Clinical Cancer Research

Twist of Fate for Acute Promyelocytic Leukemia: TRIB3–TWIST1 Interaction Promotes Resistance

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While acute promyelocytic leukemia has a good prognosis with all-trans retinoic acid (ATRA) treatment, ATRA resistance is a major obstacle. It is now demonstrated that TRIBBLES 3 (TRIB3) stabilizes TWIST1, leading to ATRA resistance. Peptides that disrupt this interaction lead to the degradation of TWIST1 and overcome ATRA resistance.

See related article by Lin et al., p. 6228

In this issue of *Clinical Cancer Research*, Lin and colleagues uncover a new mechanism of resistance of acute promyelocytic leukemia (APL) cells to the differentiation therapy all-trans retinoic acid (ATRA) involving the interaction between TRIB3 and TWIST1, and design a novel therapeutic strategy to overcome this resistance (1).

APL, first classified in 1957, was known as one of the most dangerous acute leukemias due to a high early death rate (within 30 days of diagnosis), specifically from fatal hemorrhage, with a median survival of less than 1 month (2). APL is characterized by a translocation between chromosomes 15 and 17, which juxtaposes the gene promyelocytic leukemia (PML) with the retinoic acid receptor alpha (RARα). RARα is both a transcription factor and nuclear receptor, while PML is a tumor suppressor involved in the organization of nuclear domains that helps to regulate cell division and apoptosis. The PML-RARα fusion protein causes a block in myeloid differentiation at the promyelocyte stage.

The arrival of ATRA for the treatment of APL, which promotes myeloid differentiation and leads to degradation of the PML-RARα fusion, heralded a sea change for treatment and prognosis. Early studies showed impressive single-agent activity with complete response rates of 70%–80% for first-line induction comparable with multi-agent chemotherapy (2). Improvement in relapse rates was seen with the addition of anthracyclines (2). The introduction of arsenic trioxide (ATO) in the 1990's further shifted the paradigm, and its combination with ATRA and anthracyclines in first-line treatment resulted in further improvement in disease-free and failure-free survival (2). While these results are encouraging, relapse still occurs in 5%–20% of patients (2). Furthermore, an early death rate of 17% has been reported in the Surveillance Epidemiology and End Results dataset outside of clinical trials, and elderly patients are particularly affected (3). Another issue is extra-medullary disease; 3%–5% of patients will relapse in extramedullary sites, with the central nervous system (CNS) being the most common location, and this can occur even in the setting of sustained hematologic and molecular remission (2). Multiple mechanisms for acquired treatment resistance to ATRA have been described, including increased ATRA metabolism leading to reduced cellular ATRA concentrations, and mutations in the RARα ligand binding domain affecting ATRA and ATO binding sites (4). As ATO therapy has moved into first-line regimens, the treatment options for patients with relapse after ATRA or ATO have narrowed (4).

The interesting study by Lin and colleagues in this issue proposes a novel mechanism for ATRA resistance in APL. The authors previously reported the contribution of TWIST1, a basic helix-loop-helix transcription factor involved in the epithelial–mesenchymal transition (EMT), to extramedullary disease progression in acute myelogenous leukemia (AML; ref. 5). In this study, the authors found that TWIST1 RNA and protein levels are particularly high in patients with APL, compared with other subsets of AML. Because TWIST1 does not appear to be a direct transcriptional target of PML-RARα, the authors investigated alternative mechanisms for how the protein levels of TWIST1 could be regulated in APL.

It has been demonstrated previously that TRIBBLES 3 (TRIB3), a pseudokinase protein and member of the TRIBBLES family, interacts with the selective autophagy substrate p62, which can promote tumorigenesis (6). Other studies have suggested that TRIBBLES family proteins can reduce the expression of CEBPα, an important transcription factor in myeloid differentiation (7). It has also been shown that TRIB3 promotes APL progression by stabilizing PML-RARα (8). Now Lin and colleagues report that TRIB3 stabilizes PML-RARα indirectly via the binding and stabilization of TWIST1 (Fig. 1). This leads to the decreased ubiquitination and degradation of TWIST1 in APL cells. The authors show that knockdown of TRIB3 decreases protein levels of TWIST1 in APL cells, leading to differentiation and apoptosis, and leads to prolonged survival in several different mouse models of APL. Furthermore, they demonstrate that knockdown of TRIB3 also prevents CNS infiltration of APL cells. More significantly, they show that knockdown of TRIB3 also confers ATRA sensitivity to an ATRA-resistant APL cell line, both *ex vivo* and in mouse xenograft studies.

The authors go on to identify the domains of TWIST1 that are critical for the physical interaction with TRIB3, and design interfering peptides to disrupt this interaction. They demonstrate that treatment with one of these peptides can cause differentiation of

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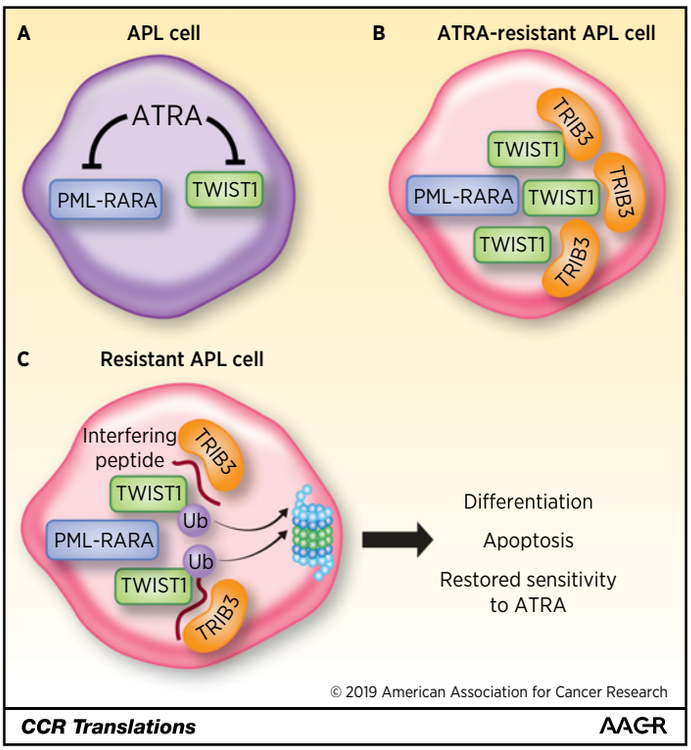

Figure 1.
Interference with the TRIB3–TWIST1 interaction overcomes ATRA resistance in APL. **A,** In APL cells, ATRA treatment leads to degradation of PML-RARα and also decreased expression of TWIST1. **B,** In ATRA-resistant APL cells, TWIST1 protein levels increase due to stabilization by TRIB3, which physically interacts with TWIST1 and prevents its degradation. **C,** Interfering peptides, which disrupt the interaction between TRIB3 and TWIST1, lead to ubiquitination (Ub) and proteasomal degradation of TWIST1, which ultimately leads to restored sensitivity to ATRA, differentiation, and apoptosis.

the resistant APL cell line and of APL patient samples *ex vivo*. Treatment with this peptide can also confer sensitivity to ATRA and prolong survival in mice xenografted with the ATRA-resistant APL cell line. This study uncovers a novel mechanism of APL resistance, and proposes a novel therapeutic approach, which could have translational applications for patients with resistant APL in the future.

This study raises several important questions. First, how does TWIST1 promote APL progression and extramedullary infiltration? This transcription factor is best known for its role in inducing EMT in epithelial cells, thereby promoting transformation and metastasis. The roles of TWIST1 in hematologic malignancies are more enigmatic, but perhaps it could also promote the acquisition of invasive properties to leukemic cells to facilitate infiltration through the blood–brain barrier? If this is the case, then perhaps TWIST1 inhibition could be a useful therapeutic approach for other hematologic malignancies that invade the CNS. Second, is the only role of TRIB3 in APL to stabilize TWIST1? Could the effects of TRIB3 knockdown on myeloid differentiation in APL cells also be partially because of reduced CEBPα expression, or to the interaction with p62?

From a clinical perspective, if peptides that interfere with the TRIB3–TWIST1 interaction could be designed with acceptable pharmacologic properties for use in humans, several potential therapeutic approaches could be envisioned. The authors demonstrate that the interfering peptide can confer ATRA sensitivity to ATRA-resistant APL cells, so this therapeutic modality could be used to treat patients who relapse after initial ATRA treatment. The authors also suggest that this approach could be combined with ATRA or other differentiating therapies upfront for patients who have a high risk for CNS infiltration, such as those with a high white cell count at diagnosis, to prevent early treatment failures. Finally, because the TRIB3–TWIST1 interaction has also been demonstrated for other malignancies, this therapeutic approach could potentially be applied to solid tumors to prevent metastasis.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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