Curing APL: Differentiation or Destruction?

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In a recent issue of *Nature Medicine*, Nasr et al. show that the effectiveness of all-*trans* retinoic acid and arsenic trioxide in acute promyelocytic leukemia is independent of their ability to cause differentiation. Targeted destruction of the PML-RARα oncoprotein appears key to eliminating the cells from which relapse can arise.

In 1987, all-*trans* retinoic acid (RA) was reported to induce clinical remissions in acute promyelocytic leukemia (APL) (Huang et al., 1987), and it was subsequently shown to greatly increase cure when combined with chemotherapy. The bone marrow of patients with APL differentiates in response to RA, and it was believed that the curative effect of RA reflected the ability of this vitamin A derivative to cause maturation of leukemic cells. Since that time, RA treatment of APL has been described as the first example of successful differentiation therapy of a human cancer, and much effort has gone into developing differentiation therapies for other malignancies. Recent work by Nasr et al. (2008) seems to pull the rug out from under these efforts by suggesting that differentiation does not in fact underlie the remarkable effectiveness of RA in APL.

A series of studies published in 1998 compared the PML-RARα fusion common to APL with the rare and clinically RA-resistant PLZF-RARα fusion (Grignani et al., 1998; Guidez et al., 1998; He et al., 1998; Lin et al., 1998). These studies showed that pharmacologic doses of RA could induce release of transcriptional corepressor complexes, activate transcription, and cause differentiation of cells expressing PML-RARα, but that RA did not have these same effects on cells expressing PLZF-RARα because PLZF interaction with corepressors was not reversed by RA. Following these observations, the widely accepted view of APL pathogenesis has been that PML-RARα fusions repress transcription of genes required for myeloid differentiation and that RA has a therapeutic effect by reversing this repression, thereby stimulating myeloid maturation (Melnick and Licht, 1999). The relative importance of transcriptional activation through PML-RARα versus degradation of PML-RARα permitting gene expression has been an area of continuing controversy, but the hypothesis that RA is effective because it induces differentiation had moved into the realm of accepted "fact."

The current work by Nasr et al. cracks open this view of how RA cures APL. Working with primary cells transduced with PML-RARα, the authors made the interesting observation that although RA caused differentiation and suppressed myeloid colony formation, when RA was removed, the cultures retained the ability to form colonies of immature myeloid cells. To follow up on this observation, the authors moved to in vivo murine models of APL and examined how cells able to transfer disease from one animal to another were affected by RA treatment. (In the Nasr et al. manuscript, these cells are referred to as leukemia-initiating cells or "LICs"; such cells have been given varied names including "leukemic stem cells" and "leukemia-repopulating cells.") The authors observed that although RA therapy converted leukemic promyelocytes into differentiated granulocytes, the LICs persisted, albeit at reduced numbers. Next, the authors showed that differentiation could occur without any significant impact on LICs. Low doses of RA induced differentiation without reducing LICs in the PML-RARα model, and higher doses of RA induced differentiation of PLZF-RARα mouse APL cells despite the clinical ineffectiveness of RA in human patients with the PLZF-RARα fusion and no decrease of LICs in mice. Thus, differentiation per se does not correlate with reduction of LICs.

In order to understand how LICs might be eliminated, Nasr et al. turned to studies of RA in combination with arsenic trioxide (AS), an agent that, like RA, is selectively effective for APL treatment. At the cellular level, AS has been observed to cause partial differentiation as well as apoptosis of APL cells; at the molecular level, AS causes a sumoylation of the PML-RARα fusion that targets it for ubiquitin-mediated degradation. In both mice and humans, the combination of RA and AS causes a rapid disappearance of APL cells and is frequently curative. The authors showed that although RA and AS do not synergize as far as inducing differentiation, the combination causes rapid disappearance of LICs. Since RA and AS synergistically caused degradation of the PML-RARα fusion, the authors assessed whether such degradation was required for the elimination of LICs. Bortezomib, a proteasome inhibitor, was given to mice receiving RA and AS. Although differentiation of APL cells was robust in these triply treated mice, bortezomib blocked PML-RARα degradation and reversed the curative effect of the RA + AS combination. As the authors note, bortezomib may influence other pathways in the cells studied, but their results nevertheless link PML-RARα destruction to the elimination of LICs. Further experiments exploring the impact of cyclic AMP on APL response, including the importance of a site of PKA phosphorylation within the RARα moiety, provided additional support for their hypothesis that PML-RARα degradation, but not myeloid differentiation, underlies the elimination of LICs.

As illustrated in Table 1, the data of Nasr et al. can be placed in a broader context for advancing our understanding of cure in APL. Although at present, RA + AS appears to be the most effective

Table 1. Impact of Therapies in Acute Promyelocytic Leukemia

Treatment	Effect on PML-RARα	Effect on Promyelocytes	LIC Elimination	Outcome
RA	transactivation > degradation	differentiation	no	relapse
RA + arsenic	degradation > transactivation	differentiation	rapid	cure
Liposomal RA	transactivation and degradation	differentiation	slow	cure or relapse
RA + anthracycline		death and differentiation	slow	cure or relapse

Retinoic acid (RA) can induce gene transcription through PML-RARα as well as degradation of this fusion protein. The relative contributions of these activities to the effects of RA on acute promyelocytic leukemia have not been clear. This table provides a summary interpretation of data available from human and mouse studies. The work of Nasr et al. (2008) supports the idea that transactivation underlies RA-induced differentiation but shows that RA does not eliminate cells from which relapse can arise (LICs). The curative RA + arsenic combination causes both rapid degradation of PML-RARα and elimination of LICs. How exposure to persistent high levels of RA, as is possible with liposomal RA, or therapy with a combination of RA + anthracycline may be curative is not clear; these treatments could act through PML-RARα degradation or might eliminate LICs over time through alternative mechanisms (see text).

treatment for APL, APL can be cured with other regimens. Liposomal RA can be curative in human patients with APL (Tsimberidou et al., 2006). The authors imply that higher and prolonged plasma levels of RA may cause a greater catabolism of PML-RARα that effects LIC elimination, but the possibility that such levels of RA reduce LICs through transactivation of target genes is not excluded. How the combination of anthracycline and RA is often curative is a question for which even greater speculation is required. Is it possible that the combination results in greater destruction of the PML-RARα fusion than RA alone? Does RA alter gene expression in such a way as to make APL LICs more sensitive to the chemotherapeutic agent? A larger question is how complex curative regimens of induction, consolidation, and maintenance are able to eventually eradicate all cancer-repopulating cells despite such cells persisting after initial therapy.

In the work of Nasr et al., LICs were studied at a functional level but were not identified as a population distinct from the bulk of the leukemic population. The authors state that such isolation would facilitate "elucidation of the actual cellular basis for LIC clearance," speculating that LIC clearance may be based on loss of self-renewal or apoptosis. What are likely to be features of these LICs? Studies of human leukemias have suggested that for many APLs, the LICs might be present in a myeloid committed rather than a stem cell compartment. The poor transplantability of human APL cells as xenografts has hampered progress in identifying the LICs of human APL, but recent systems show promise for permitting such studies (Ishikawa et al., 2007). Some transplantable mouse APL models show remarkable morphologic and immunophenotypic homogeneity, raising the possibility that the LICs of APL are cells phenotypically similar to promyelocytes. Are the LICs of APL a distinct separable cell type, or might they be cells at a particular stage of the cell cycle or localized to a particular microenvironment?

The work of Nasr et al. further extends our understanding of retinoids in normal and malignant hematopoiesis. An underappreciated aspect of retinoid signaling is that retinoids not only are inducers of myeloid differentiation but also can serve as critical signals that facilitate self-renewal of stem cells (Purton et al., 2006). The particular phenotype of APL may arise because the RARα fusions of this leukemia dysregulate both the self-renewal and differentiative roles of RA and its receptors.

The discovery of RA and AS as therapeutics for APL was based on clinical observations, not on rational drug design. Conceptually, the idea that these drugs work by targeting a critical abnormal protein for degradation fits well with the notion that malignancies are dependent upon their oncogenes. Nevertheless, the possibility that agents can be discovered or designed that restore maturation to immature malignant cells should not be abandoned, even if the former leader in differentiation therapy might not be so grown up after all.

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