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Chronic Lymphocytic Leukemia: Keeping Cell Death at Bay

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Chronic lymphocytic leukemia (CLL) is a common adult leukemia caused by abnormal accumulation of B cells. Raval et al. (2007) now implicate dowregulation of the expression of the kinase DAPK1 both genetically and epigenetically in familial and sporadic CLL.

Chronic lymphocytic leukemia (CLL) was thought to be a homogeneous disease of immature immune-incompetent B cells with minimal proliferative capacity, which accumulate because they are unable to undergo apoptosis. With increasing knowledge of the genetic lesions and signaling pathways involved, CLL is now considered a more heterogeneous leukemia that originates from antigen-stimulated B cells that escape normal cell death mechanisms and/or undergo increased proliferation (Chiorazzi et al., 2005) due to aberrant activity of ZAP70, a Src family protein tyrosine kinase normally only expressed in T cells. Interestingly, CLL is a familial disease in a relatively high proportion (10%–20%) of patients indicating an underlying genetic susceptibility for developing malignant lymphoproliferation. However, genome-wide screening of families with CLL has not identified clear candidate genes (Sellick et al., 2006).

Epigenetic regulation of gene expression—which is crucial for both normal and malignant development of cells—has been implicated in CLL (Calin et al., 2005). Epigenetics affects all aspects of tumor cell biology including cell growth, cell cycle control, differentiation, DNA repair, apoptosis, and cell death. Epigenetic regulation by, for example, promoter methylation, microRNAs, or histone modifications not only influences expression of individual genes but also may affect whole cellular programs such as hematopoietic differentiation (Chen et al., 2004). Consequently, epigenetic modulation of gene expression has become a target for therapeutic intervention (Bhalla, 2005).

In this issue of *Cell*, a consortium led by Christoph Plass and Albert de la Chapelle presents results from their search to find genes involved in aberrant cellular proliferation, differentiation, and apoptosis in CLL (Raval et al., 2007). They identified DAP kinase 1 (DAPK1) as a candidate gene silenced in all cases of CLL. Epigenetic silencing of DAPK through promoter methylation occurred in almost all sporadic cases of CLL. Furthermore, in a large family with several affected individuals, a mutation/polymorphism in the promoter of DAPK1 was identified as causing persistent downregulation of DAPK1 expression due to increased binding of the HOXB7 transcriptional repressor. This study identifies a single gene as being affected by genetic and epigenetic alterations in each case of CLL analyzed and provides evidence for the genetic susceptibility of this disease.

Several functions for DAPK1 in differentiation and cell death have been described (Bialik and Kimchi, 2006). Initially, DAPK was cloned as a serine/threonine kinase associated with the cytoskeleton and identified as a mediator of cell death induced by interferon γ. DAPK1 is

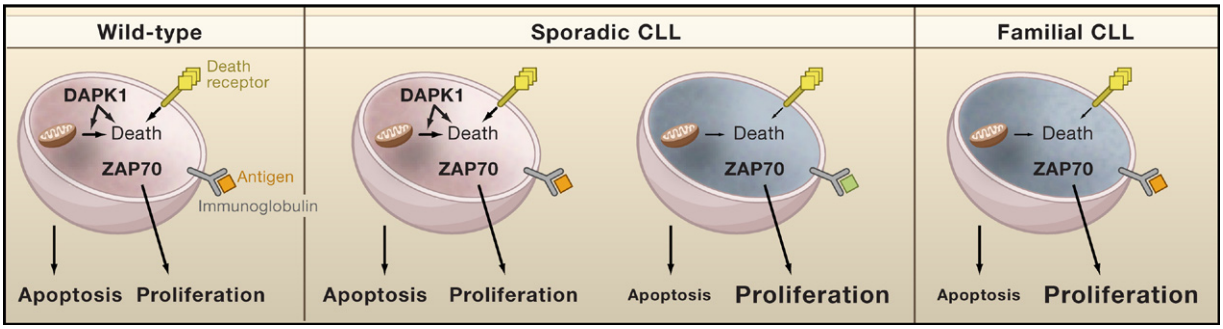

Figure 1. DAPK1 in Homeostasis of Normal and CLL B Cells

Homeostasis in B cells that are triggered by the interaction between immunoglobulin (Ig) receptor and antigen is maintained by a delicate balance between proliferation and apoptosis. The serine/threonine kinase DAPK1 increases and/or synergizes with cell death signaling to ensure the balance between proliferation and cell death in individual B cell clones in a wild-type situation. Although aberrant expression of ZAP70 (observed in CLL) may drive increased proliferation, this event is opposed by intact death receptor and mitochondria-dependent apoptosis pathways. In sporadic cases of CLL, the B cell population is heterogeneous with normal B cells (yellow) as well as a few (clonal, premalignant?) B cells in which DAPK1 is epigenetically silenced. When these B cells are activated by antigen, proliferation is not effectively offset by apoptosis and increased expansion of the clone ensues. In the case of familial CLL, the intrinsic apoptosis defect mediated by downregulated DAPK1 expression—caused by increased binding of the HoxB7 transcriptional repressor to a mutated *DAPK1* promoter—is already present in the germline. Thus, B cells are more susceptible to clonal expansion and secondary hits associated with malignant transformation.

also involved in several pathways mediating apoptosis, although its precise function in the extrinsic (death receptor-dependent) or intrinsic (mitochondria-dependent) pathways remains unclear. Also, no direct physical interactions between DAPK1 and adaptor proteins (such as FADD and APAF1), inititator caspases, or effector caspases have been shown. Other functions of DAPK1 include suppression of invasion and metastasis, which may be related to its association with the cell cytoskeleton (Inbal et al., 1997). Thus DAPK1 may act as an initiator of signaling pathways that suppress a malignant phenotype and facilitate triggering of programmed cell death.

How does one incorporate the results from Raval et al. into pathogenesis and treatment of CLL? Resistance to treatment is a hallmark of CLL, and resistance to chemotherapy has been attributed to deregulated expression of apoptosis regulators. Increased expression of the apoptotic inhibitor Bcl2 found in CLL may be due to lack of expression of microRNAs suppressing Bcl2 expression (Calin et al., 2005). CLL cells were also found to be resistant to induction of apoptosis by the apoptosis-inducing ligand TRAIL, considered of high therapeutic value in many tumors. Epigenetic modification of gene expression by HDAC inhibitors is able to revert apoptosis resistance, and HDAC inhibitors have already been introduced into clinical studies in CLL (Inoue et al., 2006). The observation that a proapoptotic cell death modifier such as DAPK is epigenetically silenced in CLL and may be responsible for the deathless phenotype, and resistance to apoptosis provides a new twist. The fact that DAPK1 expression is downregulated in a variety of different tumors supports the general concept that evasion from death programs is a general hallmark of cancer cells (Calmon et al., 2007; Holleman et al., 2006).

However, we are left with two questions. First, where do we place DAPK in the process of malignant transformation of precursors en route to overt "benign" or "malignant" CLL? Most likely, silencing of DAPK1 occurs at an early stage of the malignant process, because CLL cells have to escape cell death control and apoptosis during the sequential steps of acquisition of the malignant potential (Figure 1). Thus, DAPK1 deficiency—either through a genetic mutation or aberrant epigenetic regulation—is probably a prerequisite for further aberrant regulation of cellular programs. The fact that epigenetic regulation, for example by altered promoter methylation, are inherited like any other gene function, also provides a new perspective on genetic susceptibility to tumor formation with the potential of a deeper understanding of cancer susceptibility genes. The second question that remains is whether or not restoration of DAPK1 function will reinstate the cell death sensitivity of CLL cells irrespective of the "benign" and the aggressive subtype. The answer to this question remains to be determined experimentally and may prove to be effective in treating this disease.

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Stopping Ras in Its Tracks

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Ras interacts with many downstream effectors that regulate complex cytoplasmic signaling networks. In this issue, Gupta et al. (2007) use mouse models of Ras-mediated tumorigenesis to show that the interaction of Ras with a single isoform of phosphatidylinositol 3-kinase (PI3K), called p110α (PIK3CA), is critical for tumor formation. This result will stimulate re-evaluation of pharmacological approaches to target Ras for cancer treatment.

At least 11 distinct functional classes of proteins—including the Raf serine/threonine kinases and the p110 catalytic subunits of class I PI3Ks—have been implicated as effectors of the small GTPase Ras (Repasky et al., 2004). Because of the frequent mutational activation of Ras in cancer and the vast number of its effectors (Figure 1), there has been much debate regarding the most effective strategies to develop anti-Ras inhibitors. In this issue, Gupta et al. (2007) further validate the importance of p110α and the PI3K-Akt signaling pathway in Ras-induced tumorigenesis.

The majority of studies analyzing a role for effectors in Ras-mediated oncogenesis have been performed in cultured cells. For example, experiments in immortalized human epithelial cells ectopically expressing oncogenic Ras have shown that concurrent activities of Raf, PI3K, and Ral-GEF (guanine nucleotide exchange factor) effector pathways are required for Ras-mediated initiation of tumor growth. The PI3K pathway is also crucial for tumor maintenance (Lim and Counter, 2005). One recent study used RNA interference to demonstrate the necessity of the Ral-GEF-Ral pathway in tumorigenesis, invasion, and metastasis of *KRAS* mutation positive pancreatic carcinoma cell lines (Lim et al., 2005).

Genetically engineered mouse models have also validated the importance of Ras effectors (Repasky et al., 2004). Mice deficient for the effectors Tiam1, PLCε, or Ral-GDS showed impaired tumor initiation when tested in skin tumorigenesis—induced by the carcinogen dimethybenzanthracine (DMBA)—in which H-Ras activation is causal and frequent. Genetic ablation of other Ras effectors, in particular of p110α and p110β, results in defective development, thus complicating analyses of their contributions to Ras-mediated cancer development. Furthermore, deficiency studies can only assess the requirement of a given factor for tumorigenesis and not the specific interactions involved. Thus, Gupta et al. (2007) engineered mice in which the endogenous *PIK3CA* gene encodes a mutant p110α protein that is enzymatically active, but cannot interact with Ras, to evaluate the role of this effector in endogenous normal and mutant Ras signaling.

To selectively ablate p110α interactions with Ras, Gupta et al. (2007) introduced missense mutations into the germline sequence specifying the Ras-binding domain of p110α. Viable mice homozygous for this *PIK3CA* mutant allele were obtained, indicating that this partial loss-of-function mutant of p110α was sufficient to support development. They then analyzed these *PIK3CA* mutants in two well-characterized Ras-driven tumor models. In the K-Ras LA2 mouse model (Johnson et al., 2001), tumorigenesis is driven by endogenous levels of mutant K-Ras (G12D) expression, and these mice develop lung adenocarcinomas at a high frequency. When the K-Ras LA2 mice were crossed with mice homozygous for mutant *PIK3CA*, a striking 95% reduction in lung tumor forma-