Synthetic lethality: General principles, utility and detection using genetic screens in human cells

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A B S T R A C T
Synthetic lethality occurs when the simultaneous perturbation of two genes results in cellular or organismal death. Synthetic lethality also occurs between genes and small molecules, and can be used to elucidate the mechanism of action of drugs. This area has recently attracted attention because of the prospect of a new generation of anti-cancer drugs. Based on studies ranging from yeast to human cells, this review provides an overview of the general principles that underlie synthetic lethality and relates them to its utility for identifying gene function, drug action and cancer therapy. It also identifies the latest strategies for the large-scale mapping of synthetic lethali- ties in human cells which bring us closer to the generation of comprehensive human genetic interaction maps.

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1. Introduction

Synthetic lethality was first described by the American geneticist Calvin Bridges in the early 20th century [1]. He noted that when crossing fruit flies (Drosophila melanogaster), certain nonallelic genes were lethal only in combination even though the homozygous parents were perfectly viable. The term “synthetic lethality” was coined some 20 years later by his colleague Theodore Dobzhansky who observed the same phenomenon in Drosophila pseudoobscura [2]. Indeed, synthetic is used here for its ancient Greek meaning: the combination of two entities to form something new.

Synthetic lethality is thus defined as a type of genetic interaction where the co-occurrence of two genetic events results in organismal or cellular death (Fig. 1) [3,4]. Similarly, genetic combinations that yield a non-lethal growth impairment are called synthetic sick but are usually grouped together with synthetic lethal interactions, as is done in this review. Although best known in the context of loss-of-function mutants, combinations of other types of perturbations can also result in synthetic lethality, including overexpression of genes, the action of a chemical compound or environmental change [5–8].

Geneticists have been interested in synthetic lethal interactions because they reveal information about the functional relationships between genes and are relatively easy to score in genetic screens [9]. In addition, as many basic cellular processes are ultimately required for viability, synthetic lethality screens can be used to study a wide range of processes. In recent years, synthetic lethality has attracted attention from cancer biologists as it provides a new angle for therapy and may explain the sensitivity of cancer cells to certain drugs [7,10]. This review intends to provide the conceptual framework and utilities of synthetic lethality and highlights the main strategies to identify them in human cells using functional genetics.

2. The principles underlying synthetic lethality involve robustness and buffering

Synthetic lethal genetic interactions exist because of the mechanisms employed by cells and organisms to maintain homeostasis in the face of diverse genetic and environmental challenges [4,11]. This robustness of cells and organisms is thought to play a critical role in evolution, developmental canalization (i.e., the ability to produce the same phenotype regardless of genotype) and multifactorial diseases such as cancer [12,13]. Genetically, robustness is highlighted by the fact that while ~80% of budding yeast (Saccharomyces cerevisiae) genes are not required for proliferation in rich medium [14], most single mutants are sensitive for an additional perturbation (i.e., a synthetic lethal interaction) such as knockout of a second gene, a change in culture conditions or exposure to a chemical compound [8,15]. Thus synthetic lethality can be thought of as a feature of genetic robustness.

Genetic robustness is established via various buffering mechanisms, such as functional redundancy and proteins known as capacitors. In diploid organisms, redundancy is the simplest type of buffering due to the presence of two alleles in the genome. Indeed, many heterozygous knockout mice are not discernable
from their wild-type littermates. Redundancy is also provided by genes that have a common ancestor and can still partially perform the same task (e.g., AKT1 and AKT2), although in yeast this type of redundancy is relatively rare, explaining only a small fraction of genetic interactions [15]. Instead, robustness is mostly provided by non-homologous genes operating in the same cellular process or in back-up pathways (Fig. 2). For example, a large scale RNA interference (RNAi) screen to identify human genes that were synthetic lethal with low concentrations of the mitotic inhibitor paclitaxel found some coding for proteins of the mitotic spindle apparatus itself as well as others coding for proteins of the proteasome, which is involved in protein-degradation, indicating that mitosis is buffered at several different levels [16].

Another interesting type of buffering is provided by so-called "capacitors" like heat shock proteins and chromatin regulators that can mask the effects of many different mutations (Fig. 2) [15,17,18]. Therefore, capacitors display many synthetic lethal interactions. The role of heat shock proteins in the buffering of genetic variations is believed to stem from their ability to promote proper folding of mutated proteins. Capacitors may also explain the effectiveness of HSP90 and histone deacetylase inhibitors as anticancer drugs, a topic that is discussed below [19].

Thus, cellular systems maintain homeostasis partly by ensuring that processes do not depend on any single component, which could easily be perturbed by mutation or environmental effects, setting the scene for synthetic lethal interactions [4,11,13].

3. Synthetic lethality provides insights into gene function and drug action

The true potential of synthetic lethality has been most extensively realized in yeast [9]. Here, the large-scale quantitative mapping of synthetic lethal interactions has reached genome-wide scale and the resulting comprehensive genetic interaction networks are a rich source for the functional annotation of genes [8,15]. This is because genes that act in the same process often buffer each other, so they also tend to cluster together in these networks and gene function or even enzyme–substrate relationships can be inferred [3,20]. Clearly, the elucidation of similar comprehensive synthetic lethal interaction networks in human cells would be very attractive, particularly as the majority of genes have still not been assigned a function a decade after the first draft of the human genome was generated.

Drugs also display synthetic lethal interactions with genes, which can be used to study their effects on cells [6]. Indeed, drug–gene interaction screens in yeast have been used extensively to study the mechanism of action of drugs and similar studies in human cells would be invaluable [21–24]. This is particularly important because for many drugs that are used in the clinic not all protein targets are known, which is essential for understanding their unwanted side effects.

Knowledge of drug–gene synthetic lethal interactions may also be used to design combination therapies and predict synergistic/sensitizer drugs [16,25–28]. This is particularly important in cancer and infectious diseases, as drug combinations with distinct cellular targets can limit treatment resistance and synergistic drugs can be used at much lower concentrations to achieve the same biological effect, thereby limiting side effects [29].

4. Synthetic lethality provides a framework for the mechanism of action of chemotherapy

The notion that conventional chemotherapeutics work by targeting fast dividing cells is not the whole story and a molecular understanding of why certain cancer cells respond to a specific chemotherapeutic while others are resistant, remains largely unexplained [7]. It is also not apparent why some cancers are sensitive for drugs such as Bortezomib, Vorinostat and Geldanamycin that inhibit the proteasome, histone deacetylases and HSP90 respectively, since these cellular targets are not directly implicated in tumorigenesis. Instead the effectiveness of these chemotherapies may be due to synthetic lethality [5,7]. For example, given that genomic instability, defective DNA repair, and deregulated transcription are some of the hallmarks of cancer, drugs that further perturb these processes may be specifically toxic (i.e., synthetically lethal) in cancer cells. In other words, the molecular changes in the cancer cells have strained the buffering capacity of these systems.
Identifying new synthetic lethal interactions found in specific cancer types and individual tumors, particularly a map of the synthetic interactions between DNA repair pathways and DNA damage-based cancer therapies, would be an invaluable aid for guiding therapy choice.

5. Synthetic lethality and new opportunities for cancer therapeutics

Although much is now known about the large number of molecular aberrations that are found in cancer it can be difficult to directly translate this knowledge into new therapies. A major hurdle in drug discovery is that most oncogenes are not easily accessible for inhibition by small molecules and the restoration of loss-of-function changes in tumor suppressor genes in patients is nearly impossible [5]. In their seminal paper, Lee Hartwell and Stephen Friend proposed that synthetic lethality may provide a solution [30]. They reasoned as follows: Cancer cells have acquired molecular changes that distinguish them from their wild-type counterparts. Consequently, cancer cells have been “re-wired”, exposing new genetic vulnerabilities. Synthetic lethal interaction partners of cancer-associated molecular changes should therefore offer excellent therapeutic opportunities.

This notion is somewhat related to oncogene addiction, the observation that tumorigenesis cells become “addicted” to their genetic changes and thus vulnerable for the sudden inactivation of oncogenes or reactivation of tumor suppressors [31]. To discriminate between synthetic lethal interactions and oncogene addiction, the former has therefore also been called “non-oncogene addiction” [7].

The great potential of synthetic lethality-based cancer therapy is best illustrated by a landmark achievement concerning the tumor suppressor and DNA repair genes BRCA1 and BRCA2, mutations in which can cause breast and ovarian cancer. The BRCA genes display synthetic lethality with another DNA repair enzyme called PARP and the tumors of patients carrying these mutations could be successfully treated using a chemical PARP inhibitor with remarkably mild side effects [32–35]. Interestingly, recent studies have suggested various other synthetic lethal partners of PARP, including the tumor suppressor PTEN [28,36–38].

The BRCA/PARP case has become the paradigm for a new class of rational cancer therapies based on the synthetic lethality concept [39–41]. Furthermore, it calls for a comprehensive effort to identify synthetic lethals in human cells and the challenge now remains to adapt newly-available methods to identify them.

6. In silico prediction and evolutionary conservation of synthetic lethality

Based on yeast studies it is known that some characteristics make two genes more likely to display a genetic interaction. For instance, shared localization, physical interaction and co-expression are some of the predictors of synthetic lethality [42]. But even with the high-quality data from yeast, prediction algorithms are unreliable, and empirical testing remains the only way to confidently and comprehensively identify genetic interactions [9].

As a large number of yeast genes have human orthologs, and genome scale genetic networks are available in yeast, it is interesting to explore the evolutionary conservation of genetic interactions. Remarkably, S. cerevisiae and Saccharomyces pombe, yeast species that diverged 300–600 million years ago and differ in many biological properties, share approximately 30% of their genetic interactions, suggesting significant conservation [43,44].

The utility of yeast for the accurate prediction of synthetic lethality in higher eukaryotes remains unclear. Although a study of spindle assembly checkpoint genes found that about 25% of yeast synthetic lethals were conserved in the nematode Caenorhabditis elegans [45], several larger-scale studies found as few as 1% of interactions conserved between these two organisms, questioning the general conservation of synthetic lethality [46,47]. Some of the weak conservation may be explained by technical limitations or perhaps genetic interactions are more likely to be conserved only in certain biological processes such as chromosome segregation. Indeed, the few studies that have successfully used yeast genetic interactions to predict interactions in mammalian cells suggest there is limited conservation [23,48–50]. Future studies will undoubtedly shed more light on this important question but in the meantime, the only way to systematically identify synthetic lethals in higher eukaryotes, including humans, is to use an empirical approach.

7. Synthetic lethality screen approaches in human cells

Until recently, synthetic lethality screens were mostly limited to screens using chemical compounds but a scalable approach to identify gene-gene interactions was lacking [27,51,52]. With the discovery of RNAi it has become feasible to systematically identify synthetic lethal interactions in human cells, and a variety of different screening strategies have recently been developed.

The two general approaches in classical genetics that are used to link phenotype with genotype (referred to as forward and reverse) can also be used to classify synthetic lethality screens in human cells (Fig. 3). Traditional forward genetics starts with a phenotype of interest, for instance obtained by screening a collection of mutants, and aims to identify the responsible gene. Conversely, reverse genetics takes a defined mutant, for example a knockout mouse, and looks for the functional consequence of this genetic change. In synthetic lethality screens, the forward approach uses the genetic variability in a collection of cancer cell lines whereas in the reverse tactic a single specific genetic change is engineered resulting in an isogenic cell line pair. In both cases the next step is to identify genes that are required for cell viability using RNAi-mediated knockdown and linking these to the genotypic differences.

Both approaches have been successfully employed but have strengths and weaknesses. A caveat of the forward approach is that cell lines typically show a plethora of heterogeneous aberrations, making it challenging to correlate specific genetic changes with the identified sensitivity to a certain knockdown. Reverse genetic approaches have the advantage that every sensitivity is most likely a true genetic interaction. However, as the isogenic cell line was artificially created the genetic background and cellular state does not strictly reflect a “normal” cancer cell. Therefore, the genetic interactions do not necessarily mirror those in cancer cells that naturally harbor the genetic defect.

A second methodological distinction is based on the screening format. To circumvent the practical limitations of screening individual short hairpin (shRNA) vectors in a single-well, several groups have developed pooled methods that are inspired by technologies pioneered in yeast to genetically barcode knockout clones so they can still be accurately identified even within a mixed population of cells [53–56]. Such screens are much easier to handle than large-scale single well screens and thus have a higher throughput (Fig. 4).

8. Examples of synthetic lethality screens

In the last year, several RNAi screening approaches were reported used to identify synthetic lethal interactions with oncopogenic RAS, which is frequently mutated in a variety of cancers but difficult
to target directly using small molecule inhibitors and therefore attractive for a synthetic lethal therapy. As well as being some of the most comprehensive human synthetic lethality screens to date, these studies also effectively highlight the different screening approaches that are currently available. In addition, they offer an opportunity to discuss some of the current limitations and challenges of synthetic lethality screens in human cells.

In two papers published by the groups of William Hahn and Gary Gilliland, a forward, single-well single-gene approach was employed [57,58]. In the larger of the two screens, kinases, phosphatases and known cancer-relevant genes (~1000 in total) were inhibited by RNAi in a panel of 19 RAS mutant and wild type cell lines [58]. ShRNAs were selected by supervised clustering for follow-up experiments and the hits after a second round of validation in a panel of lung cancer cell lines were RAS itself and the NF-κB activator TBK1, which is consistent with previously reported observations that RAS transformed cells require active NF-κB signaling and that RAS activates TBK1 [59,60]. In addition, the therapeutic potential of targeting the newly identified synthetic lethal interaction between the NF-κB pathway and oncogenic RAS was demonstrated in a mouse model of lung cancer [61].

In another study, Stephen Elledge and colleagues undertook an alternative reverse pooled approach to identify synthetic lethal RAS interactors [62]. Using a clone of the DLD1 colorectal cancer cell line differing only in the mutational status of RAS, a so-called isogenic clone, they screened almost 75,000 shRNA vectors (covering

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**Fig. 3.** Forward screen approach to identify synthetic lethality. Cell lines (A–H) are grouped based on mutant status of a single gene (X). Essential genes that are common in the mutants but not in the wild type cell lines are potential synthetic lethal (SL) interactions with gene X and are selected for validation in an independent panel of cell lines.

**Fig. 4.** Overview of a pooled shRNA screen set-up. Cell lines (A and B) are infected with short hairpin RNA libraries targeting thousands of gene products by RNAi. Cells are cultured to allow the depletion of those containing shRNAs that target essential genes. Genomic DNA is isolated and the vectors are quantified using so-called barcode sequences (short stretches of DNA) that are unique for each shRNA vector. By comparing the genes that are required in one cell line but not the other by custom micro-array or deep sequencing, potential synthetic interactions can be identified.
most of the genes in the human genome) for differential sensitivities (Fig. 4). Interestingly, the putative RAS synthetic lethal interactors that were identified are involved in diverse processes including proteasome function, suggesting that RAS-induced transformation requires substantial buffering. In particular, the authors found that RAS mutant cells are hypersensitive to inhibition of the mitosis checkpoint protein PLK1. In a similar effort, the laboratory of Julian Downward recently performed a screen in isogenic colorectal carcinoma HCT116 cells with a pooled library targeting 2500 genes and identified the transcription factor SNAIL2 to be required only in RAS mutant cells [63].

9. Limitations of RNAi screens and outlook

The overlap between the synthetic lethalities in the mentioned RAS synthetic lethality screens is very limited. Some of this may be due to the use of different cell lines for the screens and technical approach but given that \(~75\%\) of the interactions identified in the genome-wide DLD1 screen also displayed an effect in HCT116 cells this is unlikely to be the complete explanation [62]. Rather, the use of different shRNA libraries that target only partially overlapping genes and inhibit gene expression with different efficiencies, provides a technical explanation and indicates a large number of false-negatives in the screens. Indeed, the variability in knockdown efficiency and the fact that RNAi typically only results in \(70–90\%\) inhibition of expression highlights a weakness of this tool. In addition, the fact that RNAi screens are plagued with off target effects provides an explanation for false positives. Therefore, the availability and further development of powerful screening technologies, including better characterized knockdown libraries, will continue to be a driving force behind human functional genetics. Particularly, new technologies such as a method for the large-scale generation of human knockout cells will add to the current tool-box and may finally provide yeast-like genetics in human cells [64].

Although the mentioned new and other known synthetic lethal interactions may provide handles for novel therapies, most of the described genetic interactions in cancer cells do not strictly adhere to the definition of synthetic lethality which demands that only the combination of two perturbations results in lethality. Frequently the perturbations are also toxic on their own, which would limit the specificity of the drug and likely introduce unwanted side-effects, or the interaction is more accurately described as synthetic sick so the cancer cells would not be completely eliminated. Analogous to the therapeutic window for drugs, i.e., the safe and effective dose range, “the synthetic lethality window” of the genetic interaction will be important for its potential as a therapy (Fig. 5). For example, with the BRCA-PARP synthetic lethal interaction, BRCA mutant cells are some two orders of magnitude more sensitive to PARP inhibitors than non-mutant cells and this strong responsiveness is probably key to its clinical success. It is currently unclear how common this type of strong genetic interaction is in human cells.

In summary, synthetic lethality is a valuable concept to understand the functions of genes, the mechanism of action of drugs and their interactions, and has immediate relevance for cancer therapy. In recent years it has brought inspiration to search for a new class of anti-cancer therapies and future treatment strategies will increasingly consist of combination therapies based on personalized tumor vulnerabilities, including synthetic lethality. Aided by powerful genetic tools, there is little doubt that we are at the brink of an explosion of synthetic lethality information in human cells.

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