PRAMEing a Picture of Differentiation Therapy for AML?

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Addition of retinoic acid to chemotherapy improves survival of patients with acute myeloid leukemia. This effect is more pronounced in leukemias that express high levels of PRAME. PRAME is an inhibitor of retinoic acid signaling, which may prove to be an important marker for retinoic acid response. Clin Cancer Res; 19(9): 2277–9. ©2013 AACR.

In this issue of Clinical Cancer Research, Bullinger and colleagues (1) present data that suggest that PRAME overexpression modulates the retinoic acid signaling pathway in acute myeloid leukemia (AML) to increase sensitivity to all-trans retinoic acid (ATRA).

PRAME (preferentially expressed antigen in melanoma) was first identified as a gene encoding an HLA-A24–restricted peptide able to stimulate tumor-specific cytotoxic T lymphocytes in a patient with melanoma (hence the name; see ref. 2 for review). It is a member of the family of cancer testis antigens and is expressed at high levels in germinal tissues and malignancies but is barely detectable in other normal tissues. It is expressed in the majority of melanomas and has been detected in non–small cell lung cancer, head and neck cancer, renal carcinoma, myeloma, sarcomas, breast cancer, acute lymphoblastic leukemia, chronic lymphocytic leukemia, chronic myeloid leukemia, and AML.

Epping and colleagues (3) first recognized that a C-terminal leucine-rich repeat in the C-terminus of PRAME contains a consensus LXXLL-binding domain that mediates interaction with the ligand-binding region of the retinoic acid receptor (RAR). RAR is a member of the nuclear hormone receptor family. It binds as a heterodimer with the retinoid-X receptor (RXR) to consensus retinoic acid response elements (RARE) in promoters of target genes (4).

In the absence of ligand, it attracts a corepressor complex containing histone deacetylase activity that modulates chromatin structure and suppresses transcription (Fig. 1A). Upon binding ligand, RAR undergoes conformational changes that result in decreased affinity for the corepressor complex and exposure of an interaction domain that binds a histone acetyl transferase–containing coactivator complex. Recruitment of the coactivator complex results in local remodeling of chromatin and initiation of transcription (Fig. 1B). Epping and colleagues (3) showed that PRAME is a ligand-dependent repressor of RAR: In the presence of ligand (but not in its absence) PRAME binds to RAR and recruits EZH2, a polycomb protein that represses transcription through modulating histone methylation (Fig. 1C).

Many of the RAR target genes are involved in differentiation and apoptotic pathways, and silencing retinoic acid signaling inhibits differentiation and apoptosis. Indeed, expression of a dominant-negative RAR construct in murine hematopoietic progenitors generates a phenotype of myeloid maturation arrest (5) similar to that seen in AMLs. The key role of retinoic acid in differentiation has led many investigators to focus on it as a potential target in malignancies. The paradigm for differentiation therapy is acute promyelocytic leukemia (APL; ref. 4). In APL, the characteristic t(15;17) translocation leads to expression of a PML–RAR fusion protein, which retains the RAR DNA binding, ligand binding, and protein interaction domains and is capable of binding to RAREs. PML–RAR fails to undergo the ligand-induced conformational changes necessary to release the corepressor complex at physiologic levels of retinoic acid and hence RAR target genes remain silenced. Pharmacologic levels of ATRA initiate degradation of PML–RAR and activation of transcription of RAR targets, leading to repression of the silenced differentiation pathways and maturation and subsequent apoptosis of the leukemic blasts. The introduction of ATRA as therapy for APL has revolutionized outcomes in this formerly highly fatal disease, and when combined with chemotherapy, generates remission rates of up to 90% (4).

There has been much interest in translating the success of ATRA differentiation therapy to other malignancies. AML is an attractive target, as the RAR pathway is commonly suppressed in AML. Indeed, src-family kinases (6), the MN1, SKI, or AML-ETO oncogenes, or aberrant expression of PRAME have all been shown to affect the RAR pathway. However, therapeutic trials in AML using ATRA have yielded inconsistent results. Estey and colleagues (7) randomized high-risk patients with AML to receive fludarabine/idarubicin/ cytarabine with or without ATRA and found no significant difference in the 2 arms. Similarly, the MRC AML12 trial (8) of daunorubicin/cytarabine/thioguanine with or without ATRA failed to show significant change in...
remission rate, event-free survival (EFS), or overall survival. Belhabri and colleagues (9) also found no significant difference when adding ATRA to idarubicin/cytarabine induction. Conversely, the German-Austrian AMLSG HD98B trial (10) randomized elderly patients to ± ATRA beginning on day +3 in combination with idarubicin/cytarabine/etoposide induction and consolidation with cytarabine/mitoxantrone. They reported a statistically significant difference in complete response (52% vs. 39%) and overall survival (11.3 vs. 7.0 months). The reason for the discrepancy with the other ATRA trials is unclear but may be related to time of initiation of ATRA or the target population. Nevertheless, subgroup analysis of the HD98B trial suggested that the enhanced response was limited to those patients who manifested the good prognostic indicators of mutation of nucleophosmin (NPM1) and lack of mutation of FLT3 (11). On the basis of this study, the German-Austrian group developed AML 07-04, targeting a younger population of patients with NPM1 mutation; preliminary analysis presented at the 2011 meeting of the American Society of Hematology (12) indicates a statistically significant increase in complete response, EFS, and overall survival in the ATRA-treated cohort.

In this issue of *Clinical Cancer Research*, Bullinger and colleagues (1) present a further analysis of patients in the HD98B and 07-04 trials, focusing on the potential role of PRAME as a predictor of response. In Fig. 5 of their article, tantalizing data that suggest that expression of PRAME is associated with improved response to ATRA are presented. Oddly, this was seen only in the younger population studied in the 07-04 trial; PRAME expression was not a factor in the ATRA response of older patients. The reason for the age differential is not clear: Perhaps it is a result of seemingly minor differences in the treatment regimens, perhaps it has to do with age-dependent physiologic differences, perhaps it is a function of the relatively small PRAME-positive cohort analyzed.

Is PRAME a biomarker for response, or truly involved mechanistically? The data presented in Fig. 4, indicating similarity of changes in gene expression in K562 cells when PRAME is silenced or when cells are treated with ATRA, suggest a mechanistic role for PRAME in the retinoic acid pathway. While consistent with the known antagonistic effects of PRAME on ATRA signaling, this finding does not fully illuminate the observed improved clinical outcome associated with PRAME. If PRAME truly is an inhibitor of the retinoic acid pathway, then one would expect that PRAME expression should negatively impact the clinical outcome. However, Figs. 2 and 3 indicate that even cells that express high levels of PRAME are still able to respond to high levels of ATRA. One could speculate that leukemic cells that express high levels of PRAME preserve an active (although silenced) retinoic acid signaling pathway capable of responding to pharmacologic doses of ATRA. Moreover, Bullinger suggests that the ATRA response signature presented in Fig. 1 indicates that the PRAME-expressing leukemias might be more sensitive to chemotherapy, similar to NPM1-mutated patients. It would be important to assay the PRAME-expressing leukemic cells themselves in vitro to further determine the mechanism of ATRA response and to determine whether the cells undergo differentiation, apoptosis, or enhanced susceptibility to chemotherapy. Follow-up experiments are needed to afford full interpretation of this very provocative article.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

**Grant Support**

R.L. Redner receives research grant funding from the Leukemia and Lymphoma Society (6028-10).

Received February 26, 2013; accepted March 15, 2013; published OnlineFirst March 26, 2013.

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