IN THE SPOTLIGHT

Activating TCR Signaling to Thwart T-ALL

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Summary: Thymic negative selection is a process that aims to eliminate autoreactive T cells by inducing the apoptosis of thymocytes expressing a T-cell receptor (TCR) with high affinity for self-MHC. In this issue, Trinquand and colleagues demonstrate that TCR engagement or anti-CD3 stimulation of TCR-expressing T acute lymphoblastic leukemia cells results in their apoptosis. This cell death is reminiscent of thymic negative selection and has the potential for therapeutic exploitation. *Cancer Discov; 6(9); 946–8. ©2016 AACR.*

See related article by Trinquand et al., 972 (10).

T-cell malignancies can be divided in two groups: peripheral T-cell lymphomas (PTCL), which arise from postthymic mature T cells, and T-cell acute lymphoblastic leukemias or lymphomas (T-ALL), which originate from thymic immature T cells. These two disease groups differ profoundly in terms of their oncogenesis, clinical presentation, treatment, prognosis, and epidemiology.

With respect to PTCL, multiple molecular alterations have recently been described in genes involved in T-cell receptor (TCR) signaling pathways. Whole genome/exome sequencing has identified mutations in TCR signaling elements in 84% of Sézary syndrome samples (1) and in more than 90% of adult T-cell leukemia/lymphoma samples (2). Similarly, a targeted sequencing study has described mutations in TCR signaling in half of angioimmunoblastic T-cell lymphomas and other T follicular helper–related lymphomas (3). Functional studies have demonstrated the activating character of many of these mutations, suggesting that activation of TCR signaling supports PTCL lymphomagenesis. Such activation could be an important driver of tumor T-cell survival, just as B-cell receptor (BCR) signaling is crucial for cancerous B-cell survival in some B-cell malignancies. Accordingly, disruption of BCR signaling by application of the BTK inhibitor ibrutinib has an antitumor effect (4).

T-cell lymphoblastic leukemias and lymphomas are derived from immature T cells blocked at various stages of thymic development. The 2008 World Health Organization classification scheme differentiates pro-T, pre-T, thymic, and mature T-ALL based on expression by the tumor cells of CD2, CD5, CD7, CD8, CD1a, and surface CD3 (sCD3; ref. 5). Mature T-ALL cells characterized by sCD3 and TCRαβ or γδ expression represent ~20% of T-ALL cases in adults, and up to 50% of T-ALL cases in children (6). The molecular events involved in T-ALL oncogenesis have been deciphered by genome-wide profiling studies, including gene expression and sequencing analyses. The overexpression of certain transcription factors, including TAL1, TLX1, TLX3, LMO1, LMO2, MEF2C, HOXA, and NKX2.1, is a key driver of T-ALL and is involved in the maturation block. This overexpression can result from various mechanisms, including translocations involving TCR genes. NOTCH pathway activation, via *NOTCH1*-activating mutations or inactivation of *FBXW7*, a component of a ubiquitin ligase complex involved in NOTCH degradation, is present in 60% of T-ALL cases. Lastly, mutations affecting key intracellular signaling components, especially RAS, PTEN, or the IL7R–JAK–STAT pathway, can cooperate with other events and precipitate T-ALL (7). Epigenetic and cell-cycle regulators may also be altered in T-ALL cells, but no recurrent mutation in any TCR signaling component has been identified to date in this malignancy, highlighting a critical difference between T-ALL and PTCL. Indeed, the involvement of the TCR pathway in T-ALL oncogenesis was unknown.

The cell-of-origin of T-ALL is the T-cell progenitor, which undergoes a maturation process that wends its way across the thymus. This process is mediated by both cell-intrinsic factors (expression of specific transcription factors) and cell-extrinsic factors (cytokines and cellular interactions with cortical or medullary thymic epithelial cells, thymic dendritic cells, and fibroblasts). The earliest thymic progenitors are double-negative ($CD4^-CD8^-$) cells that rearrange their TCRδ, TCRγ, and TCRβ genes. Candidate TCRβ chains are synthesized that associate with the invariant pre-Tα chain (encoded by *PTCRA*) and appear on the thymocyte surface as a pre-TCR that can be tested for the functionality of the TCRβ chain. Thymocytes with successfully rearranged TCRβ genes expand and start to express both CD4 and CD8, becoming double-positive thymocytes that undergo TCRα gene rearrangements and eventually express functional TCRαβ on the surface. Thymocytes undergo positive and negative selection at this point. Cells expressing TCRs able to bind to self-MHC with at least moderate affinity survive (positive selection), whereas cells expressing TCRs with high affinity for self-peptide MHC undergo apoptosis (negative selection) to avoid autoimmunity (8). The molecular mechanisms that control these processes are only

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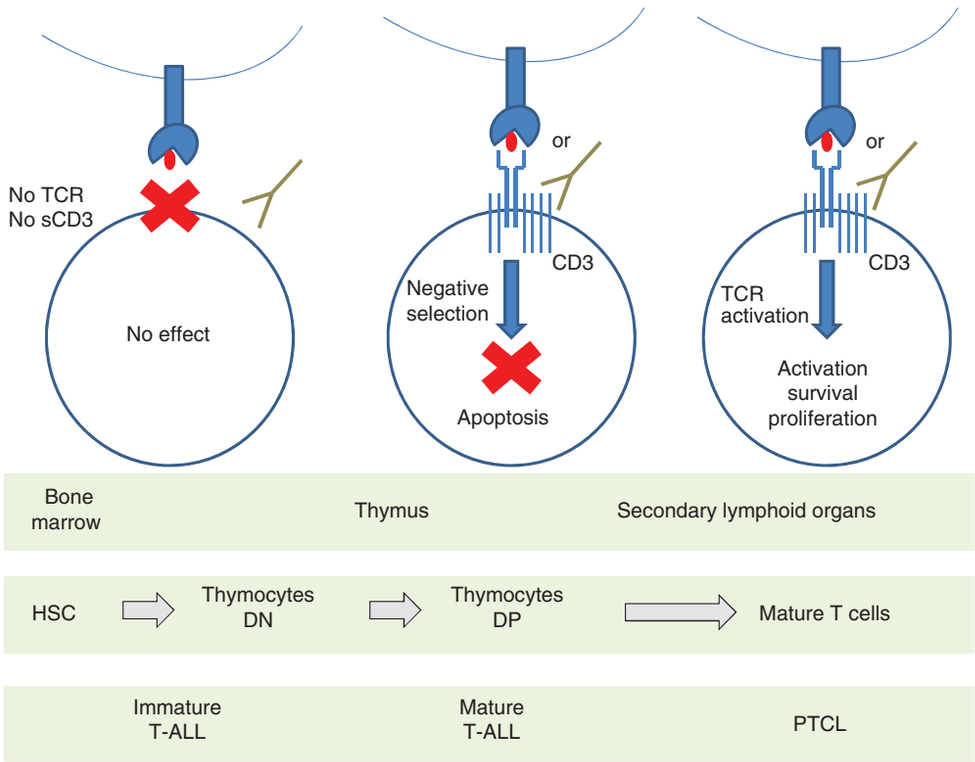

Figure 1. During normal T-cell development, multipotent progenitors emerge from the bone marrow and migrate into the thymus, where T cells mature. These developing progenitors start to express a TCR and undergo positive and negative selection. During negative selection, engagement of a TCR with high affinity for antigen presented on self-MHC results in apoptosis, helping to eliminate autoreactive T cells before they are released to the secondary lymphoid organs. Once T cells are mature, however, TCR engagement induces T-cell activation, survival, and proliferation. The work of Trinquand et al. (10) suggests that this paradigm can be recapitulated in T-cell malignancies, because TCR-positive T-ALL cells that experience TCR engagement or CD3 stimulation by anti-CD3 antibody undergo apoptosis in a manner reminiscent of negative selection. In contrast, PTCL cells often show the presence of an activating mutation in the TCR signaling pathway, indicating that TCR signaling in this context may be oncogenic. These opposing results highlight the importance of cell and disease context when assessing the potential of new anticancer therapies. HSC, hematopoietic stem cell; DN, double negative; DP, double positive.

partly understood, particularly related to how a difference in affinity leads to opposing cell fates: survival versus apoptosis. It is thought that differences in binding strength and kinetics of activation are involved, as well as the cellular localization of TCR signaling components such as ERK (9). Such complexities suggest that manipulation of TCR signaling could have unpredictable effects on T-ALL cells, depending on signaling strength and stage of thymocyte maturation, but this issue had not been extensively studied in human T-ALL prior to the paper by Trinquand and colleagues in this issue (10).

In this article, Trinquand and colleagues (10) demonstrate using genetically modified mice and T-ALL cells that stimulation of TCR signaling either via presentation of an MHC-restricted TCR-specific antigen or via CD3 stimulation with anti-CD3 antibody results in the apoptosis of TCR-positive T-ALL cells through a program mimicking thymic negative selection.

Trinquand and colleagues first transfected a TCR-negative human T-ALL cell line, SIL-ALL, with the Marilyn TCR-HY (Vα1.1Vβ6) that recognizes the male antigen HY (peptide DBY). Coculture of this engineered cell line with syngeneic splenocytes pulsed with DBY peptide induced massive cell death, in contrast to coculture with splenocytes pulsed with OVA peptide or treatment with DBY peptide alone. Importantly, this effect was not observed in the parental cell line, indicating that the observed cell death was related to TCR activation by a peptide/MHC complex. A parallel effect occurred *in vivo* when the same TCR-HY complex was examined using the TEL-JAK2 transgenic T-ALL mouse model. Double transgenic male TEL-JAK2/HY mice failed to develop T-ALL and survived longer than the corresponding females, confirming that TCR engagement by a cognate peptide/MHC complex can have antileukemic consequences.

Trinquand and colleagues then demonstrated that treatment with anti-CD3 monoclonal antibody induced phosphorylation of TCR signaling components followed by apoptosis in both TCR-transfected cell lines and primary human T-ALL samples with various mutational profiles. These data indicated that anti-CD3 antibody treatment can successfully recapitulate TCR engagement in this context. Gene expression profiling revealed that TCR stimulation by anti-CD3 antibody in T-ALL cells induced a transcriptional program featuring a thymic negative selection signature, suggesting that the negative selection program is still functional in these tumor cells and is responsible for the apoptosis induced by anti-CD3 treatment. Finally, Trinquand and colleagues xenotransplanted the above

cell line and primary human T-ALL cells into NSG mice to show *in vivo* that anti-CD3 treatment can impair TCR-positive T-ALL development. Only a marginal effect on CD3-negative T-ALL was observed with this approach.

The results from Trinquand and colleagues are convincing and clearly demonstrate that treatment with anti-CD3 can have an antileukemic effect that is mediated by the activation of a process mimicking thymic-negative selection. Notably, this process still operates in these tumor cells despite the fact that they bear numerous oncogenic alterations. However, although the use of anti-CD3 as T-ALL therapy is an attractive notion, one pitfall might be the high risk of selection of a TCR-negative clone as a mechanism of tumor escape. Indeed, in Trinquand and colleagues' experiments with the TCR-HY/TEL-JAK2 model, female mice, which did not experience TCR stimulation, quickly developed fatal T-ALL. Moreover, although the survival of most male mice was extended, some developed B-ALL, TCR-negative T-ALL, or endogenous TCR-expressing T-ALL. Similarly, leukemic cells recovered from mice transplanted with TCR-positive human T-ALL cells that had been treated with anti-CD3 antibody displayed low or absence of TCR expression. These latter results suggest that the leukemic-initiating cells of T-ALL may be resistant to anti-CD3 treatment, which could limit the efficacy of this therapeutic approach.

Nevertheless, the discovery that mature T-ALL cells can be induced to undergo apoptosis by TCR activation is very exciting. Dissecting the molecular mechanism involved in this process may allow the therapeutic exploitation of this T-ALL Achilles' heel by means other than anti-CD3 antibody. Furthermore, it would be interesting to determine if this proapoptotic program can be activated in immature forms of T-ALL, because adult T-ALL tends to feature mature cells less frequently. Finally, the findings of Trinquand and colleagues highlight the importance of cell context, which can dramatically modify the biological significance of a unique signal. Their data indicate that TCR signaling, which is thought to support oncogenesis in PTCL, can have an antioncogenic effect in T-ALL (Fig. 1). Such considerations must be kept well in mind when contemplating novel anticancer approaches.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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