No T without D3: A critical role for cyclin D3 in normal and malignant precursor T cells

A definitive knockout reported in this issue of *Cancer Cell* by Sicinska et al. reveals an unsuspected role for cyclin D3 in normal T cell development and suggests new therapeutic possibilities in precursor T cell leukemia.

Regulation of cell cycle progression occurs at several checkpoints, and genetic alterations that affect the expression or function of various regulator proteins have been implicated in virtually every type of cancer cell, which characteristically exhibit growth rates higher than those of their normal cellular counterparts. The G_1/S checkpoint is of particularly broad importance in cancer, as it determines whether or not a cell will commit to a round of cell division.

Regulation of the G_1/S checkpoint by D cyclins, cyclin-dependent kinases (CDKs), CDK inhibitors (CKIs), and the retinoblastoma tumor suppressor protein pRB has been discussed in detail recently in the pages of *Cancer Cell* (Evans et al., 2003) and will be reviewed here only briefly. pRB plays a pivotal role by sequestering the transcription factor E2F, whose transcriptional targets include genes needed for S phase entry. Active CDKs 4 and 6 relieve this block by phosphorylating pRB, which disrupts its association with E2F. CDK4/6 activity is dependent upon interaction with D cyclins, which are normally regulated during the cell cycle at multiple levels, including transcription, protein stability, posttranslational modifications, and subcellular localization. D cyclins also promote S phase entry by sequestering $p21^{CIP1}$ and $p27^{KIP1}$, which are negative regulators of CDK2.

Mammals have three D cyclins (cyclins D1–D3), polypeptides of ~34 kDa that are thought to be functionally interchangeable within a given cell (Ciemerych et al., 2002); however, not all cells express multiple D cyclins. In this issue of *Cancer Cell*, Sicinski and coworkers (Sicinska et al., 2003) report that cyclin $D3^{-/-}$ mice suffer from an isolated defect in thymocyte development characterized by a marked deficit in $CD4^+CD8^+$ "double-positive" (DP) T cells. This phenotype stems from a nonredundant role for cyclin D3 in the maturation of $CD4^-CD8^-$ "double-negative" (DN) T cells, the precursors of DP T cells. In the mouse, developing DN T cells successively pass through four stages (DN-1–DN-4) defined by differential expression of CD44 and CD25 (Figure 1). Cyclin $D3^{-/-}$ thymocytes fail to undergo the proliferative burst that normally accompanies the DN-3 to DN-4 transition. Pro-proliferative signals depend on the pre-T cell receptor complex (pre-TCR), which contains TCRβ, a "surrogate" α chain called pre-Tα, CD3ε, γ, and ζ, and the tyrosine kinase $p56^{LCK}$. As would be predicted from the cyclin $D3^{-/-}$ phenotype, normal DN-3 to DN-4 transition coincides with a dramatic upregulation of cyclin D3, and this upregulation fails to occur in $p56^{LCK-/-}$ mice, placing cyclin D3 downstream of pre-TCR signals.

These findings add cyclin D3 to a cohort of proteins that appear to participate in a coordinated set of signaling events required for the DN proliferative burst (Figure 1). An initiating event appears to be engagement of Notch1 by yet-to-be clearly defined ligand(s), which produces signals that upregulate pre-Tα (Reizis and Leder, 2002), setting the stage for assembly of the pre-TCR signaling complex. Pre-Tα signals mediate multiple events during β-selection, which include in addition to proliferation, TCRβ allelic exclusion, induction of differentiation, and rescue from apoptosis. Sicinska et al. suggest signals produced by the pre-TCR complex upregulate cyclin D3, which provides the essential proliferative drive needed for expansion of the DN-4 pool, the immediate precursors of DP thymocytes. While this picture has been mostly delineated from work in the mouse, it is believed that similar signals and mechanisms apply to human T cell development.

Given the role for cyclin D3 in normal thymocyte proliferation, Sicinska et al. asked if cyclin D3 might be specifically required for the growth of at least some forms of precursor-T cell acute lymphoblastic leukemia/lymphoma (T-ALL) tumors derived from immature T cells. The authors first pursued this by studying the effect of cyclin D3 deficiency on murine T-ALL models initiated variously by transgenes encoding constitutively active $p56^{LCK}$, retroviruses expressing activated forms of Notch1, or p53 deficiency. All of these models produce

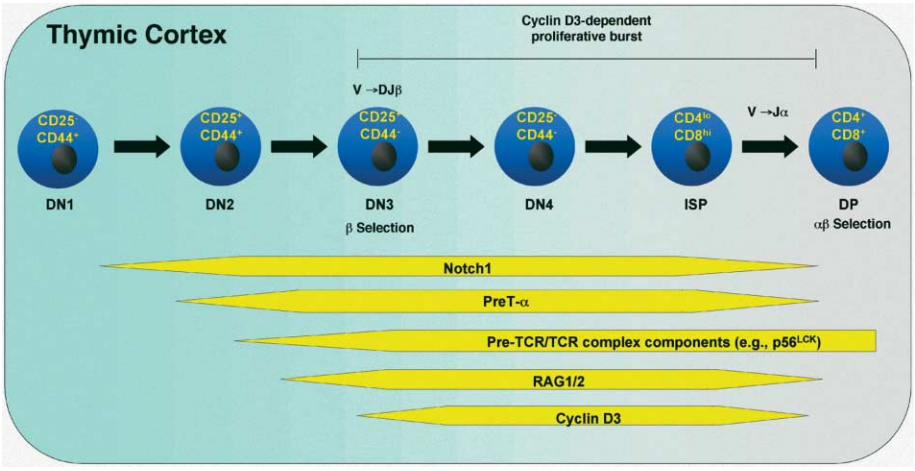

Figure 1. Expression patterns of proteins required for the proliferative burst during $CD4^-CD8^-$ "double-negative" pre-T cell maturation

Activation of Notch1 signaling in early thymic T cell progenitors initiates a coordinated series of events that lead to the assembly of the pre-TCR signaling complex and upregulation of cyclin D3. The resultant proliferative burst is eventually terminated by expression of αβ TCR complexes.

mainly $CD4^+/CD8^+$ T-ALLs, but only those tumors caused by Notch1 and $p56^{LCK}$ signals require pre-TCR function for their development. Consistent with the pathway outlined in Figure 1, cyclin D3 deficiency prevented Notch1 leukemias and delayed the onset of tumors caused by $p56^{LCK}$. In contrast, the development of T-ALL in the $p53^{-/-}$ background was unaffected by cyclin D3 deficiency. The authors' analysis was then extended to primary human T-ALLs by nearest neighbor analysis, which revealed a strong positive correlation between cyclin D3 and $p56^{LCK}$ mRNA expression. Finally, the authors showed that RNAi-mediated knockdown of cyclin D3 causes a G_0/G_1 growth arrest in a number of established human T-ALL cell lines, including one (SUP-T1) known to require Notch1 signals for growth and survival.

These studies support cyclin D3 as a rational target in certain forms of T-ALL, adding it to a list of neoplasms in which specific D cyclins are sometimes solely responsible for the G_1 cell cycle drive, such as cyclin D1 in breast carcinoma (Yu et al., 2001). Other tumor types (e.g., mantle cell lymphoma), which almost always have dysregulation of particular D cyclins, could also prove sensitive to specific inhibitors. Because most cells express multiple D cyclins, inhibitors of individual D cyclins could, in principle, disrupt the growth of tumors expressing single D cyclins while causing less toxicity than general cell cycle inhibitors. But can such inhibitors be developed? Short of a dramatic breakthrough in RNAi delivery, this approach needs to rely on small molecule inhibitors of specific cyclin D-CDK4/6 interactions. While D cyclins show some sequence divergence (50%–60% of amino acid residues are identical across the family), it is likely the key contacts with CDK4/6 are highly conserved, which might make this approach untenable. A high-resolution structure for cyclin D-CDK4/6 complexes (currently unknown) is needed to clarify this issue.

A strategy more likely to be fruitful is targeting of pathway(s) that promote D cyclin activity in T-ALL, which characteristically have a high growth fraction. In this regard, it is worth asking whether the same signals that drive proliferation during the DN to DP transition contribute to the growth of human T-ALL cells. Human T-ALLs are genetically and phenotypically heterogeneous, but a sizable fraction ($\sim$60%) express pre-Tα and TCRβ (Asnafi et al., 2003). Multiple pre-TCR components (pre-Tα [Bellavia et al., 2002], TCRβ, and the adaptor molecule SLP76 [Allman et al., 2001]) are essential for Notch-mediated leukemogenesis in the mouse, and some T-ALLs require continued Notch signaling for growth (Weng et al., 2003); together with the cyclin D3 knockdown data of Sicinska et al., these findings suggest the signals required for the normal proliferative burst may interact in analogous ways to promote tumor growth. However, many issues have yet to be explored. It will be of interest to see if enforced expression of cyclin D3 is sufficient to cause murine T-ALL, even in Notch/pre-TCR-deficient backgrounds. Of broader significance, the signals that operate upstream of D cyclins in established T-ALLs are unknown; Notch- and pre-TCR-mediated signals appear to be excellent candidates for such a role, but direct evidence is lacking at this point. Further, human T-ALLs often harbor deletions, mutations, and/or epigenetic aberrations that result in defective CKI expression (Batova et al., 1997), indicating that problems with braking mechanisms controlling G_1/S progression may be as important as pro-proliferative signals. More detailed knowledge of the events that promote cyclin D activity in various T-ALL subtypes will be needed if rational antiproliferative therapies are to be developed successfully.

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Selected reading

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