Pulling the Pin on NPMc+ acute myeloid leukemia

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Title: Pulling the Pin on NPMc+ acute myeloid leukemia

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In this issue of Haematologica, Hleihel et al.\textsuperscript{1} identify the propyl isomerase Pin1 as a key target of retinoic acid (RA, also known as all-trans-retinoic acid) in NPMc+ acute myeloid leukemia (AML). AML is an aggressive cancer with few effective treatment options and extremely poor outcomes in the majority of cases. Approximately one third of AML patients carry mutations in the \textit{NPM1} gene that encodes the multifunctional protein nucleophosmin. The mutations, collectively termed NPMc+, cluster at the 3′ end of the \textit{NPM1} open reading frame and introduce a nuclear export signal that causes relocalization of nucleophosmin from the nucleolus to the cytoplasm\textsuperscript{2}. Evidence from clinical trials, had suggested that RA treatment may enhance the efficacy of intensive chemotherapy in a subset of NPMc+ patients\textsuperscript{3}. Excitingly, two concurrent studies in 2015 implicated RA as a degrader of mutant nucleophosmin\textsuperscript{4,5}, but did not elucidate the molecular target of RA responsible for this effect. Herein, Hleihel and colleagues provide novel mechanistic insights using AML cell lines and patient samples. They further demonstrate synergy between RA and chemotherapy or arsenic trioxide (ATO) in NPMc+ AML that is dependent on expression of the protein PML.

RA is a hormone that at physiological concentrations regulates a wide-array of biological processes by activating gene expression via retinoic acid receptor (RAR) transcription factors. Seminal studies in the 1980s, initially \textit{in vitro} and subsequently in clinical trials, identified the potent efficacy of RA against acute promyelocytic leukemia
(APL), a sub-type of AML most often characterized by the oncogenic fusion protein PML-RARα. Although the molecular details remain debated, at pharmacological concentrations RA promotes both the transcriptional activation of PML-RARα target genes and the degradation of the fusion protein itself, driving differentiation of promyelocytic blasts to mature neutrophils⁶. Notably, a 2014 study by Wei and colleagues uncovered that as well as its effects on RAR signaling, RA is also a potent inhibitor of Pin1⁷, a unique enzyme that binds to phosphorylated Ser/Thr-Pro motifs within target proteins and catalyzes their cis/trans conformation thereby altering their stability or activity. Known Pin1 targets include RARα, PML and PML-RARα, CyclinD1 and NF-κB⁸.

In the present study, Hleihel et al.¹ began by expanding on earlier observations that RA treatment of NPMc+ AML cells leads to NPMc+ proteolysis, P53 activation, differentiation and apoptosis⁴⁵. They initially tested whether PML, the essential protein component of PML Nuclear Bodies (PML NBs), is required for RA activity in NPMc+ AML. PML NBs are small nuclear matrix-associated structures that provide a molecular docking station for a wide-array of interacting proteins. Although seemingly dispensable for life (Pml knockout mice are viable), PML NBs are detectable in most cell types, are regulated by cellular stress and are associated with numerous biological processes and disease states⁹. PML NB formation is dysregulated in NPMc+ AML cells compared with NPM1 wild-type cells, and the authors found that PML knockout in the NPMc+ AML cell line OCI-AML3 abrogates its sensitivity to RA. Analyzing the kinetics of the RA response in OCI-AML3 cells and NPMc+ primary AML blasts, they unexpectedly found that P53 activation can be untangled from NPMc+ degradation, with the former evident within two hours of treatment and the latter occurring only after 24-48 hours.

These observations in turn prompted the authors to investigate the role of Pin1 in the RA response. Both RA and a structurally distinct Pin1 inhibitor AG17724 triggered
stabilization of PML and P53 proteins, solely in NPMc+ cells. These effects could be abrogated by shRNA-mediated Pin1 knockdown, though it is perhaps surprising that Pin1 knockdown itself is tolerated in the NPM1 mutant context. Importantly, the team found that OCI-AML3 cells and NPMc+ primary AML blasts have increased expression of Pin1 compared with NPM1 wild-type controls providing a potential explanation for the selective effects of RA on mutant cells. They went on to validate their findings in vivo using an OCI-AML3 xenograft model. As was previously demonstrated in vitro, RA synergized with both ATO and DNA damaging chemotherapy, with therapeutic efficacy and NPMc+ degradation dependent on PML expression. Excitingly, two NPMc+ AML patients treated with ATRA/ATO combination on a compassionate basis, demonstrated a significant albeit incomplete response.

Altogether, the findings reported in this issue by Hleihel et al., as well as earlier work from their group and others demonstrate the potential for expanding the clinical use of RA beyond APL. The data support a model whereby RA induces multiple anti-leukemic effects in NPMc+ AML cells, most of which are initiated by and dependent on re-assembly of PML NBs triggered by inhibition of Pin1. A number of important questions remain, however. The mechanism of NPMc+ degradation, nor the significance of this phenomenon for the therapeutic response beyond OCI-AML3 cells, remains unclear. Likewise, the role for RAR signaling in potentiating (or opposing) the Pin1/PML/P53 axis, or indeed AML differentiation in the NPMc+ context, has not been explored. Unbiased methodologies such as pooled CRISPR screening could identify essential nodes of the various aspects of the RA response such as PML stabilization, NPMc+ proteolysis, differentiation and cell death. Further validation using genetically engineered mouse models of NPMc+ AML as well as patient derived xenografts (PDX) will also be important in building confidence in the
strategy. Collectively, these studies would bring us closer to extending the application of a safe existing drug to an area of unmet need.
References:


