therapy. In the not-too-distant future, it may be possible to link the different pathways controlling apoptosis, differentiation, and proliferative capacity in hematopoietic cells, providing a more comprehensive picture of the genetic basis of acute leukemia and perhaps new molecular targets for the design of effective therapy.

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Integrating Genetic Approaches into the Discovery of Anticancer Drugs

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The discovery of anticancer drugs is now driven by the numerous molecular alterations identified in tumor cells over the past decade. To exploit these alterations, it is necessary to understand how they define a molecular context that allows increased sensitivity to particular compounds. Traditional genetic approaches together with the new wealth of genomic information for both human and model organisms open up strategies by which drugs can be profiled for their ability to selectively kill cells in a molecular context that matches those found in tumors. Similarly, it may be possible to identify and validate new targets for drugs that would selectively kill tumor cells with a particular molecular context. This article outlines some of the ways that yeast genetics can be used to streamline anticancer drug discovery.

The recent remarkable progress in identifying molecular alterations in human tumor cells has unfortunately not been paralleled in the field of anticancer drug discovery. The shortage of effective anticancer drugs is due in part to the fundamental difficulties associated with the development of any safe effective drug. For example, it remains a formidable task to design small molecules that alter the function of macromolecules with both sensitivity and specificity (for example, an enzyme with a small active site). It is even more difficult to inhibit protein-protein interactions mediated over a large surface, or to restore function to a defective protein (such as an inactive tumor suppressor protein). Even when successful, massive efforts are required—often measured in years to decades—from dozens of chemists, biochemists, and toxicologists.

There are also many difficulties specific to anticancer drug discovery programs. An effective chemotherapeutic must selectively kill tumor cells. Most anticancer drugs have been discovered by serendipity, and the molecular alterations that provide selective tu-

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mor cell killing are unknown. Even understanding the detailed molecular mechanism by which a drug acts often provides little insight into why the treated tumor cell dies. For example, we do not understand why cisplatin, a DNA cross-linking agent, is an effective chemotherapeutic for most germline testicular tumors (*1*).

The discovery of novel, more effective anticancer drugs is likely to require both an awareness of sensitizing molecular contexts (that is, how the tumor cell differs genetically from the normal cell) and which patients have these sensitizing defects. Here we examine the advantages and hazards of engaging the power of genetics in anticancer drug discovery.

Applying Genetics to Drug Discovery

The use of a genetic approach for drug discovery can potentially improve on current paradigms in two important ways. First, a mutation is a model of an ideal drug. By disabling a single gene in a cell or organism the function of one and only one protein is eliminated, as though one had a perfect drug for that target. Second, one of the most powerful aspects of carrying out a genetic screen (a search for mutations anywhere in the genome that produce a desired phenotype) is that we approach biology with humility—we allow the organism to tell us which are the important functions. By identifying genes whose mutations produce the desired therapeutic outcome, we will have simultaneously identified and validated appropriate new drug targets.

The current state of human cell genetics does not permit the exploitation of genetics for drug discovery, so it is necessary to use "model organisms" for which genetic manipulation is facile. Many of the genes that are frequently altered in tumors have structural or functional homologs in model genetic systems, including the yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, the nematode *Caenorhabditis elegans*, and the fruit fly *Drosophila melanogaster* (Table 1). If the potential drug targets are components required for cell division or DNA repair, where there is significant conservation of function between humans and yeast, then yeast would be the organism of choice. Fruit flies and nematodes are also potentially valuable models, especially when conservation from human to yeast is weak or when the target components are present only in the multicellular context. Finally, an increasing number of single gene knockouts in murine embryonic stem cells offer opportunities for working with drug targets that are even more closely related to homologous targets in humans (*2*).

Uncovering therapeutic advantages. Identification of drug targets that would achieve a high therapeutic advantage requires knowledge of how the tumor cell differs from the normal cell. Cancer cells are genetically different from their normal cell counterparts, often having undergone at least a half-dozen mutations (*3*, *4*). Which of these mutations makes the tumor cell different from the normal cell in a way that can be exploited to kill the tumor cell selectively?

We believe that the genetic changes that give rise to the genetic instability of tumor cells may provide the key to tumor cell sensitivity. Tumor cells universally exhibit genetic instability. Perhaps the best single documentation of this assertion is that many tumor cells from different origins have been examined for their ability to undergo gene amplification and all exhibit high rates of gene amplification in comparison with normal untransformed cells (*5*). Other indications of genetic instability in tumor cells are their frequent karyotypic abnormalities, multipolar mitoses, and nucleotide repeat instability [(*3*, *6*); reviewed in (*7*)]. Some of the genetic changes underlying this genetic instability have been identified and they fall into three categories: defects in DNA repair pathways [for example, patients with xeroderma pigmentosum (XP) and hereditary nonpolyposis colon cancer (HNPCC) show alterations in nucleotide excision repair (*8*) and DNA mismatch repair (*3*, *9*), respectively], defects in cell cycle checkpoints [the *p53* gene in the Li-Fraumeni syndrome (*10*) and the *ATM* gene in the hereditary cancer-prone syndrome ataxia telangiectasia (*11*, *12*)], and defects that cause inappropriate transition from the G_1 to the S phase of the cell cycle [for example, RAS activation, MYC activation, or Cyclin D amplification (*13*)]. We will use the term "DNA damage response element or pathway" as a general term to cover all three categories.

The reasons for thinking that the genetic changes underlying genetic instability are valuable for drug discovery are as follows. First, because all cancers are genetically unstable, this is a general context in which to consider cancer therapy. Second, genetic instability is probably necessary for the evolution of the cancer cell to a metastatic state. Third, it is well known that defects in many DNA damage response elements resulting in genetic instability also create vulnerability to killing by certain damaging agents. For example, XP mutations cause sensitivity to ultraviolet light, and mutations in *ATM* and the breast cancer susceptibility gene *BRCA2* cause sensitivity to ionizing radiation (see Table 1 and references therein). While these strategies at-

Table 1. Human genes altered in tumors and their relatives in model genetic systems. Genes that are not structural homologs but act in analogous pathways (such as human *p53* and *S. cerevisiae RAD9*) are shown in brackets. *Saccharomyces cerevisiae* genes are designated with superscript Sc, *S. pombe* with Sp, *C. elegans* with Ce, and *D. melanogaster* with Dm. Because of space limitations, this is only a representative list of genes mutated in tumors that have genetic analogs in model systems. Comprehensive lists of model system genes analogous to human genes mutated in tumors can be found in the references listed herein and in (*34*).

Function	Human genes	Model system analogs: Structural homologs or related biological roles	References
DNA damage checkpoint	*p53*	[*RAD9*^{Sc}, *rad1*$^{+Sp}$]	*10, 11, 15, 17*
	ATM	*MEC1*^{Sc}, *TEL1*^{Sc}, *rad3*$^{+Sp}$, *mei-41*^{Dm}	*11, 12, 15, 17, 35*
DNA mismatch repair	*MSH2, MLH1*	*MSH2*^{Sc}, *MLH1*^{Sc}	*3, 9, 36*
Nucleotide excision repair	*XP-A, XP-B*	*RAD14*^{Sc}, *RAD25*^{Sc}	*8, 15*
O^6-methylguanine reversal	*MGMT*	*MGT1*^{Sc}	*15, 37*
Double-strand break repair	*BRCA2, BRCA1*	[*RAD51*^{Sc}, *RAD54*^{Sc}]	*15, 16, 24, 38*
DNA helicase	*BLM*	*SGS1*^{Sc}, *rqh1*$^{+Sp}$	*39*
Growth factor signaling	*RAS*	*RAS1*^{Sc}, *RAS2*^{Sc}, *let-60*^{Ce}	*20, 40, 41*
	NF1	*IRA1*^{Sc}, *IRA2*^{Sc}	*41, 42*
	MYC	*dMyc*^{Dm}	*32, 41*
	PTH	*patched*^{Dm}	*41, 43*
Cell cycle control	Cyclin D, Cyclin E	*CLN1*^{Sc}, *CLN2*^{Sc}, Cyclin D^{Dm}, Cyclin E^{Dm}	*19, 26, 44*
	p27^{kip1}	[*SIC1*^{Sc}]	*19, 26, 45*
	Rb	*Rbf*^{Dm}	*26, 46*
Apoptosis	*BCL-2*	*ced-9*^{Ce}	*10, 27, 41*

tempt to turn genetic instability into an asset for therapeutic advantage, the tumor cell heterogeneity that results from this instability could compromise the effectiveness of antitumor drugs identified by this or other means.

Screening for drugs and screening for targets. We have modeled DNA damage response defects in the yeast *S. cerevisiae* by constructing a panel of about 70 isogenic strains (*14*), each harboring a single mutation affecting different damage response elements, including DNA repair (*9*, *15*, *16*), cell cycle checkpoints (*11*, *15*, *17*, *18*), and cell cycle regulation (*19*, *20*). These yeast mutants can be used as models of the cancer cell to screen directly for drugs or for drug targets. By screening directly for drugs that are more lethal to mutant than to wild-type yeast, we have the opportunity to identify drugs with a therapeutic advantage. Because the cancer context being modeled is vulnerability to DNA damage, the drugs identified in this screen probably damage DNA either by interacting directly with DNA or by interacting with proteins responsible for DNA metabolism. Alternatively, genetic screens can be used to identify protein targets that would create a therapeutic advantage in a mutant compared with a wild type by screening for second site mutations that are lethal in the mutant strain but not in the wild-type strain (see later discussion of synthetic lethality).

Screening drugs against the yeast damage response mutant panel. About 50 U.S. Food and Drug Administration (FDA)–approved anticancer chemotherapeutic agents have been evaluated in the hope that the yeast mutants might reveal insights into the mode of action of agents that are widely used in the clinic. After excluding agents that are not toxic to yeast for obvious reasons (for example, hormones, growth factors, and pro-drugs requiring metabolic activation), we obtained dose response curves on the panel of yeast mutant strains for 22 (50%) of the remaining agents or close structural or functional analogs. Sensitivities of yeast mutants to some of the drugs have already been described, but the comprehensive panel of isogenic strains allows quantitative comparison between strains so that the most vulnerable mutant can be easily assessed. Two examples of drug sensitivity patterns are shown in Fig. 1. The cross-linking agent cisplatin (*15*, *21*) causes many types of damage, reflected by the wide variety of yeast mutants that are hypersensitive, but nevertheless it displays fairly high specificity for mutants defective in postreplication repair. Mitoxantrone, a topoisomerase II poison (*21*, *22*), is highly specific for defects in DNA double-strand break repair, a finding compatible with the expected effect of topoisomerase II inactivation (*23*). Thus, in principle, cisplatin and mitoxantrone might exhibit high therapeutic advantage for tumors defective in postreplication repair or double-strand break repair, respectively.

The mutant yeast panel is also being used for rapid screening of chemical libraries. This strategy may uncover new chemotherapeutic compounds with a high therapeutic index in tumors with a common genetic alteration. Such compounds would have been missed in previous searches for chemotherapeutic agents that relied on the response of cancer patients whose only common feature may have been the tumor site.

Extrapolating back to clinically useful data. Knowledge that a topoisomerase poison is more toxic to a yeast cell that is defective in the DNA double-strand break repair pathway is clinically relevant only if an analogous defect occurs in human tumors and this defect determines sensitivity to topoisomerase poisons. In many respects, the most difficult aspect of the genetic approach to drug discovery is the lack of knowledge about mammalian biological pathways.

Three steps need to be completed before the yeast compound sensitivity data can be exploited clinically. First, it must be shown that mammalian cell lines defective in analogous damage response pathways are more sensitive to the compounds identified in the yeast strains. This can be done by testing matched pairs of cell lines, usually murine knockout cell lines. For several damage response pathways, this has already been demonstrated; for example, as predicted by the sensitivities seen in yeast double-strand break repair mutants, murine cells lacking RAD54 are sensitive to ionizing radiation, methylmethane sulfonate, and the cross-linking agent mitomycin C (*24*). Second, tumor cell lines must be assayed for their sensitivity to the particular compound. Finally, assays for the sensitizing damage response defects must be done to validate that it is these particular defects that determine tumor sensitivity. Six of the FDA-approved drugs [cisplatin, mitoxantrone, streptozotocin, camptothecin, cytarabine, and hydroxyurea (*21*)] are now being assayed in both matched pairs of cell lines and in tumor cell lines. Biochemical assays are also being performed on tumor cell lines to see if analogous damage response defects are present.

Some of the drug sensitivities identified in the yeast panel may not be extrapolated to tumors for a number of reasons. Mammalian cells may have different damage response pathways not present in yeast (for example, oncogene-mediated apoptosis in response to DNA damage), or a defect that sensitizes yeast to a drug may not be a defect that occurs in tumors. Further complica-

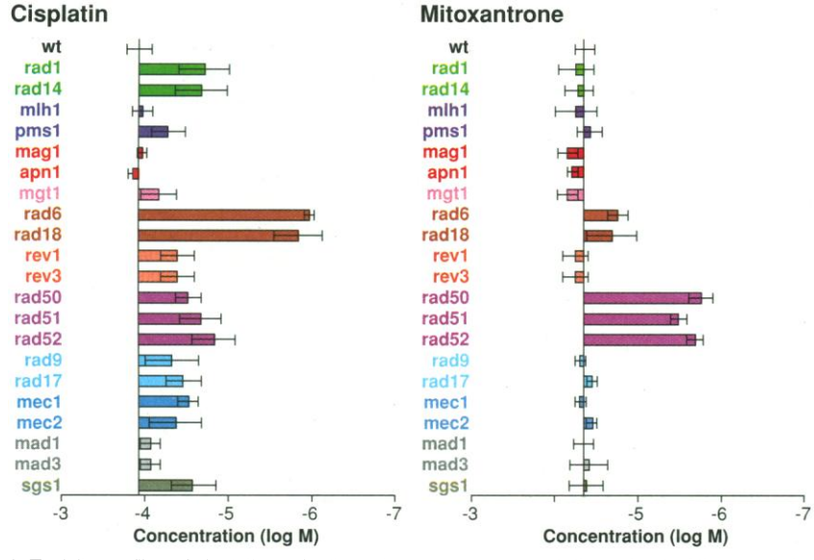

Fig. 1. Toxicity profiles of cisplatin and mitoxantrone on a representative subset of the yeast damage response mutant strain panel (*14*). The yeast mutants are color-coded according to the damage response pathway disrupted. The rad1 and rad14 mutants are defective in nucleotide excision repair (*15*); mlh1 and pms1, mismatch repair (*9*); mag1 and apn1, base excision repair (*15*); mgt1, reversal of O6-methylguanine (*15*); rad6 and rad18, postreplication repair (*15*); rev1 and rev3, mutagenic replication bypass (*47*); rad50, rad51, and rad52, double-strand break repair (*15, 16*); rad9, rad17, mec1, and mec2, DNA damage checkpoint (*11, 17*); mad1 and mad3, spindle assembly checkpoint (*11, 18*); sgs1, BLM-related DNA helicase (*39*). The median inhibitory concentration (IC_{50}) values are given as the logarithm of the molarity and are averages of three independent experiments. The vertical bar is set at the respective wild-type IC_{50} value.

tions include tissue-specific contexts, as well as the complexity of multiple genetic alterations present in tumors. Finally, independent mechanisms of resistance, such as the expression levels of P-glycoprotein or cytochrome P-450, might also modify the cumulative sensitivity of a particular tumor to a drug (*25*). Despite these potential pitfalls it is expected that in many cases it may be possible to "uncover" the sensitizing molecular defects that allow certain patients to respond to drugs by extrapolating from the yeast experiments. This knowledge might allow identification of patients who are more likely to respond to chemotherapy because of the specific molecular defects in their tumors.

Genetic Screening for New Drug Targets

Many of the genetic alterations frequently found in tumors are loss-of-function mutations in tumor suppressor genes and thus do not constitute ideal drug targets, because it is difficult to develop drugs that restore the function of a missing or altered protein. It may be possible to achieve this indirectly by inhibiting the activity of a protein that acts downstream of the missing tumor suppressor gene product along a signaling pathway [for example, inhibiting CDK4 activity may correct for the loss of p16INK4a (*26*)]. However, our limited knowledge of mammalian signaling pathways makes this at best a limited and risky approach. An alternative, broader strategy is to exploit the loss of these functions in the development of new therapies through the use of a genetic technique available in *S. cerevisiae* called synthetic lethal screening [reviewed in (*27*)]. This approach identifies second site mutations that by themselves are not lethal, but in combination with the primary defect cause lethality. In the setting of anticancer drug target identification, the primary defect would be a mutation in a gene conserved from yeast to humans that is frequently inactivated in tumors (for example, defects in DNA mismatch repair; see Table 1). Gene products with mutations that specifically kill cells with the primary defect would constitute putative "secondary drug targets" (that is, secondary to the primary defect) whose inactivation in tumors may yield great therapeutic advantage.

In principle, synthetic lethality can result when two mutations have an additive negative effect on a single essential biological pathway, or when the mutations inactivate two different but functionally overlapping pathways (*27*). One form of genetic instability shows how synthetic lethality could be applied to cancer therapy. All cells use two pathways to eliminate mistakes made during DNA replication: a 3′→5′ proofreading exonuclease activity in DNA polymerase, which eliminates incorrect bases immediately after they are added to the growing chain (*15, 28*), and the mismatch repair system, which eliminates mistakes in the newly replicated DNA that have escaped the proofreading activity (*9*). In budding yeast, cells can survive without one of the pathways, albeit with an increased mutation rate. Eliminating both pathways kills yeast cells, presumably because of an excessively high mutation rate (*29*). A hypothetical drug that inhibited the proofreading activity of DNA polymerases delta (*29*) or epsilon (*30*) would specifically kill a yeast cell that lacked the mismatch repair system, but not a normal yeast cell. The overlapping functions of the mismatch repair and proofreading are conserved from yeast to humans, as are the proteins that carry them out. Therefore, the antiproofreading drug may be effective in killing tumors with defects in mismatch repair but not affect normal proliferating cells.

Synthetic lethality can be detected by candidate crosses and genome-wide screening. The first method uses prior knowledge to make and test predictions about which combination of mutations will kill cells. This method is applicable to any organism in which mutations can be constructed to order, including budding and fission yeasts, nematodes, flies, and mice, and was the method used to show the synthetic lethality of defects in proofreading and mismatch repair. The second method is to perform genetic screens for new synthetically lethal mutations. A strain that carries a single mutation is mutagenized and subjected to various screening programs that will reveal new mutations that are synthetically lethal with the original mutation. This approach requires no prior knowledge but depends on the ability to conduct large-scale genetic screens and is currently restricted to microorganisms, nematodes, and *Drosophila*. Once synthetically lethal mutations have been identified, cloning of the corresponding wild-type gene allows identification of the mutated protein and assessment of the suitability of the protein as a target for drug discovery. The complete sequence of the budding yeast genome will permit comprehensive and automated screening for synthetic lethality. Over the next several years, all of the approximately 6250 yeast genes will be disrupted, eventually allowing for comprehensive synthetic lethal screening through the construction of double mutants containing disruptions of each gene in combination with the primary mutation of interest.

Extrapolating to mammalian targets. Figure 2 outlines the steps involved in identifying and using synthetic lethal screens in model genetic organisms to identify secondary targets. Examples of primary tumor defects that can be modeled in facile genetic systems include *S. cerevisiae* mutants lacking the *MSH2* DNA mismatch repair gene (*9*), *C. elegans* mutants defective for the *BCL-2* homolog *ced-9* (*31*), and fruit flies overexpressing *Myc* (*32*). Once secondary targets have been identified in the model systems, there are several conditions that must be met before it is reasonable to initiate high-throughput screens for inhibitors of the mammalian homologs of these gene products. It is first necessary to validate that the synthetic lethality also occurs in mammalian cells (both matched pair cell lines and tumor cell lines) in which the primary and secondary targets are inactivated. This will require the use of mammalian inducible gene disruption techniques such as ribozymes, antisense molecules, or dominant-negative strategies (*33*). The pharmacological feasibility of each putative drug target must be determined simultaneously, because the secondary targets most amenable to the inhibition by small molecules (for example, enzymes with well-defined substrates) will be the obvious first choices for further analysis. Only after these tests have been completed can the standard high-throughput screens for inhibitors of these validated mammalian secondary targets be initiated.

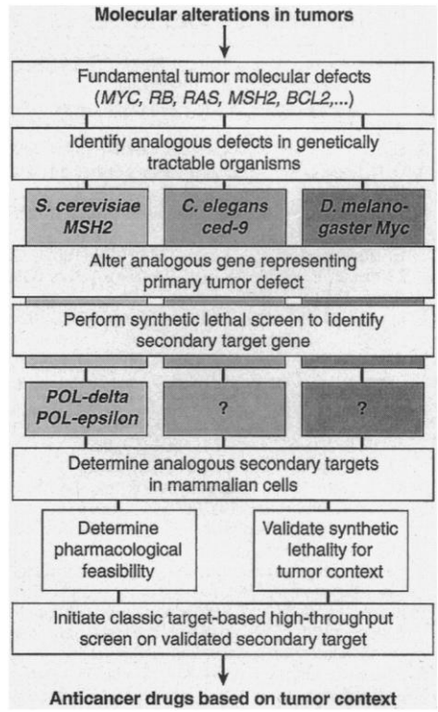

Fig. 2. Outline of a synthetic lethal screening strategy for identifying secondary targets. For further explanation see text and Table 1.

Summary

Whether drug-based or target-based screens are used, it is possible to exploit the detailed information gathered for several model organisms that are genetically tractable. Such approaches are well suited to identifying drugs that have a selective killing capacity for the tumor context. They allow us to escape from strategies that are based on inhibiting the activities of oncogene products, or attempting to restore the lack of activity resulting from the inactivation of a tumor suppressor gene product. Because such genetic approaches allow an alignment of particular molecular defects with "specific" drugs, there is a high probability that the serious side effects associated with many currently used chemotherapeutics will be less problematic. Although the utility of genetics and model organisms is potentially quite broad, three inadequacies will continue to limit clinical applications. The first stems from the current difficulties in understanding the complexities of the mammalian cell signaling circuitry, the second stems from our still limited methods of assessing molecular alterations in tumors, and the third stems from relatively ineffective ways of conditional gene inactivation in mammalian cells. Finally, as more therapies are developed for particular molecular defects, there will be increased need as well as incentive to improve methods for detecting these alterations.

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Environment and Cancer: Who Are Susceptible?

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Acting in concert with individual susceptibility, environmental factors such as smoking, diet, and pollutants play a role in most human cancer. However, new molecular evidence indicates that specific groups—characterized by predisposing genetic traits or ethnicity, the very young, and women—may have heightened risk from certain exposures. This is illustrated by molecular epidemiologic studies of environmental carcinogens such as polycyclic aromatic hydrocarbons and aromatic amines. Individual genetic screening for rare high-risk traits or for more common, low-penetrant susceptibility genes is problematic and not routinely recommended. However, knowledge of the full spectrum of both genetic and acquired susceptibility in the population will be instrumental in developing health and regulatory policies that increase protection of the more susceptible groups from risks of environmental carcinogens. This will necessitate revision of current risk assessment methodologies to explicitly account for individual variation in susceptibility to environmental carcinogens.

Most cancer results from the interaction of genetics and the environment (*1–3*). That is, genetic factors by themselves are thought to explain only about 5% of all cancer (*3*). The remainder can be attributed to external, "environmental" factors that act in conjunction with both genetic and acquired susceptibility. This is an optimistic message for cancer prevention in that exposure to environmental carcinogens—tobacco smoke, dietary constituents, pollutants (in the workplace, air, water, and food supply), drugs, radiation, and infectious agents—is theoretically preventable. But it challenges scientists to document environment-susceptibility interactions and policy-makers to rapidly