Anticancer Therapy SMRT-ens Up: Targeting the BCL6-SMRT Interaction in B Cell Lymphoma

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Transcription factors have proven to be difficult targets for the development of small-molecule drugs. In this issue of *Cancer Cell*, Cerchietti et al. identify and characterize a specific, small-molecule inhibitor of BCL6, an oncogenic transcriptional repressor, that has high clinical promise for treating diffuse large B cell lymphoma.

Personalized cancer medicine offers the hope that by identifying cancer-causing mutations in critical regulatory genes, we can target these mutant proteins to cure cancer while limiting the side effects. A major roadblock to this has been our inability to find specific, bioavailable small-molecule inhibitors of nonenzymatic proteins, especially transcription factors (Arkin and Wells, 2004). Enzymes have been amenable therapeutic targets because active sites have a structural topology conducive to specific inhibition by small molecules (Arkin and Whitty, 2009) and as a result many currently available drugs target enzymes. Although these drugs have proven effective, little progress has been made toward identifying inhibitors of oncogenic transcription factors. In this issue of *Cancer Cell*, Cerchietti et al. provide a road map to circumvent this roadblock for the most common form of diffuse large B cell lymphoma (DLBCL) by identifying a specific small-molecule inhibitor of BCL6, an oncogenic transcriptional repressor that is responsible for the majority (40%–70%) of this malignancy (Cerchietti et al., 2010).

During the rapid expansion of activated B cells in germinal centers of lymphoid tissues, immunoglobulin gene loci undergo recombinations and somatic hypermutations in order to generate diversity in antibodies against various foreign antigens (Jardin et al., 2007; Lossos, 2005; Parekh et al., 2008). Germinal center B cells rapidly proliferate despite a state of physiologic genomic instability because BCL6 represses a cadre of genes involved in regulation of the DNA damage response

and cell cycle checkpoints, such as *ATR*, *CHK1*, *TP53*, and *CDKN1A* (Figure 1A). After clonal diversity has been accomplished, BCL6 is downregulated at the mRNA and protein levels. Suppression of BCL6 allows engagement of cell cycle checkpoints and further B cell differentiation and maturation. Oncogenic overexpression of BCL6, whether via chromosomal translocation, promoter mutation, or gene amplification, permits continued B progenitor cell proliferation and acquisition of additional mutations. Not surprisingly, this leads to the formation of an aggressive B cell lymphoma.

Cerchietti et al. used structural analysis of BCL6 and indentified a pocket within the BTB repression domain that is required for recruitment of the SMRT corepressor that links BCL6 to histone deacetylases to repress transcription. Computer modeling allowed the identification of a set of 1000 small molecules predicted to bind the target pocket. These compounds were organized by structural similarity into 100 groups and one to two compounds were selected for further study from each group based on favorable drug properties. Of the nearly 200 compounds selected, 100 were commercially available, circumventing the need for chemical synthesis, which is costly and time consuming. These 100 compounds were then screened for their ability to block BCL6-mediated transcriptional repression. From successive rounds of screening a lead compound, "79-6," with favorable chemical composition emerged that reproducibly inhibited BCL6. Compound 79-6 bound the target pocket of the BTB domain of BCL6 and

prevented the recruitment of corepressor complexes to the *ATR* locus without affecting BCL6 binding to the DNA. 79-6 appears to selectively inhibit BCL6 because it did not affect transcription repression caused by several other BTB-containing proteins. The addition of 79-6 to DLBCL cell lines reactivated BCL6-regulated genes only in DLBCLs expressing BCL6, but had no effect on DLBCL lines that did not express BCL-6. This effect translated into 79-6 specifically inducing apoptosis in BCL6-dependent DLBCLs transplanted into SCID mice, but not in BCL6-independent tumors. As hoped for a targeted therapeutic drug, only minor toxicities (mild leucopenia) were identified in mice administered many rounds of drug.

This work represents one of the few true examples of "transcriptional therapy." Although histone deacetylase inhibitors and DNA methyltransferase inhibitors have traditionally been thought of as acting through altering transcription, it has been difficult to pin down a commonly regulated target (or even a pathway) that would explain the ability of these drugs to affect a wide variety of tumor types. Therefore, it is possible that these epigenetic regulators, perhaps through chromatin effects, cause problems with DNA replication or repair, that kills rapidly cycling tumor cells (Stimson et al., 2009). By contrast, 79-6 specifically targets the recruitment of corepressors and HDACs to allow the expression of key regulatory factors that engage checkpoints and kill DLBCLs. This type of targeted transcriptional inhibition, via structures to fit small molecules
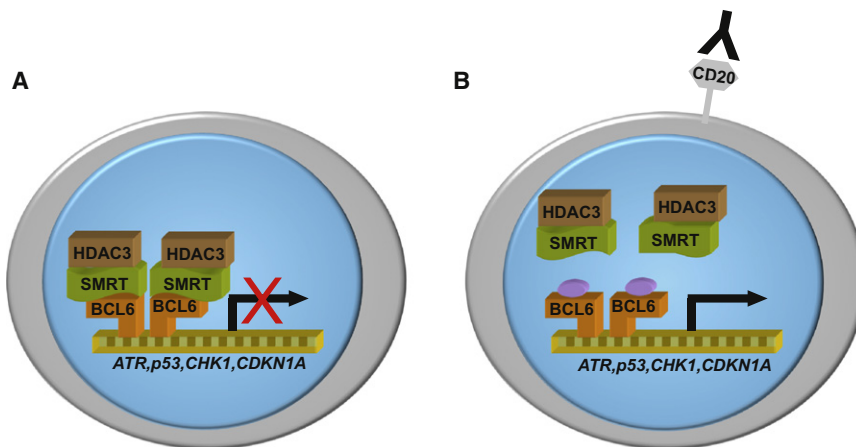
A

B

Figure 1. Schematic Model of How the BCL6 Inhibitor 79-6 Blocks Transcriptional Repression by BCL6
(A) BCL6 binds to its target loci (yellow line) and recruits SMRT and histone deacetylase 3 (HDAC3) that mediate transcriptional repression. As a result, the expression of key regulators of the DNA damage response and checkpoint activation, such as *ATR*, *CHK1*, *TP53*, and *CDK1NA*, is repressed. This allows for rapid B cell proliferation in germinal centers during a state of physiologic genomic instability necessary for recombination and somatic hypermutation of immunoglobulin loci. Oncogenic overexpression of BCL6 permits continued B cell proliferation and accumulation of DNA damage leading to formation of DLBCL. (B) A small-molecule inhibitor of BCL6 identified by Chercietti et al. (depicted by purple ovals) blocks recruitment of corepressor complexes to BCL6 target genes. The resulting expression of DNA damage response proteins and checkpoint regulators promotes cell cycle arrest and apoptosis in DLBCLs. This approach may be especially useful in conjunction with rituximab, an anti-CD20 monoclonal antibody.

into critical motifs, is a model for how other high-value cancer targets might be attacked, even old standards such as c-Myc that have failed high-throughput screening methods.

One of the remarkable characteristics of 79-6 is its specificity for BCL6 over highly related BTB domain-containing factors. By comparison, tyrosine kinase inhibitors that have had great success in chronic myelogenous leukemia (CML) and efficacy in solid tumors (e.g., lung, breast) are ATP analogs that affect multiple kinases. Given that kinase signaling cascades ultimately affect transcription factors that control many genes, kinase inhibitors that are commonly thought to be targeted therapeutics probably have far more wide-ranging effects than would a compound such as 79-6.

Chemotherapy and radiation are the mainstay therapies for many types of cancer, including DLBCLs. The regimen

for DLBCL includes cyclophosphomide, doxorubicin, vincristine, and prednisone with the recent addition of rituximab (R-CHOP therapy) (Kahl, 2008). Alkylating agents and topoisomerase inhibitors, such as cyclophosphamide and doxorubicin, respectively, induce DNA damage whereas vincristine inhibits microtubule functions essential for mitosis. These drugs all target rapidly cycling cells and thus have numerous toxic side affects. Rituximab is a monoclonal antibody against CD20, a B cell-selective antigen, which elicits complement-mediated B cell destruction (Figure 1B). Given that BCL6 recruits corepression complexes containing HDAC3, HDAC inhibitors have been applied to DLBCL in clinical trials. However, these agents have been largely disappointing as single-agent therapies in phase II trials. This may be due to the nonselective action of the agents used to date that inhibit multiple HDACs, and

more selective inhibitors (e.g., HDAC3 inhibitors; Figure 1A) may be more useful. However, as our ability to identify BCL6-overexpressing lymphomas is refined, the personalized approach of using a very selective BCL6 inhibitor in combination with a second, highly selective agent such as rituximab may be very effective with only limited side effects (Figure 1B).

We have gained significant insight into the genes, molecules, and pathways that cause or contribute to the pathogenesis of many diseases. Although this has led to more informed selection of therapeutic targets for drug development, the identification of therapeutic compounds with biologic specificity, adequate bioavailability, and favorable pharmacokinetics remains an enormous challenge. Cherchietti et al. have provided an eloquent example of investigators collaborating to meet this challenge. As more cancer genomes are sequenced and the price of a human genome approaches affordability, additional inhibitors, similar to 79-6, will be needed to attack the tremendous genetic diversity that underlies tumor development and win the war against cancer.

REFERENCES

Arkin, M.R., and Wells, J.A. (2004). Nat. Rev. Drug Discov. *3*, 301–317.

Arkin, M.R., and Whitty, A. (2009). Curr. Opin. Chem. Biol. *13*, 284–290.

Cerchietti, L.C., Ghetu, A.F., Zhu, X., Da Silva, G.F., Zhong, S., Matthews, M., Bunting, K.L., Polo, J.M., Farès, C., Arrowsmith, C.H., et al. (2010). Cancer Cell *17*, this issue, 400–411.

Jardin, F., Ruminy, P., Bastard, C., and Tilly, H. (2007). Pathol. Biol. (Paris) *55*, 73–83.

Kahl, B. (2008). Semin. Hematol. *45*, 90–94.

Lossos, I.S. (2005). J. Clin. Oncol. *23*, 6351–6357.

Parekh, S., Prive, G., and Melnick, A. (2008). Leuk. Lymphoma *49*, 874–882.

Stimson, L., Wood, V., Khan, O., Fotheringham, S., and La Thangue, N.B. (2009). Ann. Oncol. *20*, 1293–1302.