

# Knockout drug screens

Cell lines that differ by a single genetic change show promise in drug screens to identify compounds with gene-selective properties.

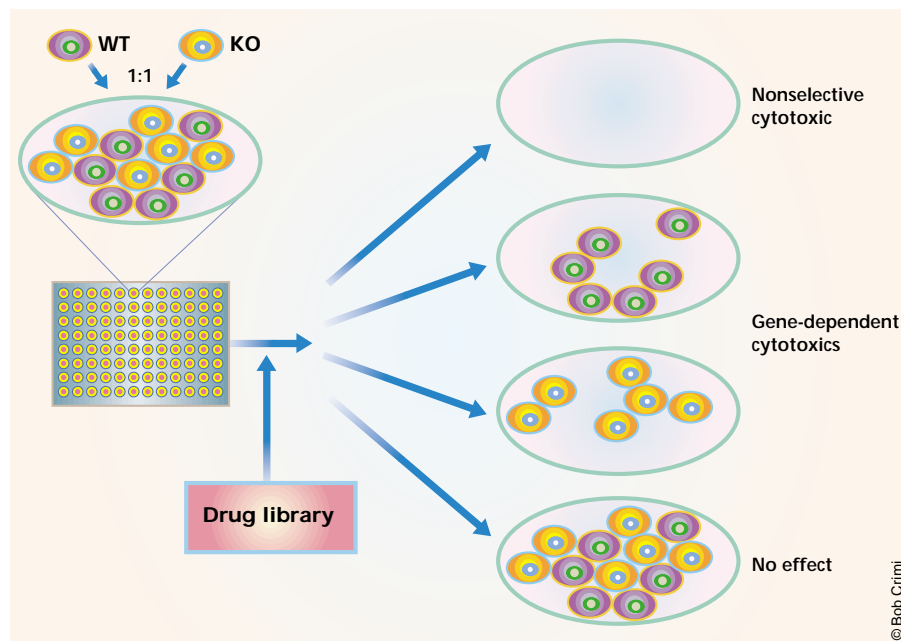
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How does one discover drugs to treat diseases whose pathophysiology is poorly understood? This daunting question faces many researchers interested in alleviating disease, especially those working on cancer. Perhaps more than any other disease, cancer has frustrated efforts to understand and manage it, and there continues to be a great need for better therapeutics. In this issue, Torrance *et al.*<sup>1</sup> introduce a new twist to top-down drug screening strategies, one that integrates fluorescence protein technology with somatic cell genetics, thereby capturing some of the appeal of molecular approaches.

As far as cancer drug discovery goes, three general strategies have been taken that might be termed engineering, bottom-up, and top-down strategies. Engineering strategies seek to improve upon existing therapeutics that have clinical efficacy, for example, by developing targeting principles to deliver cytotoxic drugs to tumor cells in a specific manner<sup>2</sup>. The most common way to discover new therapeutic agents is to screen libraries of lead compounds by high-throughput, plate-based assays. Bottom-up screening strategies are biochemically based, seeking to identify agents that affect molecules thought to be critical to disease. In contrast, top-down screening strategies are biologically based, seeking to identify agents that affect cellular processes thought to be critical to disease.

During the past decade, many cancer drug discovery groups have turned to bottom-up screening strategies, because of the significant advantages to subsequent optimization by medicinal chemistry efforts that derive from knowing the drug target<sup>3</sup>. However, it is notable that virtually all cancer drugs used in the clinic today were discovered through top-down screening. Moreover, as we do not yet know all the biochemical targets in the cell, nor their physiological functions, there is still an important niche for top-down strategies.

In their study, Torrance *et al.* exploit fluorescent protein technology to illustrate how



**Figure 1.** Co-culture drug screening with isogenic “knockout” cells. Cells that are wild-type (WT) or nullizygous (KO) for the gene of interest are modified with spectrally distinct blue or yellow fluorescent proteins (BFP or YFP) and co-cultured at a 1:1 ratio in multiwell plates. Relative cell numbers are read on fluorescence plate readers at different times after drug addition. The method identifies cytotoxic or growth-inhibitory compounds that are gene-selective in their action.

isogenic cell lines that differ by a single genetic change can be used to identify compounds with gene-selective properties. Briefly, they performed a drug screen in which they co-cultured two isogenic colon tumor cell lines, one that expressed a mutant *K-Ras* allele and the other in which that allele was deleted by homologous recombination. These cell lines were also tagged with yellow or blue fluorescent proteins that were expressed at similar levels. This scheme allowed relative cell numbers to be monitored easily in mixed cultures by measuring fluorescence signals unique to each cell line. In this way, compounds that preferentially targeted the growth or survival of cells with the mutant *K-Ras* allele were identified in a well-controlled fashion, on the basis of the relative reduction in fluorescence signals of the mutant cells in co-cultures.

The elegant combination of somatic cell genetics and fluorescence protein technology is what makes the report from Torrance *et al.* interesting. Using fluores-

cence protein technology to measure relative cell numbers saves time and money. Traditionally, cell growth assays used to screen cancer compounds in high-density formats relied on protein stains (e.g. sulfarhodamine B) or metabolic stains (e.g., MTT assay; see Torrance *et al.*, Experimental Protocol) to measure relative cell number. These methods entail significant reagent expense and work-up time, which are avoided. By utilizing isogenic “knockout” cell lines, Torrance *et al.* also address a major shortcoming of top-down screening strategies, which is how to relate biological readout to molecular mechanism. While still facing such challenges, the method at least introduces a rational boundary condition that circumscribes the area in which mechanistic questions can be considered. An additional advantage of the method comes from co-culturing the control and experimental cell lines together. This feature not only addresses major sources of variation that arise in counter-

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screens, but also saves resources by halving the number of wells to be screened. Taken together, the combined benefits of somatic genetics and reductions in time and cost generates a nice package, especially to investigators in academia or small companies who seek to do cell-based screens on a tight budget.

Under the conditions used by Torrance *et al.*, the hit rate from the initial screen of ~30,000 compounds was ~0.6%. Further analysis pared down the initial hits to just four compounds that reproducibly suppressed cells expressing mutant K-Ras at least twofold, at multiple concentrations, and against other pairs of isogenic tumor cell lines. Two of the four compounds were known. One was a wortmannin analog, which as a likely inhibitor of phosphoinositidyl-3'-kinase could be rationalized on the basis of its key requirement in mediating signals downstream from *Ras* (ref. 4). The second was the DNA minor groove-binding drug mithramycin, which may affect transcription of *K-Ras* (or perhaps downstream target genes), based on reports that it can bind the *H-Ras* promoter and block transcription<sup>5</sup>. The remaining compounds were novel, but only one displayed the same pref-

erence for cells expressing mutant K-Ras in a rodent model system. This compound was a novel cytosine derivative that was about sixfold more cytotoxic to mutant K-Ras-expressing cells. The proof of concept offered by this compound might be consistent with a recent report that the presence of oncogenic *Ras* alleles in human tumor cell lines correlates with heightened sensitivity to the cytosine derivative 1- $\beta$ -D-arabinofuranosylcytosine (Ara-C)<sup>6</sup>.

The method offered by Torrance *et al.* to identify selective growth-inhibitory compounds is simple, rapid, and accessible. It requires only a fluorescent plate reader and a few pairs of "knockout" cell lines with matching robust expression levels of yellow fluorescent protein or blue fluorescent protein from identical vectors. The main entry requirement for the cells is that the targeting event not affect the rate of monolayer cell proliferation itself. Torrance *et al.* cleverly exploited such a quirk in the colon tumor cell system used, which displays a reduction in anchorage-independent growth but not anchorage-dependent growth following *K-Ras* deletion<sup>7</sup>. This issue may present some challenges for other cancer genes, but with appropriate

experimental designs the simplicity and accessibility of the method still make it attractive.

Top-down screens are unbiased to molecular targets so they can produce biologically interesting drug leads. While following these up can be quite challenging—optimization of lead compounds suffers without knowledge of the target—such screens still occupy an important niche because not every important target or its function is known yet. For basic researchers, the availability of easier, cheaper, and more focused screening methods may aid the discovery of research tools that promote new concepts and directions. Thus, enlisting somatic cell genetics in the cause of cancer drug discovery is a laudable advance, one that may promote conceptual breakthroughs as well as discovery efforts.

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