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Desperately Seeking Genotype-Selective Anticancer Agents

An approach has been described to identify new agents that selectively kill mammalian cells with multiple genetic abnormalities. Compounds of this type could lead to innovative cancer treatments based on tumor genotype rather than by histology or anatomical location.

Small molecules have historically been valuable reagents to probe biologically intractable pathways and to identify important cellular proteins. Consider the role aspirin had in the discovery of cyclooxygenases, norepinephrine and epinephrine in α and β adrenergic receptors, bioamines in histamine receptors, nicotine in the acetylcholine receptors, or rapamycin in mTOR.

Conventional drug discovery is dominated by approaches that exploit selected molecular targets and in vitro assays. Cell-based or animal-based assays have lagged substantially behind. Nonetheless, integrated cell- and animal-based assays will undoubtedly become increasingly valuable as we begin to recognize that much remains to be discovered about the complex biological networks that are responsible for human diseases and that many human diseases involve multiple rather than single genes. This is especially true for cancer models, as genetic instability generates multiple and dynamic genomic alterations.

Many cancer researchers have emulated the drug discovery strategies taken by their nononcology brethren and looked for compounds that affect a specific protein, often an oncogene, with obvious enzymatic activity that is overexpressed or inappropriately activated. Nonetheless, many of these protein targets are found in normal tissues, and, thus, disrupting their activity could lead to serious untoward effects and lack of disease specificity. An attractive alternative approach inspired by studies with yeast and popularized by Hartwell [1] and Kaelin [2] involves searching for genotype-selective antitumor agents that become lethal to tumor cells only in the presence of specific oncogenes or the absence of specific tumor-repressor genes. This synthetic lethal strategy in an ideal situation should yield compounds with exquisite selectivity against tumors with the altered genotype. The principle of synthetic lethality in its simplest form is illustrated in Figure 1A. Cells with intact and fully functional genes A and/or B are viable, while cells in which genes A and B are altered or deleted die. By analogy, in a synthetic lethal chemical screen one looks for killing only when cells have a specific genotype and are exposed to a small molecule (Figure 1B). A number of compounds have already been reported that exhibit synthetic lethal properties [3, 4].

Recently, Stockwell and colleagues [5] have taken this elegant synthetic lethal approach to a higher level. Specifically, they have generated isogenic pairs of cell lines from two primary normal human fibroblasts expressing up to six genetic elements that have been implicated in human cancers: the human catalytic subunit of the enzyme telomerase (hTERT), the Simian Virus 40 large T (LT) and small T (ST) oncoproteins, the Human Papilloma Viral protein E6 and E7, and an allele of H-RAS ($RASV^{12}$). They then screened the isogenic pairs with over 23,000 compounds for selective cytotoxicity. While individuals who perform traditional in vitro high- and ultra high-throughput screens might not view this number of compounds as especially large, it is substantial for the more labor intensive cell-based assay, even one simply measuring cell killing. Interestingly, the authors identify and describe nine compounds that have at least 4-fold greater cytotoxicity to the TERT/LT/ST/ RAS^{V12} cells compared with the parent primary fibroblasts. While the identified compounds obviously reflect the chemical elements in the screened libraries, it intriguing that four of the nine compounds are currently used in the clinic as antineoplastic agents. This validates the notion that the general approach has the potential to identify newer agents that may have clinical utility. In addition to the known agents reported, the authors disclosed a new compound, erastin, which caused selective, rapid, nonapoptotic death to cells harboring both ST and oncogenic RAS^{V12} and not to normal fibroblasts or isogenic cells with other genotypes. A secondary byproduct of this screening system was the observation, revealed by the identified compounds, that overexpressing hTERT and either E7 or LT increased expression of topoisomerase 2α and that overexpressing RAS^{V12} and ST both increased endogenous expression of topoisomerase 1.

As with all provocative articles, this contribution raises several interesting and unanswered questions. For example, the mechanism by which hTERT and E7 or LT increased topoisomerase 2α expression is left unanswered. The authors observed that the rate of cell proliferation increased when each genetic element was introduced into fibroblasts. One class of identified compounds, group 1, had no clear genetic basis for their tumorigenic cell selectivity, and the authors hypothesized that group 1 compounds were simply selective for dividing cells, in part because they targeted DNA or protein synthesis. If this hypothesis is true, the number of compounds initially identified in the comparative isogenic screening assays would seem rather small when one considers that it is conventional wisdom that most clinically used anticancer drugs act preferentially on rapidly cycling cells. Moreover, the commonly used 1,990 compound National Cancer Institute diversity collection has a substantial number of agents that interact with DNA or have been examined as anticancer agents. Thus, it would be interesting to evaluate all of the commonly used anticancer agents with these isogenic lines to see how many are selective for the tumorigenic phenotype. Further clarification of the molecular target of erastin would be valuable. While many investigators have fo-

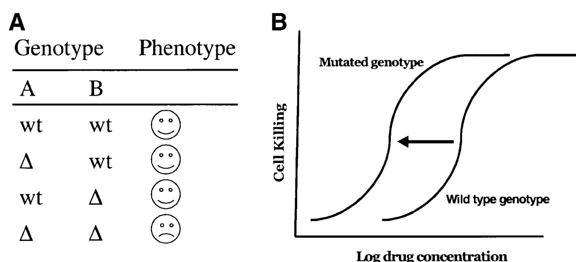

Figure 1. A Schematic Representation of the Traditional Synthetic Lethal Strategy Used in Yeast Genetics Studies

(A) A and B represent different genes. When both normal genes are present, the yeast are viable, signified by the smiling face. When either gene A or B are altered or deleted (indicated with Δ), the organism remains viable; when both genes are mutated, the lethal phenotype, signified by the frowning face, occurs.

(B) In a synthetic lethal chemical screen, the lethal phenotype occurs only when the organism has the altered gene or genes and is exposed to a specific chemical agent. Thus, the concentration/dose-response curve is shifted to the left in the cells that have the altered or deleted gene(s). In some cases, the small molecule might inhibit function of the product of a second gene—for example, gene B—that is essential for survival only when the product of gene A is mutated or deleted.

cused on exploiting apoptotic death as a target for anticancer agents, a good case can be made that the resulting immunological recruitment associated with selective nonapoptotic death of tumor cells might actually be a desirable attribute for any drug. Thus, understanding how erastin selectively kills cells would seem to be a worthy undertaking. Another unanswered question is why only partial resistance to camptothecin was seen when small interfering RNA to topoisomerase 1 was introduced into TERT/LT/ST/RAS^{V12} cells. Perhaps there are other as yet unknown targets for the camptothecins that exist. We also do not know how important the transfected gene expression stoichiometry is for the ultimate sensitivity to particular agents. Nonetheless, Dolma et al. [5] nicely illustrate the power of the synthetic lethal approach for exposing agents with both unique and potentially rich pharmacological properties. It seems likely that many others will emulate their approach.

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Novel Carotenoids Genetically Engineered in a Heterologous Host

Carotenoids are commercially important pigments that are essential for human health. Diverse carotenoids have been identified, but availability has impeded evaluation of their pharmaceutical potential. Molecular techniques have been developed to produce specific and novel carotenoids with improved biological activities.

Over the last few years, molecular procedures have been developed to engineer metabolic pathways. These include directed evolution by gene shuffling to alter the catalytic properties of enzymes, and combinatorial biosynthesis, i.e., coexpression of genes encoding different enzymes from related pathways. Both approaches have successfully been applied to carotenoid biosynthesis. The color of carotenoids is very helpful for the screening and selection of transformants. Typical carotenoids consist of 40 carbon atoms with an extensive conjugated double bond system. They may also carry substituted cyclic ionone end groups. These structural features are targets for modification and for mixing substituents in the same molecule.

Compounds can be synthesized in a modular way by combining genes that comprise different branches of a biosynthetic pathway, which are isolated from different organisms. Pioneering work following this approach was carried out on the synthesis of novel polyketide antibiotics [1]. This strategy only works, however, when the substrate specificity of a key biosynthetic enzyme is such that it does not need to recognize the entire substrate molecule but only certain regions of the molecule that are suitable for conversion. This is generally also the case in carotenoid biosynthesis, and therefore this combinatorial approach was very successful in generating numerous carotenoids including novel structures not found before [2]. Among the carotenoids produced by combinatorial biosynthesis were carotenoids with improved biological activity [3].

Highly homologous carotenogenic genes from related organisms may encode enzymes with modified substrate and/or product specificities [4], but this is a rare occurrence. One experimental way of acquiring enzymes with extended catalytic properties for the synthe-