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Branching NOTCH1 to DNA damage in T-cell acute lymphoblastic leukemia

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In this issue of Haematologica, Tosello *et al.* identify a very prominent role for the branched-chain amino acid transaminase 1 (BCAT1) in T-cell acute lymphoblastic leukemia (T-ALL) downstream of NOTCH1 (1). T-ALL is an aggressive hematological disorder that arises from the transformation of immature T-cell progenitors. Although T-ALL is a heterogeneous disease with mutations reported in numerous oncogenes, tumor suppressor genes, and/or transcription factors (2), activating mutations in NOTCH1 are found in over 65% of patients (2, 3). Thus, NOTCH1 is the most prominent oncogenic driver in T-ALL. Accordingly, anti-NOTCH1 therapies, including gamma-secretase inhibitors (GSIs) and others, were developed to specifically inhibit NOTCH1 signaling in T-ALL, although this approach was mostly unsuccessful in the clinic due to a combination of on-target gut toxicity and lack of clinical efficacy (4). Still, a deeper understanding of the complex transcriptional program controlled by NOTCH1 in T-ALL may reveal novel targets for its treatment. Related to this, the discovery of BCAT1 as a critical target downstream of NOTCH1 in T-ALL may be therapeutically exploited in the future (1).

BCATs are branched-chain aminotransferases with cytosolic (BCAT1) or mitochondrial isoforms (BCAT2) which facilitate the reversible transamination of the branched-chain amino acids (BCAAs) leucine, isoleucine, and valine to their branched-chain α-keto acids (BCKAs), also generating glutamine and alanine along the way (5). Beyond its normal metabolic function (or dysfunction) being involved in metabolic diseases such as diabetes, liver/kidney disease, or inborn errors of BCAA metabolism, numerous studies have also suggested that elevated BCAT1 levels are associated with

tumor aggressiveness in different types of cancer (5). To determine the role of BCAT1 specifically in T-ALL, Tosello *et al.* started by analyzing gene expression data from NOTCH1-induced mouse leukemias and discovered that *Bcat1* was highly upregulated in leukemic CD4+CD8+ double-positive cells compared to normal double-positive thymocytes. Consistently, analysis of publicly available gene expression data sets from human T-ALL patients confirmed *BCAT1* upregulation, which seemed to be more prominent in cortical T-ALL cases and/or cases with NOTCH1 mutations. Furthermore, NOTCH1 inhibition with GSIs led to reduced BCAT1 levels in both mouse leukemias and human T-ALL patient-derived xenografts (PDXs), reinforcing the idea that NOTCH1 positively regulates BCAT1. Indeed, epigenetic profiling and luciferase reporter assays confirmed that NOTCH1 binds to the *BCAT1* promoter, leading to its transactivation. Consequently, germinal loss of *Bcat1* impaired the generation of NOTCH1-induced leukemias, and shRNA-mediated *BCAT1* knockdown in human T-ALL cell lines led to reduced proliferation *in vitro* and reduced tumor burden *in vivo*.

Authors then performed gene expression RNA-seq profiling of *Bcat1*-positive and *Bcat1*-negative mouse leukemias, which revealed that BCAT1 loss results in a downregulation of pathways related to proliferation and cell cycle progression, together with a concomitant upregulation of pathways related to DNA repair and apoptosis, suggesting that BCAT1 might play a previously unrecognized role in the DNA damage response (DDR). Indeed, mouse and human T-ALL cells with loss of BCAT1 showed increased DNA damage, as measured by γH2AX staining and comet assays, together with hyperphosphorylation of DNA-PK, which controls the non-homologous end joining

(NHEJ) DNA repair pathway. On the other hand, metabolomic profiling of *Bcat1*-positive and *Bcat1*-negative T-ALLs revealed numerous changes, including an expected accumulation of leucine in the absence of BCAT1. In turn, this led to an accumulation of 3-hydroxybutyrate (3-HB), which was confirmed using ^{13}C-labeled leucine. Notably, most of these phenotypes were recapitulated with the BCAT1 inhibitor ERG245, which led to a combination of cytostatic and cytotoxic effects and accumulation of 3-HB.

Given the DNA damage effects observed upon BCAT1 loss, authors next hypothesized that the combination of BCAT1 loss/inhibition with the double-strand break (DSB)-inducing agent etoposide, which is used in the clinic to treat T-ALL patients, might enhance its cytotoxicity. Indeed, etoposide treatment synergized with *BCAT1* knockdown or ERG245 treatment in human T-ALL cells *in vitro*, leading to higher DNA damage and cytotoxic effects than with either treatment alone. These effects were further validated in T-ALL PDXs, which again showed increased cytotoxic effects *in vitro* and reduced tumor burden *in vivo* when combining etoposide with ERG245. Interestingly, the authors also demonstrated that the catalytic activity of BCAT1 is required for its effects on DDR, as overexpression of wild-type BCAT1 in otherwise BCAT1-null cells was able to rescue their increased sensitivity to etoposide, whereas overexpression of a catalytically-dead BCAT1 did not. These findings suggested that some of the metabolites accumulating upon BCAT1 loss might be responsible for the increased sensitivity to cytotoxic agents. Related to this, 3-HB is structurally similar to sodium butyrate, a well-known HDAC inhibitor (6) which also sensitized to the cytotoxic effects of etoposide, overall suggesting that the mechanism behind the increased

sensitivity to DSB-inducing agents might be linked in some way to epigenetics. Consistent with this idea, loss of BCAT1 led to increased global levels of H3K27ac, a finding that was recapitulated by direct treatment with 3-HB. In addition, the authors also found that BCAT1 loss translated into increased acetylation of several key players in the DDR, such as p53, Ku70, or Ku80. Still, whether the increased acetylation of these factors or the global changes in histone acetylation are the main mediators of the increased sensitivity to cytotoxic agents was left unaddressed and warrants further investigation.

Overall, these findings suggest that NOTCH1 inhibits the DDR in T-ALL not only directly (7) but also indirectly via activating BCAT1, which should be considered now among the growing list of metabolic enzymes that also have an impact on epigenetics (8). Another key lingering question is why BCAT1 loss leads to increased DNA damage in the first place. While this may be linked to a previously described antioxidant role of BCAT1 by counteracting ROS (9), further studies are needed to test this hypothesis. Finally, the authors took advantage of the TARGET T-ALL dataset (10) to identify the prognostic relevance of BCAT1 expression, as cases with higher levels of BCAT1 showed worse prognosis, highlighting once again the potential therapeutic benefit of inhibiting BCAT1 in T-ALL patients, especially in combination with DNA damaging agents.

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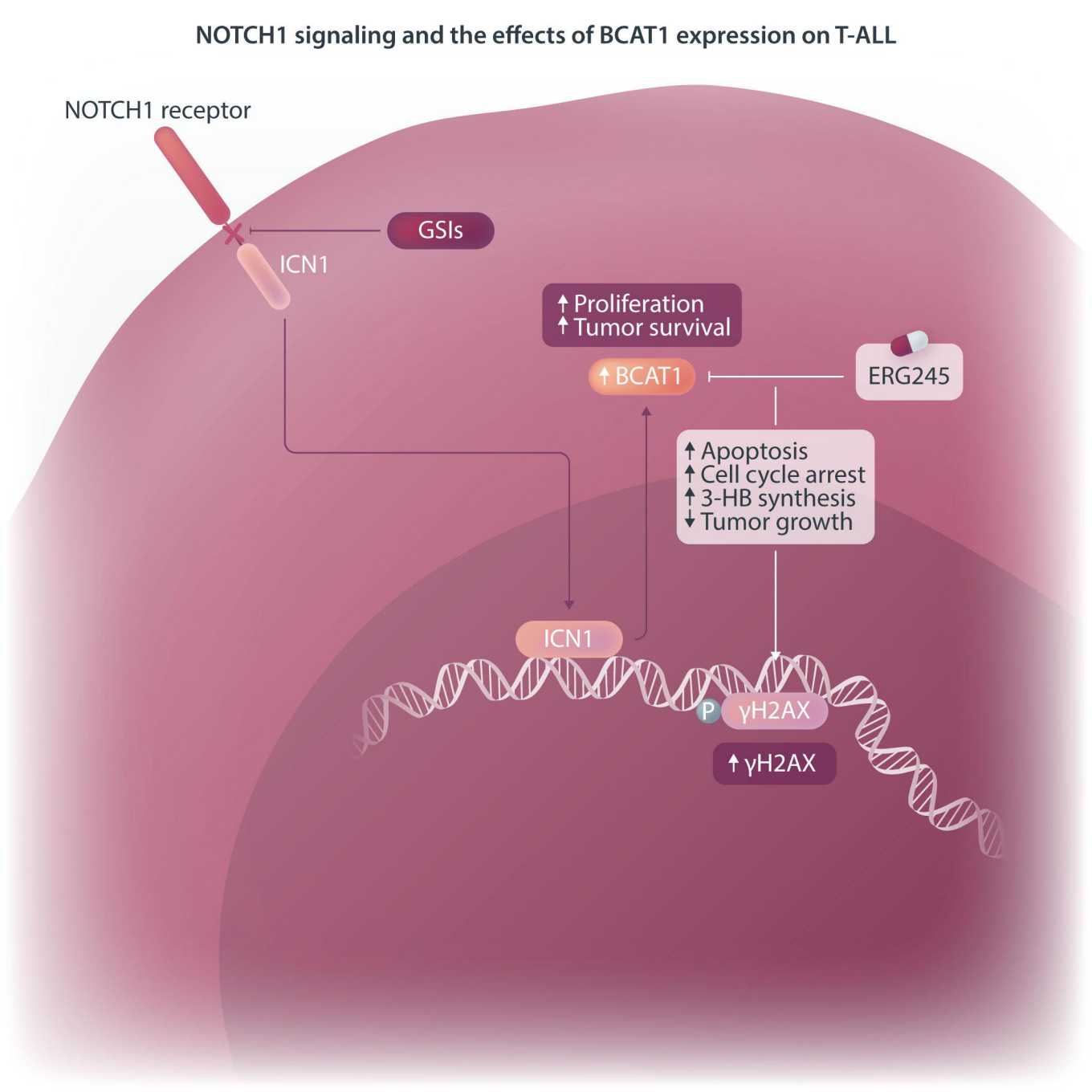
Legend to figure:

BCAT1 effects in T-cell acute lymphoblastic leukemia.


NOTCH1 signaling and the effects of BCAT1 expression on T-ALL
NOTCH1 receptor
GSIs
ICN1
↑ Proliferation
↑ Tumor survival
↑ BCAT1
ERG245
↑ Apoptosis
↑ Cell cycle arrest
↑ 3-HB synthesis
↓ Tumor growth
ICN1
P
γH2AX
↑ γH2AX