Synthetic lethality: beating cancer at its own game

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The primary focus for targeted cancer agents has typically been to counteract the oncogenic signaling that results from genetic defects. A new strategy is emerging that actually seeks to exploit the oncogenic features of tumor cells rather than overcome them. Synthetic lethality (SL) is a situation in which 2 nonlethal mutations become lethal to a cell when they are present simultaneously. If SL were to be exploited for anticancer therapy, it could lead to the development of highly selective, less toxic drugs, while expanding therapeutic targets to include those that have, until now, proven pharmaceutically intractable. Here, we discuss the idea of SL and how it can be applied to cancer therapy.

Exploiting SL in cancer treatment

There are several issues facing current cancer therapies. Traditional therapies, that seek to target rapidly proliferating cells, are extremely toxic because they kill both cancer cells and normal cells fairly indiscriminately. Newer therapies, though more targeted to cancer cells, face the significant issue of resistance, which limits their utility.

SL was first observed in genetic studies in yeast and occurs when 2 separate genes allow for a viable cell when mutated individually but are lethal to the cell when present simultaneously. The 2 genes are said to be SL partners. In the late 1990s, researchers began to examine whether the concept of SL might be applied to cancer, which is fundamentally a disease driven by genetic mutations. Thanks to improvements in genome sequencing technology, many of the genetic abnormalities underlying cancer are now known. The theory was that if SL partners for genes that were mutated in cancer could be identified, then they might present a therapeutic strategy that would specifically kill cancer cells that harbored those mutated genes.[1-3]

SL predominantly occurs because many of the molecular pathways that control cellular functions overlap with one another, so that perturbations in 1 pathway can lead to a dependency on another, "back-up" pathway. It is this dependency that could be targeted by SL-directed anticancer drugs, which would aim to knock out the secondary, back-up system to kill the cancer cell. Two synthetically lethal genes can be part of a single linear signaling pathway, part of 2 parallel pathways that direct a common cellular process, or part of 2 independent cell survival pathways that compensate for each other, each one acting as a salvage pathway in the absence of the other (Figure 1).[2]

The most significant advantage of a SL strategy for cancer therapy is that it offers exquisite specificity because it should kill only cells that harbor a certain genetic mutation. It also offers the opportunity to exploit targets that, until now, have proven challenging, such as tumor-suppressor proteins that are not necessarily readily amenable to current drug development. Finally, it provides the potential to treat more advanced, metastatic disease that has developed multiple mutations and may perhaps have become refractory to other treatments.[3]

Identifying SL interactions

SL interactions are typically identified by performing a screen in which, against a background of mutated gene A, a variety of candidate SL partner genes (gene B) are mutated to determine which cause cell death. Historically, there have been 3 major approaches to screening (Figure 2). Initially, it was performed predominantly in model organisms, such as yeast, worms, and fruit flies, because they were amenable to simple, rapid screens. The disadvantage was that an SL interaction demonstrated in a model organism may not necessarily translate into human cells, even when homologues of the genes involved exist.

In 2001, the demonstration that RNA interference (RNAi) – the major technique by which gene function can be inactivated experimentally – was also feasible in mammalian cells, meant that SL screens became possible in mammalian cancer cells. There are 2 very different approaches to testing SL interactions in mammalian cells. Knowledge-based

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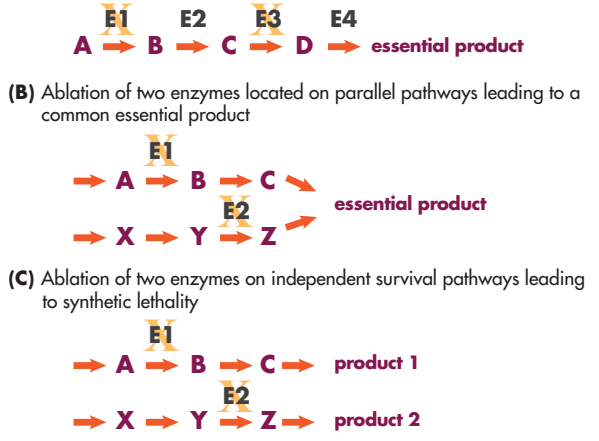

FIGURE 1 Three modes of synthetic lethality. Synthetic lethal mutations may constitute partial mutations present in one essential pathway, mutations in components of two parallel pathways that lead to the same essential product, or mutations in two independent survival pathways that compensate for one another – each one serving as a salvage pathway in the absence of the other. Reproduced with permission from Canaani, D. Application of the concept of synthetic lethality toward anticancer therapy: A promise fulfilled? [Published online ahead of print September 3, 2013]. Cancer Lett. doi: 10.1016/j.canlet.2013.08.019.

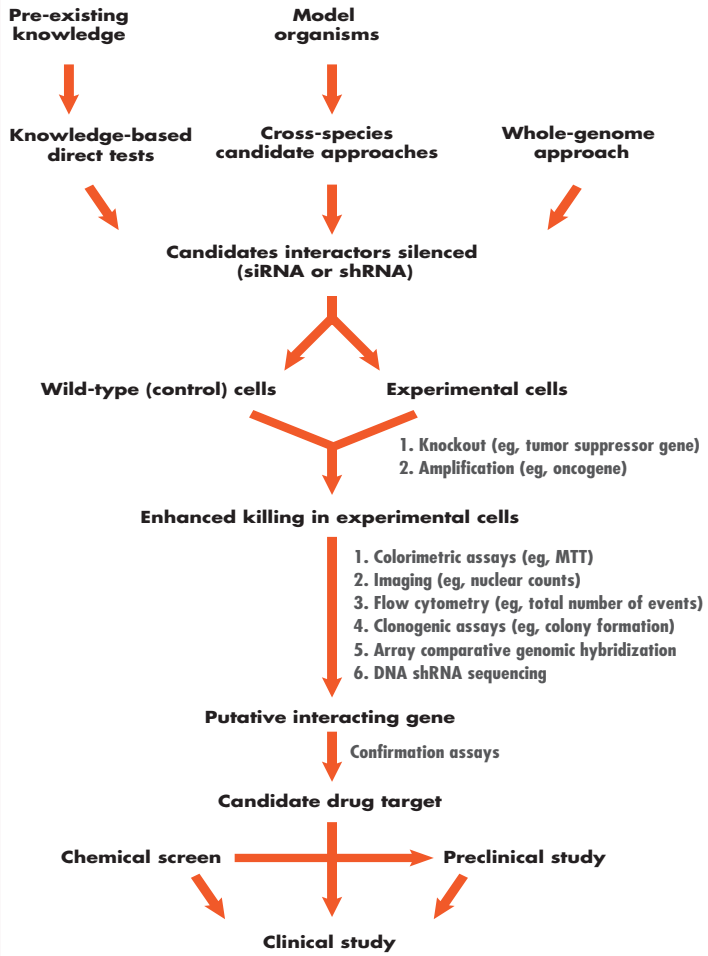

FIGURE 2 A depiction of the 3 fundamental experimental approaches typically used to identify synthetic lethal interactions: knowledge-based direct tests, an 'educated guess' approach that requires some pre-existing knowledge of the functions and interactions of the genes being tested; cross-species approaches that use animal models such as yeast, fruit flies, or worms, to test genetic interactions more simply and then infer interactions in their homologous genes in humans; and whole-genome approaches in which an entire gene or drug library is tested to identify potential 'hits.' Either siRNA or shRNA can be used to silence the target genes in these approaches. Reproduced with permission from Sajesh et al. Cancer Lett. 2013;5:739-761.

shRNA, short hairpin RNA; siRNA, small intering RNA; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide.

direct testing is essentially an "educated guess" that can be undertaken if there is a significant amount of pre-existing knowledge about genes being tested and SL is already suspected.

Alternatively, advances in high throughput technology have meant that we can now perform completely unbiased whole genome-based screening. SL interactions can be identified and validated using either an RNAi library (testing candidate genes) or a small-molecule compound library (testing candidate drugs). On the one hand, the advantage of an RNAi library screen is that it provides direct identification of a genetic target, so that we know exactly which genes are involved in the SL interaction. However, this may not necessarily lead to the development of a therapeutic compound as the target may not be amenable to drug development. On the other hand, a small-molecule compound screen directly identifies drugs that could be used to generate SL in cancer cells, the disadvantage being that we may not fully understand the mechanism of action.[1,3-5]

Targeting SL for cancer treatment

There are several scenarios in which SL can occur in cancer cells (Figure 3):

- Activated or overexpressed oncogene plus inactivated second gene.
- Inactivated tumor suppressor gene plus inactivated second gene.
- Inactivation of 2 components of same signaling network.
- Inactivation of a pair of genes involved in DNA repair or synthesis.[4]

Targeting tumor suppressors and oncogenes

The oncogene *Ras* is estimated to be mutated in about a quarter of all cancers and therefore represents a significant

target for cancer therapy but, until now, it has not proven easily druggable. SL screens are therefore underway to see if this strategy could allow us to indirectly target *Ras* in tumors. So far, the *KRAS* gene has been found to have SL with genes like cyclin A2, kinesin-like protein 2C, polo-like kinase 1 (PLK1) and the anaphase-promoting complex. Since these genes encode proteins that regulate mitotic cell division, this suggests that *KRAS*-mutant cells may be particularly vulnerable to perturbations in mitosis and could be preferentially killed by drugs that affect this process, such as paclitaxel. PLK1 inhibitors, such as BI2536, are also in clinical development and could be tested in *KRAS*-mutant patients.[3]

The epidermal growth factor receptor (EGFR) is already a validated cancer therapeutic target, with several EGFR inhibitors and monoclonal antibodies approved or in development. An SL screen of the EGFR protein network was recently carried out to determine if EGFR inhibitors could be used in an SL capacity. SL interactions included protein kinase C and aurora kinase A. As a result, phase 1 and 2 trials of an aurora kinase A inhibitor (alisertib) are underway in combination with erlotinib (NCT01471964).[6]

The most significant tumor suppressor gene is p53; mutated in half of all human cancers. However, it is not an ideal target for drug development based on conventional methods. An SL screen to identify compounds that inhibit growth of p53-deficient tumor cells identified the chemotherapeutic paclitaxel and the antidiabetic drug metformin. Researchers are trying to disseminate the molecular mechanisms underlying the SL effects of these drugs in a mutant-p53 background.[7,8]

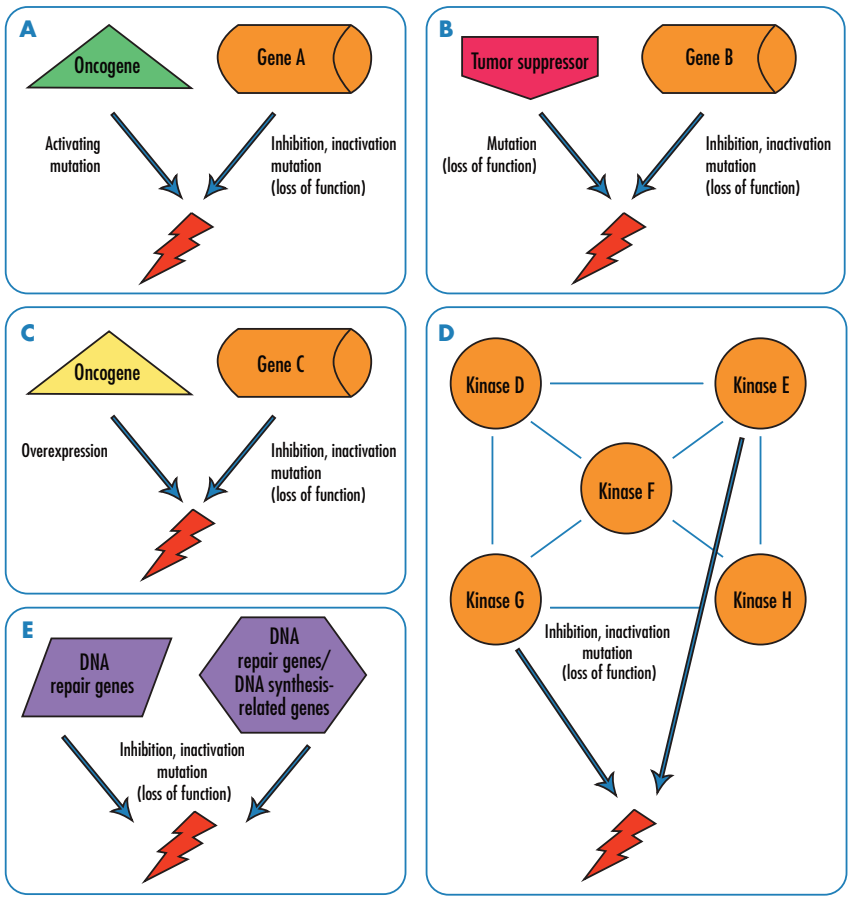

FIGURE 2 Possible molecular interactions leading to synthetic lethality (red arrows denote synthetic lethality). A, Activated oncogene and inactivation of another gene. B, Inactivated tumor suppressor gene and inactivation of another gene. C, Overexpression of an oncogene and inactivation of another gene. D, Inactivation of 2 kinases as part of a signaling pathway. E, Inactivation of a pair of DNA repair genes and DNA synthesis-related genes. Reproduced with permission from Weidle et al. Cancer Gen Proteom. 2011;8:159-172.

Targeting components of signaling pathways

Anti-EGFR therapies have demonstrated little efficacy in breast cancer and this is thought to be because the Notch pathway is hyperactivated, which may compensate for the loss of EGFR by maintaining activation of the downstream kinase Akt. In support of this hypothesis, a SL interaction was recently reported between the EGFR and Notch signaling pathways in basal-like breast cancer and could provide a target for therapy in these patients.[7]

A fraction of colon cancers have constitutive activation of the Wnt/β-catenin signaling pathway and the cancer cells have become "addicted" to this pathway. Therefore, identification of genes that are SL with members of the Wnt pathway could provide a means to kill colon cancer cells. An SL screen of Wnt identified the vascular endothelial growth factor receptor (VEGFR1) that initiates the VEGFR pathway.[9]

As well as providing potential targets for therapies to directly exploit SL, screens have uncovered SL as a previously unrecognized mechanism of action for a number of drugs approved by the US Food and Drug Administration. One example is the mammalian target of rapamycin (mTOR) inhibitor temsirolimus, which is FDA approved for the treatment of renal cell carcinoma. Tumors that are deficient in the tumor suppressor protein phosphatase and tensin homologue (PTEN) are more sensitive to mTOR inhibition. mTOR acts downstream of phosphoinositide-3-kinase (PI3K)/Akt signaling, which is upregulated in PTEN-deficient tumors. Thus mTOR is SL with PTEN as removal of both genes prevents cancer cells from compensating for the loss of PTEN by upregulating PI3K/Akt signaling.[7]

Proof-of-principle: PARP and BRCA

- Many different research groups have shown that mutation of the breast cancer susceptibility genes (BRCA1/2) is synthetically lethal in combination with inhibition of the DNA repair enzyme poly(ADP) ribose polymerase 1 (PARP1). BRCA1/2 are tumor suppressor genes that are mutated in many patients with breast, ovarian, prostate, and a few other cancers.
- Synthetic lethality is thought to result from the fact that PARP enzymes repair single-strand breaks (SSBs; a common form of DNA damage), whereas BRCA1/2 are involved in the repair of double-strand breaks (DSBs). DSBs are a lethal form of DNA damage that occurs when SSBs go unrepaired. If unrepaired, DSBs will lead to cell death. BRCA1/2 will repair the SSBs that result from PARP inhibition and, therefore in BRCA1/2 mutant cells PARP inhibition will lead to cell death.
- BRCA1/2 mutant ovarian and breast cancer cells were found to be profoundly sensitive to small molecule PARP inhibitors. As a result, a number of PARP inhibitors are in clinical trials, with the most advanced currently being niraparib (Table).
- Small molecule PARP inhibitors function in two different ways: first, through direct inhibition of PARP enzymatic activity and second, through trapping of PARP on DNA.
- PARP inhibitor development has not been without its setbacks:
- A planned phase 3 trial of olaparib in ovarian cancer patients was halted when phase 2 trials demonstrated no overall survival benefit.
- Iniparib, originally thought to be a promising PARP inhibitor, was subsequently shown not to be a true inhibitor of PARP1, and phase 2 trials in triple negative breast cancer patients were halted by Sanofi because of limited success.
- Resistance is a significant issue as secondary mutations can develop that may restore BRCA function.
- Although there are currently no FDA-approved PARP inhibitors, more than 100 clinical trials are ongoing.
- Olaparib was resurrected in April 2013, when Astra Zeneca declared that they would be pushing it forward into phase 3 trials in patients with deleterious BRCA1/2 mutations, following a retrospective analysis of this subset of patients. Olaparib conferred an 82% reduction in the risk of disease progression or death and a PFS benefit of over 7 months compared with placebo (11.2 vs 4.3 months, respectively; $P < .001$).[1-3,7,11,12]

Targeting DNA repair/synthesis pathways

The DNA in our cells can become damaged through environmental exposure or via errors introduced during its replication. Cells use a number of different mechanisms that allow them to repair damaged DNA, which include base excision repair, nucleotide excision repair, homologous recombination, and nonhomologous end-joining. Cancer is a disease of genetic instability, with tumors containing multiple genetic mutations, which are often generated by defects in DNA repair pathways. In order to thrive in spite of these defects, cancer cells become dependent on alternative DNA repair pathways and, as such, these have garnered significant interest in the development of SL therapies.[2,10]

The most significant example of SL between components of different DNA repair pathways is that between poly(ADP) ribose polymerase 1 (PARP1) and the breast cancer susceptibility genes *BRCA1/2* (see sidebar). Researchers have subsequently begun to identify other genes involved in the DNA damage response that also display SL with PARP inhibitors, a property that has been dubbed "*BRCA*ness" and which offers the potential to expand the clinical utility of PARP inhibitors. Examples include RAD51, ataxia telangeiectasia mutated (ATM), ataxia telangiectasia and Rad3 related (ATR), checkpoint kinases (CHK1/2), and cyclin-dependent kinase 1. A CHK1 inhibitor is currently undergoing phase 1 and 2 clinical trials (Table 1), and ATM, RAD51 and ATR inhibitors are in preclinical testing.[2] SL screens have also been performed for a range of other DNA repair genes. For example, SL was recently demonstrated between DNA mismatch repair proteins and DNA polymerases, both of which repair oxidative damage to DNA but via different mechanisms.[3,11]

Enhancing traditional therapy

Traditional cancer therapies, such as chemotherapy and radiation therapy, work because they induce DNA damage in cancer cells. Resistance or relapse is a significant issue with these therapies and one driving force behind this is that DNA damage can be repaired by other, compensatory mechanisms. Thus, SL therapy could potentially target these other mechanisms to enhance the efficacy of traditional therapies.

A number of DNA repair genes have been shown to be involved in repairing the DNA damage induced by chemotherapy. The base excision repair DNA repair pathway is required to repair damage caused by alkylating agents, frequently used as chemotherapy. AP endonuclease 1 (APE1) is the rate-limiting step in this pathway and therefore represents an important target for SL therapy. An APE-1 inhibitor is currently undergoing clinical trials (Table). O6-methylguanine-DNA methyltransferase (MGMT), on the other hand, repairs damage caused by another kind of chemotherapy - monomethylating and chloroethylating agents. An MGMT inhibitor is also in clinical trials (Table). PARP inhibitors also induce SL in cancer cells treated with chemotherapies that affect DNA integrity, such as alkylating agents.[3,10]

Although the concept of SL has been understood for

TABLE 1 Examples of synthetic lethal drugs in clinical trials

Agent (brand name/s)	Sponsor	Mechanism of action	Stage of clinical testing, clinical trials.gov identifiers
Veliparib (ABT-888)	Abbott	PARP inhibitor	Phase 1 in solid tumors, central nervous system tumors, metastatic breast cancer, etc. NCT00946335, NCT00770471, NCT01063816
Olaparib	Astra Zeneca	PARP inhibitor	Phases 1 and 2 in serous ovarian cancer, gastric cancer, non–small-cell lung cancer, etc. NCT00753545, NCT01063517, NCT01513174
Rucaparib (AG014699)	Clovis Oncology	PARP inhibitor	Phases 1 and 2 in ovarian cancer, breast cancer, advanced solid tumors, etc. NCT01482715, NCT01009190, NCT00664781
Niraparib (MK4827)	Tesaro	PARP inhibitor	Phase 3 ovarian cancer and breast cancer NCT01847274, NCT01905592
BMN-673	BioMarin Pharmaceutical	PARP inhibitor	Phase 1 in advanced solid tumors and hematologic malignancies NCT01399840, NCT01776437
06-benzylguanine	Various	MGMT inhibitor	Phases 1 and 2 in T-cell cutaneous lymphoma, multiple myeloma, and brain tumors NCT00275002, NCT00004072, NCT00961220
TRC102	Tracon Pharmaceuticals	APE inhibitor	Phase 1 in solid tumors and lymphomas NCT01851369
LY2603618	Eli Lilly	Chk1 inhibitor	Phases 1 and 2 in non–small-cell lung cancer, pancreatic cancer, solid tumors, etc. NCT01139775, NCT00839332, NCT01341457

APE, AP endonuclease; Chk1, DNA checkpoint kinase 1; MGMT, O6-methylguanine DNA methyltransferase; PARP, poly(ADP-ribose) polymerase

many years, technological advances and improved understanding of the molecular mechanisms underlying cancer are only now beginning to allow researchers to fully exploit this condition for the development of anticancer therapies. Although there remain significant hurdles to this strategy, the future looks promising.

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