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Hexamethylene bisacetamide as a treatment for T-cell leukemia (T-ALL)

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In this issue, Cecchinato et al. describe the effect of the hybrid polar compound hexamethylene bisacetamide (HMBA) on T-cell acute lymphoblastic leukemia (T-ALL) cell lines [1]. In general, hybrid polar compounds have been used to induce differentiation in many different tissues of all embryonic lineages [2]. HMBA is a prototype compound from this class, and has been shown specifically to differentiate the MEL murine erythroleukemia cell line [2,3], with concomitant downregulation of Notch1 [4].

Notch1 is a transmembrane receptor important in stem cell renewal and T versus B lineage differentiation, which has a unique mechanism of activation, releasing an intracellular subunit upon ligand binding, which translocates into the nucleus to induce the expression of target genes such as Deltx, Hes1 and pTalpha [5]. Mutations in its HD and PEST domains have recently been strongly implicated in the pathogenesis of T-ALL, being found in more than half of these patients [6]. The HD domain mutations are proposed to make Notch1 receptor ligand independent and the PEST domain mutations are thought to increase the half-life of nuclear Notch1 by eliminating the binding site for the E3 ligase FBW7 [7,8].

The authors show that HMBA is able to decrease cell survival significantly in all T-ALL lines tested with varying levels of effectiveness. This decrease appeared to be through apoptosis and a short delay in cell cycle kinetics rather than through differentiation, as in the MEL line. Differentiation was evaluated by FACS analysis of several T-cell markers. The authors found that Notch1, as in MEL line, decreased in all the T-ALL lines to varying extent, but was particularly evident in the Molt4 and SupT1 cell lines. In addition, they find that the Notch1 target pTalpha is downregulated in Molt4.

Next they try to dissect out the mechanism of cell cycle delay and apoptosis by examining the p53, p21 and Bcl-2 pathways. In the Molt4 line, they found a biphasic effect on p53, with early upregulation at 2–4 hours, then a downregulation at later timepoints. The authors propose that HMBA may induce p53 by causing DNA single stranded breaks, which is then followed by a feedback inhibition. This biphasic regulation of p53 was associated with a similar regulation of p21, and is consistent with the observed temporary delay in cell cycle kinetics. They also find an increase in Bax and a decrease in Bcl-2, consistent with the increased level of apoptosis observed in Molt4 cells. Analysis of other cell lines presents an inconsistent picture of these cell cycle and apoptotic regulators.

In addition, the authors evaluate the efficacy of combined γ -secretase inhibitor (GSI), which abrogates the release of Notch1 active form from the membrane, and HMBA treatment to

understand if Notch1 inhibition provoked by GSI could contribute to HMBA-induced apoptosis and increase cell cycle delay. GSI was able to induce apoptosis only in the CEM line and after the combination of the two treatments the effects were additive and not synergetic, suggesting that the apoptosis process induced by HMBA is independent from Notch1 repression.

Although this paper identifies a new class of compound that may be effective in treating T-ALL, many questions remains unanswered. For example, the authors suggest that HMBA may act by causing single stranded DNA breaks; however the authors do not show any evidence to confirm this. The mechanism and role of Notch1 downregulation has not been explored. p53 upregulation may be one possible explanation for HMBA's mechanism of action, but they are lacking direct evidence.

From a clinical standpoint, HMBA was studied in the late 80s and early 90s in phase I and phase II clinical trials for hematological and solid tumors [9,10]. Despite some clinical response, it was found to have significant hematopoietic and neurological toxicity close to its therapeutic level, and, in clinical trials, was abandoned for suberoylanilide hydroxamic acid (SAHA), a second generation hybrid polar compound. SAHA is currently in clinical trials for leukemia, lymphoma, and solid tumors [11,12]. Although both are structurally related and cause differentiation in MEL line, HMBA and SAHA have been found to have different mechanisms of action with SAHA being an inhibitor of histone deacetylase. Due to this, it is difficult to draw conclusions for one based on the other, and a similar study using SAHA would have been more clinically relevant.

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