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Back to the Future: Mechanism-Based, Mutation-Specific Combination Chemoprevention with a Synthetic Lethality Approach

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Abstract

There is an increasing recognition that the mutations accompanying carcinogenesis may provide a window of therapeutic advantage designated synthetic lethality, an example of which is reported in this issue of the journal by Huang and colleagues (beginning on page 666). First discovered and studied in yeast, synthetic lethality has basic principles that have encouraged its development for treatment and now prevention in animal models of human cancer, especially malignancies refractory to standard approaches. The pros and cons of this approach and challenges in implementing it clinically are discussed. Cancer Prev Res; 4(5); 628–32. ©2011 AACR.

Historical Perspective from Classical Combination Therapy

It has been recognized for more than 50 years that curing advanced cancer requires the simultaneous use of 2 or more drugs. Even before the acknowledgment that cancer was a genetic disease, several childhood cancers and testicular cancer were cured by using cytotoxic combinations of drugs with different mechanisms of action. These successful uses of combinations and subsequent advances in cancer biology led to the recognition that frequent relapses after response to cancer therapy are because of multiple pathways that can enable resistant cancer cell populations to survive. The early experience with the use of compounds to chemically prevent cancer also suggested that combinations of agents with different mechanisms of action would be more effective than single agents (1).

An appreciation of the genetic origin of cancer and the recognition that the major phases of cancer development (initiation, promotion, and progression) are accompanied by the sequential development of genetic alterations was a critical milestone for understanding carcinogenesis at a molecular level (2), setting the stage for the development of specific molecularly targeted therapies. The initial documented success with imatinib in targeting BCR-ABL for chronic myelogenous leukemia and in targeting c-Kit for some gastrointestinal stromal tumors showed the potential of molecular-targeted, personalized therapy (3), although other successes did not rapidly emerge. Recent successes against metastatic melanoma with a BRAF inhibitor and against some adenocarcinomas of the lung with an anaplastic lymphoma kinase (ALK) inhibitor have again heightened expectations for molecular targeting (4). It is noteworthy and relevant to the current discussion, however, that no combination targeted therapy has yet emerged clinically from the targeted therapy development paradigm, which has important implications for the clinical implementation of synthetic lethality.

Essential Features of Synthetic Lethality

The problems and challenges of developing drug therapies are complex and daunting (3, 6). Four major areas of potential influence have been identified: Genetics, synergy, lineage, and host. Synthetic lethality takes advantage of 2 of these 4 potential areas and describes a cellular condition in which 2 (or more) nonallelic and nonessential mutations, which are not lethal on their own, become deadly when present within the same cell (7). The entire idea of therapeutic (or preventive) synthetic lethality rests on the premise that neoplastic cells develop mutations that normal cells do not, and that inhibiting first one and then another critical pathway (i.e., both required to lead to an essential biochemical or molecular product) with a drug will be lethal to the malignant cells. Reference 7 presents an extensive review of this complicated topic; a simplified

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version of pharmacologically induced synthetic lethality is depicted in Figure 1A, which relates to the approach reported by Wu and colleagues (Huang and colleagues) in this issue of the journal (8) and discussed later.

Following development of genetic synthetic lethality screens in yeast (9), Hartwell and colleagues (10) suggested that such an approach could be used in developing cancer therapy. Although this suggestion was prescient, methods for genetic manipulations in eukaryotic cells did not become sufficiently robust until synthetic siRNA emerged and genome-wide screens became readily available and accessible. The involvement of a surprising number of unexpected pathways in predicting the sensitivity of established drugs was found in yeast (11) and set the stage for screening large chemical libraries in human cancers. Three major methodologies have been established for high-throughput screening for chemical synthetic lethality in mammalian cells, and the pros and cons of each methodology have been extensively discussed elsewhere (7, 12–14). These approaches have been used to screen for chemical synthetic lethality in the setting of activated oncogenes or with tumor suppressor gene deficiency. Despite an enormous amount of work, selective cancer drugs chosen for their synthetic–lethality interactions has not yet occurred. Not surprisingly, the high “hit” rate found in yeast has not been replicated in the much more complex DNA and protein damage environment of human tumors. The pathway to validating compounds with synthetic lethality in advanced cancers is likely to be long and arduous; hence, providing an overall rationale for examining this approach during earlier stages of carcinogenesis, when genetic damage is less and redundant resistance pathways are fewer.

Synthetic Lethality As a Strategy for Chemoprevention: Rationale

Why then might one pursue the synthetic lethality approach for the therapeutic prevention (chemoprevention) of cancer? Simply put, because the number of genetic abnormalities is considerably fewer, cellular heterogeneity is likely to be less extensive, and the number of operative compensatory survival pathways is likely reduced in the preneoplastic state.

Wu and colleagues have previously shown that TNF-related apoptosis-inducing ligand (TRAIL) plus retinyl

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Figure 1. A, a simplified example of synthetic lethality induced pharmacologically with TRAIL plus Smac mimic. The general synthetic lethality concept is that mutations in cancer cells prevent their ability to recover from inhibition of a reexpressed or alternative pathway; this effect potentially would lead to an enhanced risk/benefit ratio of intervention because normal cells should be unaffected or minimally affected. Theoretically, the biochemical or molecular targets could be at the RNA, RNA, protein, enzyme, or epigenetic level, and inhibitors could include siRNAs, small molecules, and targeted nanoparticles. WT, wild type. B, mutant KRAS-induced lung premalignancy. Mutant KRAS activates c-MYC (which suppresses FLICE-like inhibitory protein [cFLIP]) and downregulates decoy receptors, which sensitizes the death receptor (DR) pathway to TRAIL activation (binding DR4/5 and activating caspase 8/10). Smac mimic inhibits XIAP, thus activating caspase 3, which induces cell death by apoptosis. WT, wild type.
acacetate (which downregulates TRAIL decoy receptors) induced apoptosis in adenomatous polyposis coli (APC)-deficient cells through the activation of c-MYC and effectively prevented colorectal cancer in an APC<sup>Min</sup>/<sup>+</sup> mouse model (15). Building on this work, these investigators postulated that the frequent mutations and activation of KRAS (which results in c-MYC activation) in non–small cell lung cancer (NSCLC) might be a good target for the synthetic lethality approach. More on this topic later.

As reported in this issue of the journal, the Wu group (Huang and colleagues) conducted a series of elegant experiments showing that the combination of TRAIL and a small-molecule Smac/DIABLO (Smac) mimic [which inhibits X-linked inhibitor of apoptosis protein (XIAP) to activate caspase-3] enhanced the frequency of apoptosis in human bronchial epithelial cells transfected with a retroviral plasmid expressing a mutant KRAS (Fig. 1B; ref. 8). A novel finding of this study was that mutant KRAS downregulates decoy receptors, avoiding the need of retinyl acetate for this purpose. As shown in Figure 1A, neither agent alone induced synthetic lethality in mutant KRAS cells. These encouraging results provided the basis for treating adeno Cre virus–infected LSL-KRAS G12D mice with TRAIL plus Smac mimic. The combination treatment markedly decreased neoplastic lung lesion formation without inducing apoptosis in normal lung epithelial cells. Of particular interest, most lesions in mice receiving this intermittent combination treatment were hyperplasias, whereas control mice developed adenomas. The authors argue that their results support wider exploration of synthetic lethality, particularly that involving TRAIL and a Smac mimic, for chemopreventive activity against several human cancers. Other reasons the authors give for expanding the study of synthetic lethality are the following features of this approach:

- Eliminates preneoplastic cells via apoptosis and can be achieved using intermittent courses, whereas traditional chemoprevention requires continuous application and modulates tumorigenesis without substantially eliminating premalignant cells. Apoptotic effects could allow short-term therapy.
- Selectively targets altered cells.
- Intermittent therapy should reduce potential side effects and the costs often associated with long-term cancer prevention.

With regard to selective targeting of altered cells, the synthetic lethality approach may be particularly relevant for personalized chemotherapy and chemoprevention in cases in which no specific drugs are available for targeting an oncogenic driver or where prolonged blockade of the driver would induce unacceptable toxicities. Take the lung, for example. Notwithstanding important progress in targeting specific lung cancer alterations, such as EGFR mutations and the EML4-ALK fusion protein, there are no effective current therapies for targeting perhaps the most common oncogenic driver of lung carcinogenesis and therapeutic resistance in NSCLC, which is mutant KRAS. KRAS mutations occur in 20% to 25% of NSCLC and seem to be an early event in lung carcinogenesis. A synthetic lethality approach in the clinic, paralleling the preclinical work of Wu and colleagues, could target multiple downstream KRAS effectors, ideally inducing apoptosis in neoplastic cells while sparing normal tissues. Indeed, other synthetic lethality approaches for mutant KRAS in lung and other neoplasias are an active area of preclinical research (16).

Before jumping on this bandwagon, however, let us examine these assertions and some limitations of this approach.

**Limitations**

**Highly engineered animal models only approximate human disease**

Although some animal models are reasonable models of human disease (e.g., the ApC<sup>Min</sup>/<sup>+</sup> mouse as model for familial adenomatous polyposis), others such as the highly engineered model system in the Huang and colleagues study are more mannequin-like and are unlikely to be broadly representative of the human disease. Notwithstanding this fundamental problem, targeting mutant KRAS cells, as done by the authors in their model, via targeting the mutant KRAS pathway (there are no direct KRAS inhibitors) is extremely important in lung cancer prevention or therapy; as pointed out above, KRAS is an important clinical target in lung neoplasia. It is likely, however, that cancer in outbred humans (as opposed to inbred mice) gives rise to pathways not recognized in inbred mice that may obviate the benefit of the targeted nature of synthetic lethality. It is relevant of course that synthetic lethality has only recently been used in the treatment of advanced human cancer, even though the strategy was suggested more than a decade ago: this use involved single-agent PARP inhibitors in *BRCA1* or *BRCA2* mutation carriers with advanced breast or ovarian cancer (17, 18). Although no clinical synthetic lethality approach with combined agents has been reported to date, the presently (8) and previously (15) reported preclinical work of Wu and colleagues may be a harbinger of the clinical promise of combination synthetic lethality. One may argue that premalignant cells may be more amenable to the synthetic lethality approach because their genetic alterations are fewer than in advanced disease, but the considerable challenge remains of identifying specific treatments for specific genetic alterations underlying the disease. The recently reported high response rate of a BRAF inhibitor in a cohort of patients with metastatic melanomas, coupled with the frequent appearance of keratoacanthomas and cutaneous squamous cell carcinomas (4), suggests that many more surprises are in store when complex molecular pathways are manipulated. Possibly tolerable or acceptable in the setting of advanced cancer, such side effects are not likely to be so in the setting of earlier disease.
Daunting public and regulatory obstacles

The issue of toxicity vis-à-vis the benefit of chemoprevention is of course a critical one (19) and thus far has been a major impediment to the adoption of chemoprevention despite the demonstration of its "proof of principle" and efficacy in the breast, colon-rectum, skin, and prostate (20–22). As a combination approach, synthetic lethality faces the further obstacle of enormous scientific and regulatory concerns over agent combinations. Only 1 trial of combined targeted agents (difluoromethylornithine plus sulindac) for chemoprevention in humans (colorectal) has been reported to date (23). Although this regimen was highly successful, neither effects on apoptosis nor on synthetic lethality were operative. The combination is in further clinical development designed to meet the scientific and regulatory requirements for U.S. Food and Drug Administration approval for chemoprevention. And so, what next for synthetic lethality?

A prescription for long-term success

Huang and colleagues should be applauded for trying to develop alternative, molecularly based and targeted therapies for chemoprevention, particularly for lung cancer. Trying this technique in the lung is a superb idea as none of the classic cancer chemoprevention strategies have worked in this site. The following questions need to be addressed in the further development of the synthetic lethality approach:

1. What intermediate biomarkers or risk factors for lung cancer will be chosen to assess the potential effects of synthetic lethality? If there are none, how will they be developed?

2. Are the candidate agents available orally? If not, can they be made available through new pharmaceutical formulations such as nanoparticle encapsulations? Drugs that cannot be given orally typically are an absolute nonstarter for chemoprevention, although very short-course intermittent treatment potentially could make a synthetic lethality approach, such as proposed by Huang and colleagues, an exception to this rule.

3. What kind of short-term trials are needed to select agent dose, scheduling, pharmacokinetics, and pharmacodynamic? How would intermittent dosing be accomplished? What is the risk/benefit and efficacy trade-off of intermittent versus continuous daily dosing?

4. What cohort will be the first group, or "lowest hanging fruit," for a phase III trial? A genetic risk group? High tobacco experience? Current or prior smokers? Patients with prior (resected) early-stage lung cancer? Again, the benefit vis-à-vis agent toxicities will be a major concern in any chemoprevention trial.

The next step should be to bring these ideas about synthetic lethality to the clinic through a careful development of translational studies. Not for the faint of heart, that is for sure!

Disclosure of Potential Conflicts of Interest

F.L. Meydkens is cofounder of Cancer Prevention Pharmaceuticals, Tucson, Arizona, and holds a substantial equity interest; he is also Chief Medical Advisor for the company, but takes no remuneration. E.W. Gerner has an ownership interest in Cancer Prevention Pharmaceuticals.

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