

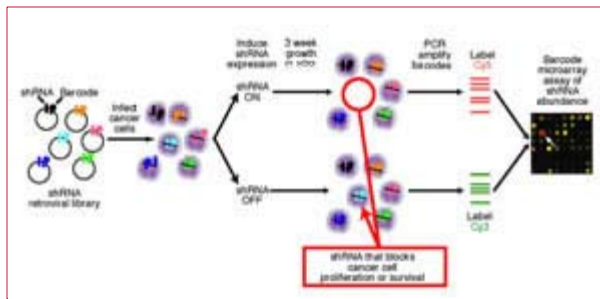
## ■ MOLECULAR BIOLOGY

**Achilles-Heel Genetic Screens for Cancer Targets**

Ngo VN, Davis RE, Lamy L, Yu X, Zhao H, Lenz G, Lam LT, Dave S, Yang L, Powell J, and Staudt LM. A loss-of-function RNA interference screen for molecular targets in cancer. *Nature* 441: 106–10, 2006.

Cancer is the consequence of genetic damage to a susceptible cell, which often deregulates signaling pathways and causes unchecked proliferation and survival. In many cases, the cancer cell becomes “addicted” to the deregulated pathway so that interference with it abrogates the transformed phenotype. Successful therapeutic targeting of a specific genetic abnormality in cancer is exemplified by Gleevec, a kinase inhibitor that is used in the treatment of chronic myelogenous leukemia. Finding molecular components of essential signaling pathways in cancer cells is therefore a rational algorithm for the development of effective cancer therapies. Recent understanding of RNA interference (RNAi)—a sequence-specific, posttranscriptional gene inactivation process—has quickly transformed this conserved cellular mechanism into a powerful laboratory tool to probe gene function. Genetic screens using RNAi are feasible because of its exquisite specificity and the relative ease with which it can be applied on a large scale. Here we describe an inducible RNAi genetic screen that can reveal genes essential for cancer cell proliferation or survival and identify molecular targets in cancer.

We constructed a retroviral vector that enabled the doxycycline-inducible expression of short-hairpin RNAs (shRNAs), which can mediate RNAi. We then created a library of shRNA vectors targeting 2,500 human genes. Each vector contained a unique 60-base-pair “barcode,” enabling the abundance of each shRNA vector to be monitored in a population of transduced cells using DNA microarray technology. Retroviral pools from this library were used to infect cell lines representing two distinct molecular subgroups of diffuse large B-cell lymphoma (DLBCL), named activated B cell–like (ABC) DLBCL and germinal-center B cell–like (GCB) DLBCL. Infected cells were divided into two groups that were either induced to express shRNA or left untreated. After allowing the induced cells to grow for 3 weeks, genomic DNA was harvested and the barcode sequences were amplified. Fluorescently labeled barcodes from uninduced and induced groups were co-hybridized to a DNA microarray containing complementary barcode sequences. The microarray fluorescent signals indicated relative abundance—depletion or enrichment—of individual shRNA vectors within the induced and uninduced populations, reflecting the effect of each shRNA on the proliferation or survival of cancer cells (Figure 1).



**Figure 1.** Inducible, barcode short-hairpin RNA (shRNA) library screen strategy for genes controlling cancer cell proliferation and survival. Infection of an shRNA retroviral library into a cancer cell line produces a “cellular library” with each cell carrying one or more shRNAs. Each shRNA is tagged by a known, unique barcode. Infected cells are divided into two subpopulations, one induced for expression of shRNAs and the other serving as the control. The inducibility of the shRNA library is important to prevent the loss of shRNA species that are acutely deleterious to infected cells. A time-dependent selective pressure is applied and genomic DNA fragments carrying the barcode representing each shRNA from each subpopulation are amplified by PCR, labeled with different fluorescent dyes, and cohybridized to a microarray containing complementary barcode oligonucleotides. The microarray is scanned and the relative abundance in the two subpopulations of an shRNA targeting a gene that influences cell proliferation or survival can be quantified.

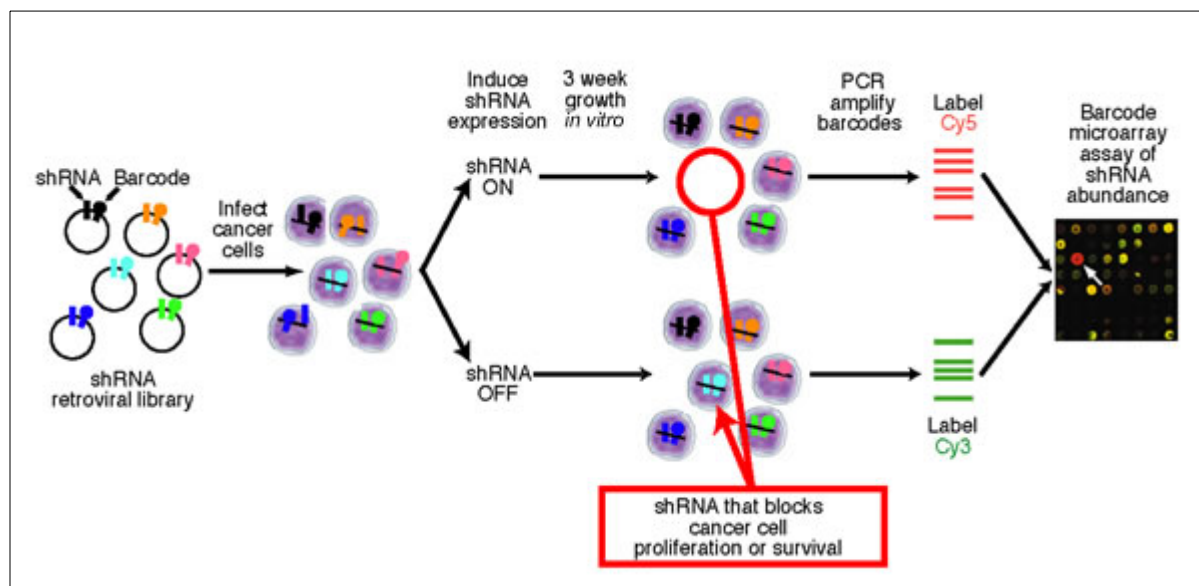
The screen that we performed was aimed at uncovering shRNAs that are selectively toxic to one lymphoma type but not the other, presumably due to the underlying molecular differences between the two lymphoma types. Remarkably, we discovered that the shRNAs that targeted genes in the NF- $\kappa$ B pathway were toxic to ABC DLBCL but not GCB DLBCL cell lines. This finding was in keeping with our previous demonstration that ABC DLBCLs depend on constitutive NF- $\kappa$ B signaling for survival. Unexpectedly, these shRNAs targeted three genes, *CARD11*, *MALT1*, and *BCL10*, which lie in a signaling pathway upstream of I $\kappa$ B kinase, the key regulator of the NF- $\kappa$ B pathway. Thus, our genetic screen has begun to unravel the mystery of constitutive I $\kappa$ B kinase activity in ABC DLBCL.

We are continuing similar screens of other forms of cancer and are consistently identifying new cancer type-specific pathways that control cell proliferation and survival. These signaling pathways could be activated by gene mutations or alterations in gene copy number that are present in particular cancer types; therapies targeting these pathways would be predicted to have a large therapeutic index. Alternatively, the pathways uncovered by our genetic screens may not be directly activated by oncogenic events but might be features of the normal cells from which the cancer develops. In this scenario, therapeutic targeting of the pathway might eliminate normal cells as well as cancer cells. For certain cancers such as lymphomas, however, the normal cellular counterparts, B lymphocytes, are dispensable for short periods of time and can be renewed.

We envision a new taxonomy of cancer centered around a cancer’s dependence on particular regulatory pathways. The Achilles-heel genetic screen that we have employed is a powerful method to achieve this end and will likely hasten the development of pathway-specific therapies.

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