Approaches to Identifying Synthetic Lethal Interactions in Cancer

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TRADITIONAL CHEMOTHERAPY, including irradiation or DNA damaging agents, relies on this principle. Most cancer cells have defects in their cell cycle checkpoints where DNA would normally be repaired before replication [5,6]. Thus, radiotherapy and DNA damaging agents target DNA in the continuously dividing cancer cells, causing cell death. Normal cells, with their intact cell cycle checkpoints, repair the damage before dividing [5,6]. However, these therapies often have serious side effects and greatly reduce a patient’s quality of life [7].

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†Abbreviations: AML, acute myeloid leukemia; APC, adenomatous polyposis coli; ATM, ataxia telangiectasia mutated; ATR, ataxia telangiectasia and Rad3-related protein kinase; BID, BH3 interacting-domain death agonist; BRCA, breast cancer early onset; BRG1, Brahma related gene 1; BRM, Brahma; CCNE1, cycline E1; CPT, camptothecin; DECODER, Deep Coverage Design shRNA Library; DISC, death-inducing signaling complex; FA, Fanconi anemia; FANCID2, Fanconi anemia group D2; gRNA, guide RNA; HR, homologous recombination; IR, ionizing radiation; KRAS, Kirsten rat sarcoma; mESC, mouse embryonic stem cells; MSH2, MutS protein homolog 2; NSAIDs, nonsteroidal anti-inflammatory drugs; PARP1, Poly (ADP-ribose) Polymerase 1; RCC, renal cell carcinomas; RNAi, RNA interference; SCNA, somatic copy number alteration; SDL, synthetic dosage lethality; SoF, survival of the fittest; SWI/SNF, switch/sucrose NonFermentable; TOP, topoisomerase; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; VHL, Von Hippel Lindau.

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Synthetic lethality represents a more targeted approach that exploits the specific changes within the cancer at a single gene level that separate it from healthy tissue.

**SYNTHETIC LETHALITY**

Synthetic lethality is defined as the interaction between two co-essential genes such that inhibiting the function of either gene separately results in cell survival, but inhibiting the function of both genes results in cell death (Figure 1) [8-10]. Inhibition of a gene may be achieved by chemical or genetic means. For instance, the genetic inhibition of a gene’s function may occur through RNAi, mutation, deletion, epigenetic changes, or perturbations of upstream regulators. Chemical inhibition of the gene’s function may be achieved by treatment with a chemical compound. The inhibition of co-essential genes can occur at any level, and the loss of function does not have to occur in the same manner for both co-essential genes. For example, one gene can be lost due to deletion and the other inhibited by a chemical compound. While synthetic lethality is best known in the context of loss-of-function mutants [9], other perturbations including gene overexpression [11,12], epigenetic changes [13], and cell extrinsic differences [14,15] can cause these interactions to occur [10] (Figure 1). In identifying genes that are synthetically lethal within each cancer type, we gain an understanding of the molecular biology of those cancers and how it can be specifically exploited. There are multiple approaches that have been successfully used to identify synthetic lethal interactions in cancer.

**DIFFERENT APPROACHES TO IDENTIFY SYNTHETIC LETHAL INTERACTIONS**

**Hypothesis-Driven Approach**

It is possible to identify synthetic lethal interactions using a candidate or hypothesis-driven approach if the alterations to the molecular pathways in the cancer are well established. For instance, if a tumor suppressor is frequently lost and the resulting changes to the gene expression profile are known, then it may be possible to use RNA interference (RNAi) or chemical compounds to inhibit the genes that are expected to be upregulated to compensate for the loss. This may result in a synthetic lethal interaction. Mutations in the breast cancer early onset (BRCA1) and BRCA2 genes are known to occur frequently in breast and ovarian cancers. Knowing that BRCA1/2 are involved in homologous recombination (HR) and DNA double-strand break repair, both Byant et al. [16] and Farmer et al. [17] hypothesized that inhibition of Poly (ADP-Ribose) Polymerase (PARP), responsible for DNA single-strand break repair, would be synthetically lethal with loss of BRCA1/2. This hypothesis is based on the knowledge that PARP deficiency results in spontaneous single-strand breaks at the DNA replication fork, which require HR for repair. Cells deficient in BRCA1/2 are unable to provide HR for repair of DNA single- and double-strand breaks caused by chemical or genetic PARP inhibition, causing synthetic lethality. The synthetic lethal interaction was extended to in vivo studies, followed by clinical studies where treatment of BRCA1/2-deficient tumors with a DNA damaging agent combined with a PARP inhibitor extended patient survival over chemotherapy alone [18,19].

Synthetic lethal interactions also may explain why particular chemical compounds have increased efficacy in cancers characterized with specific genetic alterations. While clinical trials for PARP inhibitors in ovarian cancer were successful in increasing progression free survival by 62 percent overall [20], in a follow-up analysis of the same study, it found that the BRCA1/2-deficient group had a much higher rate of 82 percent [21]. Another study by Leibowitz et al. [22] hypothesized that non-steroidal anti-in-
Inflammatory drugs (NSAIDs) are highly effective in preventing colorectal tumorigenesis due to a synthetic lethal interaction with the loss of the adenomatous polyposis coli (APC) gene. While NSAIDs were able to activate cell death pathways in both cancer and normal cells, APC deficiency triggered the activation of BH3 interacting-domain (BID), a death agonist in extrinsic apoptotic pathway, resulting in synthetic lethality [22]. In situations in which putative targets cannot be identified, a screening-based approach is necessary.

**Screening-Based Approaches**

Most large scale approaches investigating synthetic lethal interactions in cancer rely on the comparison of drug or RNAi treatment in “matched” cell lines (Figure 2). Matched cell lines are generated so that their only difference is in the expression/activation status of the gene of interest. In studying a loss-of-function phenotype (Figure 1A), the parental cancer cell line may have lost the expression of a gene, have inactivating mutations, or have been treated with an extrinsic factor (like a chemical compound) such that the activity of the gene is lost. In this model, a matched cell line could be generated from a parent cancer cell line deficient in the gene by overexpressing it (Figure 2A). Next, multiple cell lines with and without expression of the gene might be investigated (Figure 2B). Finally, one could inactivate the gene in a cell line expressing it (Figure 2C). In studying a gain-of-function phenotype (Figure 1B), the parental cancer cell line may have acquired a new gene fusion, oncogenic mutation resulting in constitutive activation, overexpression of the gene, or have been treated with an extrinsic factor (like a receptor ligand) such that the gene’s activity would increase. In this model, a matched cell line could be generated from a parent cancer cell line with an overactive gene by inactivating it (Figure 2D). Next, multiple cancer cell lines with and without expression of the gene could be compared (Figure 2E). Additionally, the gene could be overexpressed in the parent cell line, expressing it at a normal level (Figure 2F). Finally, a cell extrinsic factor like a re-
ceptor ligand could be used to treat the cells with low receptor activity such that activity of the receptor would increase (Figure 2F). These matched cell lines often differ in the activity of a single gene; one of them represents a cancer cell line characterized by a specific gene alteration, and the other represents a cancer cell line characterized by the absence of that alteration. In this respect, the latter simulates the normal tissue where genetic alterations are absent. Once the matched cell lines have been generated, they can be used in high-throughput screens. These screens can be separated into two categories: chemical libraries and genome-wide interference. Chemical libraries include both annotated and non-annotated libraries where the targets of the chemical compounds are known and unknown respectively. Genome-wide interference screens have been conducted successfully using siRNA, shRNA, and CRISPR. Many chemical library screens rely on the use of chemical compounds with unknown molecular targets. These screens usually contain tens of thousands of different compounds, which greatly increase the chances of getting a “hit.” A hit is a compound that is synthetically lethal within cancer cells containing the gene alteration being studied. The difficulty of these screens is in identifying the molecular target of the hit. Genome-wide interference screens rely on the use of siRNA, shRNA, or CRISPR libraries in order to inhibit genes involved in every molecular pathway within cells. Smaller libraries can be useful if specific pathways are established. Genome-wide interference screens suffer from the need to identify chemical compounds that inhibit the molecular targets identified before the interaction can be utilized in clinical trials. If the compounds are not available, their development can be
were plated at low densities and allowed to form colonies, mat (Figure 3A). Clonogenic assays, in which the cells treated in parallel with the chemical library in a plate for-

Yellow Fluorescent Protein (EYFP), and fluorescence was counted as a high-throughput viability assay. Following the identification of hits, an investigation of the actual drug targets and mechanism of action is required. Often an additional screen is necessary to accomplish this. If the drug class has been previously investigated and its targets have been established, then confirmation that the drug is acting “on-target” can be performed using RNAi to knock down the putative target. Annotated chemical libraries, in which the targets of the drugs are known, can be useful tools for identifying synthetic lethal interactions. Since annotated libraries are often small (hundreds of chemical compounds) in comparison to non-annotated libraries (tens of thousands of compounds), the benefit from knowing the molecular target of a compound may be offset by the reduced chance in identifying hits from a screen. Likely for this reason, the use of non-annotated chemical library screens appears to be more frequently described in the literature. Below, we discuss two papers in which screens using non-annotated chemical libraries were employed.

The first paper exploited the fact that inactivation of the tumor suppressor Von Hippel Lindau (VHL) has been shown to occur in more than 70 percent of RCC [26]. The inactivation of VHL results in abrogation of its E3 ubiquitin ligase activity and overexpression of its targets. Turcotte et al. [23] utilized a library of 64,000 chemical compounds to identify synthetic lethal interactions in VHL-deficient RCC (Figure 1A). The screen used a VHL-deficient RCC4 parent cell line and matched RCC4-VHL cell line obtained by overexpression of VHL cDNA (Figure 2A). Both matched lines stably expressed Enhanced Yellow Fluorescent Protein (EYFP), and fluorescence was used to monitor cell number. The matched lines were treated in parallel with the chemical library in a plate format (Figure 3A). Clonogenic assays, in which the cells were plated at low densities and allowed to form colonies, were used to confirm their hits. The authors observed that VHL-deficient RCC cells treated with the hit STF-62247 accumulated intracytoplasmic vesicles more readily than RCC-VHL cells, causing RCC cell death. The synthetic lethal effect of STF-62247 also was confirmed in vivo using multiple matched RCC lines. Since the molecular target of STF-62247 was unknown, the authors utilized a yeast deletion pool to identify the target. This additional screen confirmed that STF-62247 was disrupting the trans-golgi network stimulating the maturation of autophagosomes to autolysosomes selectively in the VHL-deficient RCC.

The second paper was aimed at the identification of chemical compounds that would re-sensitize cancer cells to DNA damaging agents such as ionizing radiation (IR) or Cisplatin. These agents cause DNA intrastrand crosslinks and are effective in treating a wide variety of cancers with perturbed DNA repair pathways, including ovarian cancer [27]. Eventually, the cancer cells evolve to acquire drug resistance through various mechanisms, including activation of DNA repair pathways like the Fanconi Anemia (FA) pathway [28]. For example, DNA damaging agents are initially efficacious in ovarian cancer, but most patients relapse with resistant disease that is no longer sensitive to the treatment [29]. Jacquemont et al. [25] utilized a library of 16,000 chemical compounds to identify hits that would inhibit the overactive FA pathway, thus re-sensitizing cancer cells to DNA damaging agents (Figure 1B). The parent PD20 fibroblast cell line is Fanconi Anemia Group D2 (FANCD2)-deficient, causing FA pathway disfunction. The PD20-EGFP-FANCD2 cell line utilized for the screen was obtained by overexpression of Enhanced Green Fluorescent Protein (EGFP) labeled version of the wild type (WT) FANCD2 cDNA. This overexpression made the PD20-EGFP-FANCD2 cell line resistant to DNA damaging agents. The chemical compound screen was performed using 96-well plates seeded with PD20-EGFP-FANCD2 cells treated and untreated with IR (Figure 2F, extrinsic factor). Cells were dosed with the ICCB bioactives and Commercial Diversity Set 1, Chembridge DiverSet, and NINDS II chemical compound libraries. The plates were imaged using EGFP microscopy to identify hits that selectively reduced EGFP-FANCD2 foci formation by greater than 50 percent in IR treated cells, which indicates that the hit reduces DNA repair events, causing restoration of sensitivity to the IR treatment. From the 43 hits obtained, 26 hits also successfully reduced EGFP-FANCD2 foci formation in cells treated with Cisplatin. Fifteen hits were then validated in multiple ovarian cancer cell lines. Since the FA pathway is known to be involved in DNA repair and homologous recombination (HR), in a follow-up study, the HR efficiency was tested using a GFP-based reporter system, where GFP expression was correlated with HR events. The majority of the drugs acted by inhibiting the HR process (like the compound Bortezomib) or by directly inhibiting FANCD2 foci formation.

Taken together, non-annotated chemical library screens, as described above, can be an effective means to...
identify synthetic lethal interactions within individual matched cell lines. This method is not suited to studying a large number of cell lines in parallel since it relies on the plate format. One advantage that chemical library screens have in comparison to RNAi or CRISPR-based screens (see below) is that chemical compounds tend to inhibit families of targets, whereas RNAi is specific for individual members within the family. Different interactions can be identified with both approaches since some genes within the same family might be redundant and inhibiting a single gene would not cause synthetic lethality in an RNAi screen. In contrast, inhibiting the whole family by a chemical compound might be too cytotoxic in a chemical library screen, wherein inhibiting a single member may have been synthetically lethal. Overall, this approach is suited for studying specific cancer lines, generates a large number of hits, and provides compounds that can be used in vivo, but requires establishment of molecular targets.

### siRNA Library Screens

The siRNA library screen relies on a plate format wherein each siRNA is transfected separately in its own well (Figure 3B), which makes it very similar to the format described above for the chemical library (Figure 3A). In some screens, multiple siRNAs targeting a gene are pooled into the same well (called a siRNA pool, usually comprised of four individual siRNAs). Cell Titer-Glo luminescent cell viability assay from Promega is often used to assess viability and identify hits. Both studies investigated below rely on the addition of extrinsic factors to generate matched cell lines. Once hits have been identified, in vivo studies can be conducted using RNAi constructs and chemical compounds for the established targets can be investigated.

In the first study, Kranz et al. [32] performed a genome-wide siRNA screen to sensitize the human glioblastoma cell line U251MG treated with tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) to apoptosis (Figure 1B). TRAIL is an important ligand in the death receptor-mediated apoptosis pathway, which is often inactivated in cancers, including glioblastoma [30,31]. This screen was performed in a 384-well format using matched U251MG cells both with and without TRAIL treatment (Figure 2F, extrinsic factor) and Dharmacon’s SMART-pool siRNA library targeting 5,000 genes with four pooled siRNAs per target in each well (Figure 3B). Plates were analyzed using the Cell Titer-Glo viability assay to identify hits. Validation of the hits from the screen was performed, using the same system, by transfecting individual siRNAs for every gene hit instead of transfecting siRNA pools. FAT1 was chosen for further investigation since all four individual siRNAs resulted in decreased survival in response to TRAIL. This synthetic lethal interaction was also identified in five additional cell lines. The authors found that FAT1 is linked to the extrinsic apoptosis pathway and that it impedes Caspase-8 recruitment to the Death-Inducing Signaling Complex (DISC) in an ubiquitin-independent mechanism. Synthetic lethality of FAT1 with TRAIL treatment was confirmed by knocking out FAT1 using CRISPR/Cas9-mediated genome engineering.

In the second study, Josse et al. [35] performed a siRNA library screen to identify genes that are synthetically lethal with TOPoisomerase I (TOP1) inhibition in the breast cancer line MDA-MB-231 (Figure 1A). TOPoisomerase (TOPs) are enzymes that reverse the supercoiling of DNA that occurs during replication and transcription [33]. Inhibition of TOPs has been shown to be an effective means for killing constitutively proliferating cancer cells [34]. Inhibitors trap the TOP enzyme on the DNA and result in stalled replication forks and transcriptional complexes, causing DNA double strand breaks. Importantly, TOP1 inhibitors are in clinical trials or approved for ovarian cancer, colorectal cancer, breast cancer, and more [33,34]. The siRNA screen was performed using parallel 384-well plates of matched MDA-MB-231 cells treated with the TOP1 inhibitor Camptothecin (CPT) or vehicle control (Figure 2C, chemical compound). The Qiagen human druggable genome library version 4.1, targeting 7,000 genes with four individual siRNAs per gene, was transfected into cells. Cell Titer-Glo was used to assess viability and identify hits (Figure 3B). Forty-two hits were identified that selectively killed CPT-treated MDA-MB-231 while sparing vehicle-treated cells. To validate the top hits, the effects of the siRNAs were investigated with varying CPT concentrations and additional siRNAs for more than 40 of the candidate genes. One of the top hits was Ataxia Telangiectasia Mutated (ATM). Since ATM and Rad3-related protein kinase (ATR) function in parallel, and ATR inhibitors already have been developed and are in clinical trials, the authors confirmed that ATR inhibitors are synthetically lethal with TOP1 treatment. The authors found that ATR and its downstream target Chk1 are crucial factors in repairing DNA lesions that occur when TOP1 is inhibited. This synthetic lethal interaction was confirmed in vivo in mice with COLO205 colon cancer xenografts, and combination treatment with ATR and TOP1 inhibitors was more effective than individual treatment for either drug.

In summary, the siRNA approach, like the chemical library approach, is limited by the plate format since each siRNA or siRNA pool must be transfected into an individual well (Figure 3B, plate format) as compared to the shRNA library approach (discussed below) where shRNAs are transduced in a viral pool (Figure 3C, pooled format). Thus, the plate format limits the number of cancer types that can be investigated concurrently, but the screen can be performed using a high-throughput viability assay like Cell Titer-Glo. Hits can later be validated in additional cell lines. When performing secondary siRNA screens, each gene must be targeted with multiple non-pooled individual siRNAs to avoid false positives from off-target effects. The study by Josse et al. [35] investi-
gated more than seven individual siRNAs for their top hits in order to identify the best candidates for further analysis. Overall, the siRNA approach is well suited to investigating individual matched cell lines where understanding the specific genes involved within the synthetic lethal interaction is important.

**shRNA LIBRARY SCREENS**

The use of shRNA libraries (Figure 3C) can aid in identifying the key molecular pathways that a tumor is dependent on for survival. There are a number of common strategies when performing shRNA screens. First, most screens consist of shRNA virus pools, which makes the approach applicable for studying multiple cancer lines in parallel, although a plate format with individual shRNAs per well can also be used [36], similar to siRNA screens (Figure 3B). The readout from the pooled screen is often in the form of deep sequencing [37] or a microarray [38]. The hits from the screens are then validated by performing viability assays using the individual shRNAs. Viability assays are often conducted using a kit like Cell Titer-Glo or clonogenic assays. The process of identifying the mechanism of action and chemical compounds targeting the gene hits is then different for each study.

In the first example, Etemadmoghadam et al. [38] performed shRNA library screening on 102 cancer cell lines including ovarian, colon, pancreas, lung, and other cancer types to investigate synthetic lethality with Cycline E1 (CCNE1) amplification (Figure 1B). Overexpression of the CCNE1 oncogene occurs in multiple cancers and is associated with poor prognosis in high-grade serous ovarian cancer [39,40]. CCNE1 overexpression results in increased proliferation through decreased regulation of the S phase cell cycle checkpoint [41]. The authors obtained microarray data from shRNA experiments from the Integrative Genomics Portal, and the data was analyzed using the GenePattern module ScorebyClassComp and GeneE software. The shRNA library contained a pool of 54,020 shRNAs targeting 11,194 genes. All cancer cell lines were divided into CCNE1 amplified/overexpressing or normal CCNE1 copy number/expression representing matched cell lines (Figure 2E). Each cancer type was analyzed separately. The relative abundance of each shRNA sequence in the matched cell lines was compared since shRNAs causing synthetic lethality should be eliminated from CCNE1 amplified/overexpressing cells. The screen identified 835 genes that were essential for survival in CCNE1-amplified or overexpressing cancer cell lines and 25 high confidence hits. The authors validated the results of the shRNA screen by performing an siRNA screen for their top 115 hits plus an additional 27 candidate genes. For the siRNA screen SK-OV-3 (CCNE1-unamplified cells) and OVCAR-3 (CCNE1-amplified cells) (Figure 2E) were plated in parallel, and viability was measured using the Cell Titer-Glo assay in a plate format (similar to Figure 3B). Among the best hits were cyclin-dependent kinase 2, BRCA1, and other genes involved in DNA damage repair (including HR) and cell division. Based on this screen and the chemical library screen by Jacquemont et al. [25] (see above) the authors used the chemical compound Bortezomib to target the FA pathway and disrupt HR. Bortezomib was synthetically lethal with CCNE1 amplification in multiple ovarian cancer cell lines.

In the second example, Hoffman et al. [37] identified synthetic lethal interactions in Brahma Related Gene 1 (BRG1)-deficient cancers (Figure 1A) using an epigenome-focused shRNA screen to identify epigenetic cancer dependencies. Epigenetic disregulation caused by alterations in the chromatin remodeling complex SWItch/Sucrose Non Fermentable (SWI/SNF) have been shown to be important in tumorigenesis [42]. Inactivation of BRG1, a DNA-dependent ATPase and part of the SWI/SNF complex, is associated with increased cell proliferation, dysregulation of cell cycle checkpoints, and poor clinical prognosis [43]. Matched lines for BRG1 were obtained from the Cancer Cell Line Encyclopedia and consisted of a panel of 58 human cancer cell lines differing in their BRG1 status (Figure 2B). In order to study epigenetics-based synthetic lethality, the authors constructed a Deep Coverage Design shRNA library (DECODER) containing 6,500 shRNAs, with 17 shRNAs per gene, to specifically target enzymes involved in epigenetic regulation. The DECODER lentiviral pool was transduced into each cell line in duplicate. The relative abundance of each shRNA was measured by Illumina GA2X-based next generation sequencing of each shRNA’s barcode in BRG1-deficient and expressing cell lines. Hits were shRNAs that were selectively eliminated from BRG1-deficient cells. From this screen, the gene with the strongest synthetic lethal effect was BRaHMa (BRM), a catalytic subunit of the SWI/SNF chromatin remodeling complex and paralog of BRG1. The authors hypothesized that BRM compensates for BRG1 loss in the deficient cancer cell lines. To confirm this, the authors then showed that cancer lines with homozygous loss of BRG1 are more dependent on BRM than those with heterozygous loss. To further confirm the synthetic lethal interaction between BRG1 and BRM, the authors introduced an inducible shRNA targeting BRM into several BRG1-deficient and WT cell lines. BRM knockdown in BRG1-deficient cells caused irreversible growth arrest and significant induction of repressive H3K9me3 histone methylation marks. The synthetic lethal interaction was confirmed in vivo using an inducible shRNA for BRM in BRG1-deficient lung cancer xenografts of NCI-H1299.

In the third example, Scholl et al. [36] identified a synthetic lethal interaction in Kirsten rat sarcoma (KRAS) oncogene-dependent cancer cells using a subset of the Broad Institute TRC shRNA Library that consists of 5,024 shRNAs (Figure 1B). Oncogenic KRAS mutations occur within most human cancers [44-46] and drive tumorigenesis by increasing proliferation, anabolism, evasion of apoptosis and the immune system and by stimulating metastasis [44-46]. The authors hypothesized that oncogenic KRAS mutations cause
secondary dependencies on genes that then can be exploited for causing synthetic lethal interactions. The shRNA screen was conducted in the acute myeloid leukemia (AML) cell lines NOMO-1 (mutant KRAS), THP-1 (WT KRAS), fibroblasts (WT KRAS), and human mammary epithelial cells (WT KRAS) (Figure 2E). Similar to the siRNA plate format approach (Figure 3B), the screen was conducted in a 384-well plate format in which each well was transduced with a single shRNA. Cell Titer-Glo was used to determine cell viability for each well. The top candidate gene \textit{STK33}, a member of the calcium/calmodulin-dependent protein kinase subfamily of serine/threonine protein kinases, was then validated in additional matched AML lines (Figure 2E). The authors then tested the consequence of \textit{STK33} knockdown in multiple non-AML cancer types. The synthetic lethal interaction was only observed in cancers that are dependent on mutant KRAS for viability and proliferation. The interaction was confirmed \textit{in vivo} using inducible shRNA targeting \textit{STK33} in pancreatic, breast, lung, and colon cancer xenografts. Further investigation has shown that in mutant KRAS cells, \textit{STK33} acts through S6K1-induced inactivation of Bcl-2-associated death promoter to suppress the mitochondria-mediated apoptosis and promote survival.

The shRNA approach requires redundancy in order to rule out false positive hits, since each individual shRNA has varying levels of efficacy in different cell types due to target transcript variation, shRNA design, and varying degrees of off-target effects. Overall, this approach allows for the investigation of synthetic lethality in a large number of cancer cell lines in parallel through pooled viral libraries and generates a large number of hits. These methods require the use of microarray or DNA sequencing, which can be cost prohibitive; small molecule compounds targeting the identified genes may not be readily available.

**CRISPR LIBRARY SCREENS**

A new approach to genome editing was discovered in bacteria and archaea through the identification of clustered repeat sequences in 1987 that was later named CRISPR, or clustered regularly interspaced short palindromic repeats, by Jansen et al. [47] in 2002. CRISPR serves as a bacterial immune system against bacteriophages. Invading viral DNA is incorporated into the bacterial host genome between the CRISPR repeat sequences, which can then be used to specifically target the viral DNA for degradation [48]. The CRISPR system is now a powerful tool for specifically editing the genome [49] and can be applied to identifying synthetic lethal interactions (Figure 4).

Hiroko et al. [50] performed a genome-wide CRISPR screen in mouse cells to identify genes that confer susceptibility to \textit{Clostridium septicum} alpha-toxin. The aim of this study was to identify genes that when targeted would confer resistance to the toxin. While not technically identifying synthetic lethal interactions, since the screen identified genes that resulted in survival when targeted by guide RNAs (gRNAs) (reverse of Figure 1B), this study exemplifies how a CRISPR-based approach could be conducted for studying synthetic lethal interactions in cancer. The CRISPR system generates bi-allelic mutations in each targeted gene resulting in a loss of function phenotype. Lentiviral CRISPR-Cas9 constructs that target 19,150 mouse protein-coding genes were generated using 87,897 gRNAs. The gRNA library covered 94.3 percent of the genome with at least two gRNAs per gene. Mouse embryonic stem cells (mESC) were transduced with the lentiviral CRISPR pool and treated with or without alpha-toxin for 5 days (Figure 2F). The surviving cells were analyzed by deep sequencing to detect which genes had been lost. In a synthetic lethality screen, the gRNA...
could be barcoded, allowing for hits to be identified by detecting underrepresented gRNA in the cancer population that are still present in the matched (WT) cell line (Figure 4). The authors identified 13 genes associated with alpha-toxin resistance and validated genes that were targeted by at least two independent gRNAs. Four validated hits conferring resistance to alpha-toxin were \textit{B4galt7}, \textit{1700016K19Rik}, \textit{Cstf3}, and \textit{Ext2}. The authors verified \textit{B4galt7} and \textit{Ext2} by reintroducing the full length cDNA into the corresponding gene knockout cell lines, which resulted in sensitivity to the toxin being restored. No further molecular analysis was conducted, and the results were not pursued yet with \textit{in vivo} studies or chemical compound studies.

One major advantage to using a CRISPR library is that the system provides for greater efficiency with fewer off-target effects when compared to an RNAi screen. As such, the need for redundant targeting of the same gene is reduced. CRISPR screens, like shRNA library screens, are well-suited for studying multiple cell lines in parallel since a pooled viral library can be employed. This is also a disadvantage, since the screen will then rely on costly microarray or deep sequencing to identify hits. Since CRISPR is a relatively new system, it is likely that synthetic lethal screens using this approach will become more frequent.

**THE DAISY APPROACH**

Most computational approaches to identifying synthetic lethal interactions on a global level have been performed in yeast [9,51] and then applied to human cancer by investigating the effect in their human orthologs. Combining multiple approaches into a single screen can be an effective way to computationally identify synthetic lethal interactions on a global scale in human cancer. The data mining synthetic lethality pipeline, known as DAISY, is an approach published by Arnon et al. [52]. It consists of
a data-driven computational screen for identifying synthetic lethal pairs through the analysis of cancer genomic data (Figure 1A). DAISY is a combination of three inference strategies: survival of the fittest (SoF), shRNA functional examination, and pairwise gene co-expression (Figure 5). The authors also analyzed synthetic dosage lethality (SDL), which is the overexpression of a gene that leads to the essentiality of a second gene, making a SDL pair (Figure 1B). DAISY statistically infers synthetic lethal interactions from data sets generated from cancer cell lines and patient samples (Figure 2B,E).

The first approach, SoF, is based on the assumption that cells with reduced expression of synthetic lethal gene pairs will not survive in a heterogeneous cell population. Since cancer cells with the inactivation of two synthetically lethal genes will not survive, by studying the somatic copy number alteration (SCNA) of a specific gene from the pooled populations of multiple cell lines and comparing it to the average change in all other genes, a synthetic lethal interaction can be inferred. The co-inactivation of synthetically lethal genes should occur less frequently than the average. This is similar to the shRNA screen approach, wherein microarray or sequencing is used to identify which shRNAs have been lost from the pooled population with the exception that it is on a much larger scope: each gene investigated is being compared to every other gene across multiple cell lines and patient samples. The second approach is a shRNA functional examination and relies on three separate shRNA library screens in 46, nine, and 92 cancer cell lines to identify genes that become essential due to the knockdown of another gene. Each separate screen comprised an individual data set including the SCNA and gene expression profiles for each cancer cell line examined within the screen. This relies on the hypothesis that synthetically lethal genes will be simultaneously lost from the viral pool in multiple cancer cell lines. The third approach is pairwise gene co-expression analysis, which is based on the assumption that synthetic lethal pairs are involved in closely related biological processes. The combined analysis of the three approaches analyzed more than 535 million gene pairs. A hit from the DAISY screen is defined as two genes in which the inactivation of one renders the other essential for survival.

DAISY’s effectiveness was evaluated against previously identified synthetic lethal gene pairs, including the partners of PARP1, VHL, MutS protein Homolog 2 (MSH2), and KRAS. The validation was performed on 7,276 gene pairs that were tested in six large screens. PARP1 and BRCA1/BRCA2, as well as MSH2 and Dihydrofolate reductase, were successfully confirmed as synthetic lethal pairs. Daisy’s predictive ability was also used to identify genes that become essential with VHL deficiency. Forty-four genes were predicted, and a small siRNA screen was performed to test the validity of the prediction. DAISY identified 3.83 times more synthetically lethal genes than the follow-up siRNA screen. This indicates that additional validation is needed for predicted pairs to eliminate false positives.

The predictive ability of the combined DAISY approach was then used to generate genome-wide networks of synthetic lethal and SDL interactions in cancer. More than 2,000 synthetic lethal genes and more than 3,000 SDL genes were identified in both normal and cancer cells. The identified gene pairs were also investigated in a large study based on clinical samples and cancer cell lines to test gene essentiality, clinical prognosis potential, and drug efficacy. DAISY does have its limitations. It can only be applied to cancer cells since it relies on large genomic mutation data sets, which at times can be hard to work with because of their inaccuracy. It does not account for epigenetic or posttranslational regulation that can alter synthetic lethal and SDL interactions. DAISY’s predictive power can be utilized to identify novel co-essential gene pairs, which may lead to new and improved therapeutics for treating cancer.

CONCLUSIONS AND OUTLOOK

Harnessing synthetic lethal interactions to create novel cancer-specific therapies is a rapidly growing part of cancer biology. The specificity of these interactions will allow for improved overall survival while also increasing patient quality of life. Synthetic lethality allows for the exploitation of cancer specific mutations that are not “druggable” by identifying interactions with other co-essential genes. It provides an explanation as to why some drugs have increased efficacy in specific cancer types and may even provide new clinically relevant diagnostic markers. With the discovery and implementation of CRISPR, it is very likely that studies will be conducted using CRISPR libraries. Future computational based genome-wide inference studies like DAISY could be a powerful tool for mining the myriad public data sets available to identify synthetic lethal interactions in specific cancer types. Other screens could identify synthetic lethal interactions using a microRNA library approach. As technology advances and sophisticated approaches become easier to conduct, it might be possible for synthetic lethal screens to be extended to personalized medicine.

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