remissions in vivo. Similarly, relapses appear in patients with decreased RA plasma concentrations, but that still trigger differentiation (75). This, and other evidence, has suggested that RA-induced LIC clearance was the consequence of PML/RARA catabolism, whereas induction of differentiation likely reflected reversal of PML/RARA-dependent transcriptional repression (73). Interestingly, the few patients that were actually cured with RA alone received a liposomal form, which allowed very high and prolonged plasma concentrations (76). Arsenic alone is only a modest inducer of differentiation. Yet, it is a very potent inducer of PML/RARA degradation. Thus, putting the major emphasis on degradation naturally explains the dramatic synergy between arsenic and RA. Because of distinct mechanisms of action and different target sites, no cross-resistance between RA and As is expected (40, 41). Thus, rapid proteasome-dependent degradation of PML/RARA by curative RA-As combination is
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Synergy between RA and As in APL patients.

As a follow-up to mice studies, several clinical trials were done to see if this therapeutic synergy could be translated into treatment strategies for APL patients. These studies have all shown a striking similarity in APL patients with regard to disease eradication by RA-As (7, 81–83). Patients treated with a combination of RA and arsenic obtained a quicker clinical response, had a more rapid and complete clearance of leukemic cells, and had a significantly longer period of relapse-free survival. Recent trials in de novo patients have shown that APL patients may benefit from the early use of the combination of RA-As, for both remission induction and consolidation-maintenance. LIC eradication in patients is reflected by cure and long-term follow-up of RA-As-treated patients is now available and highly supportive (81). Although larger clinical trials would further strengthen these results, the well-controlled side effects and the minimal toxicity associated with this combination therapy supports the use of this combination as a frontline therapy for newly diagnosed APL. Remarkably, a major benefit for the RA-As combination even without any chemotherapy in the treatment of APL patients was also recently reported (5, 7), suggesting that APL may be curable without any DNA-damaging therapies.

Could the APL model be generalized? Triggering oncprotein degradation could be a general therapeutic strategy in cancers in which well-defined oncogenes control the LIC maintenance. This approach could be particularly promising in leukemias or sarcomas caused by fusion proteins. As PML/RARA, they offer the unique opportunity to destabilize the protein through cooperative pathways targeting each of its moieties. In fact, in solid tumors such as breast cancers, degradation of key transcriptional activators such as the estrogen receptor, have been of clinical use (84). Targeting PML itself, out of the context of PML/RARA could also be an interesting target. Indeed, PML seems to be a critical contributor of normal or cancer stem cell quiescence (14, 15, 85).

Identifying the molecular characteristics of LIC and their oncogene-dependency should be an essential step to design new therapies. In APL, this has been facilitated by the identification of PML/RARA and the prior clinical efficacy of RA and arsenic. Although other molecular events cooperate to allow emergence of the full leukemic phenotype, PML/RARA targeting is sufficient to clear LIC. In solid tumors multiple signaling pathways need to be altered for epithelial tumors to develop. Whether, as in APL, inactivation of one single of the early affected pathways will be sufficient for tumor collapse remains a key issue.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References


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