Synthetic lethality and cancer

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Abstract | A synthetic lethal interaction occurs between two genes when the perturbation of either gene alone is viable but the perturbation of both genes simultaneously results in the loss of viability. Key to exploiting synthetic lethality in cancer treatment are the identification and the mechanistic characterization of robust synthetic lethal genetic interactions. Advances in next-generation sequencing technologies are enabling the identification of hundreds of tumour-specific mutations and alterations in gene expression that could be targeted by a synthetic lethality approach. The translation of synthetic lethality to therapy will be assisted by the synthesis of genetic interaction data from model organisms, tumour genomes and human cell lines.

Synthetic lethality
A synthetic lethal interaction occurs between two genes when a perturbation (a mutation, RNA interference knockdown or inhibition) that affects either gene alone is viable but the perturbation of both genes simultaneously is lethal.

Advances in genome sequencing are driving a paradigm shift in cancer treatment[1]: it is now possible to rapidly identify genetic and epigenetic changes that differentiate tumour cells from non-tumour cells in a patient. Tumour-specific genetic alterations reveal not only the biological changes that drive tumour progression but also the vulnerabilities that can be exploited to selectively target the tumour with therapeutics. Personalized (or precision) genotype-targeted cancer treatment has the potential to offer individualized, highly specific therapies with fewer adverse effects, as well as to reduce the overtreatment of tumours. Indeed, personalized oncogenomic approaches are currently being adopted on the front lines of cancer care and have had success in the treatment of patients with tumours that have failed to respond to standard therapies[2–4].

Currently, most genotype-targeted cancer therapeutics exploit the phenomenon of 'oncogene addiction', that is, where a tumour is dependent on an oncogene or on an oncogenic pathway for survival. However, although small-molecule and antibody-based inhibitors of oncogenes have proved to be effective for some tumour genotypes[5], not all tumours have targetable gain-of-function oncogenes, and therapeutic resistance is a common outcome. In these tumours, it may be possible to leverage both oncogenic and non-oncogenic mutations by identifying and exploiting second-site targets that, when disrupted in conjunction with a tumour-specific mutation, result in synthetic lethality (FIG. 1).

The concept of synthetic lethality was developed from genetic studies in model organisms, such as fruit flies[6,7] and yeast[8–10]. In the context of cancer, a synthetic lethal interaction that involves a cancer-specific mutation is sometimes referred to as 'non-oncogene addiction', as the mutant tumour cell requires (that is, it is addicted to) the activity of the synthetic lethal partner gene for viability[11] (FIG. 1a,b). The protein product of a gene that has a synthetic lethal interaction with a frequently occurring tumour-specific somatic mutation would be an excellent anticancer drug target, because a therapeutic that exploits the synthetic lethal interaction should result in favourable therapeutic indices, in which only tumour cells that harbour the mutation would be sensitive to the therapeutic (FIG. 1c). Furthermore, synthetic lethal interactions can expand the repertoire of anticancer therapeutic targets, as they facilitate the indirect targeting of non-druggable cancer mutations through the identification of a second-site synthetic lethal target that may be druggable.

It was first proposed 20 years ago that synthetic lethal interactions could be used to identify new anticancer drug targets[12]. Given the attractive concept of synthetic lethality-based therapeutics, it may be surprising that only one has so far progressed to the clinic. A major hurdle has been the identification of robust, clinically relevant synthetic lethal interactions. Screening for synthetic lethal interactions poses three major challenges. First, by definition, these genetic interactions result in lethality, making mutant recovery and identification difficult. Second, many synthetic lethal interactions are condition-dependent interactions and may not be conserved in all genetic backgrounds or under different cellular conditions. Third, synthetic lethal interactions are rare, and large numbers of mutant gene-pair combinations need to be queried to identify synthetic lethal interactions. For these reasons, most large-scale synthetic lethal genetic interaction screens have been carried out in budding yeast or fission yeast, as technologies that facilitate the high-throughput generation and analysis of double mutants under defined laboratory conditions are readily available. Advances in RNA interference (RNAi) and, more recently, CRISPR technology have now made it possible to carry out large-scale unbiased synthetic lethality screening directly in human cell culture.

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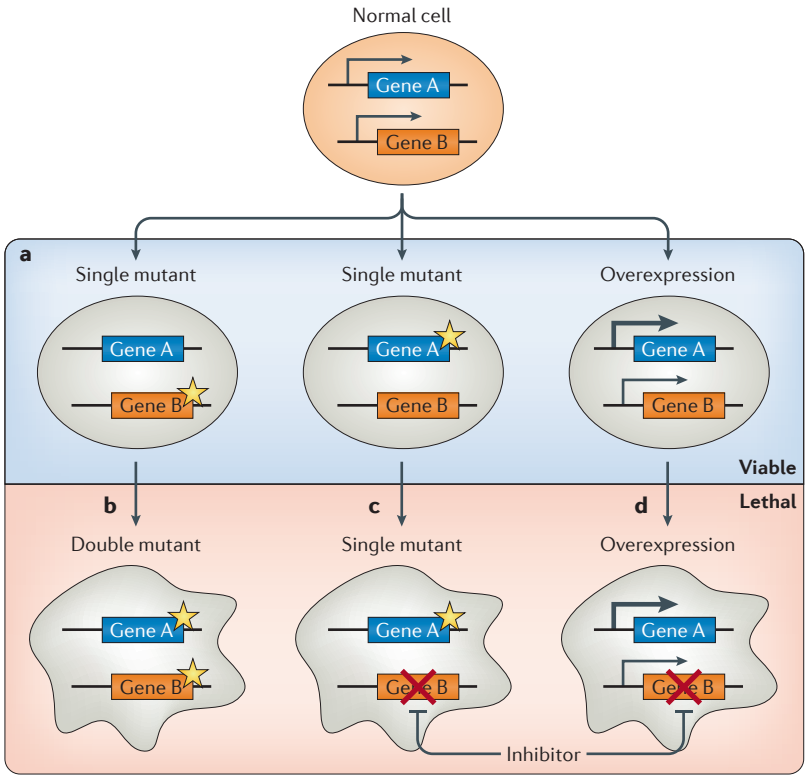


Figure 1 | **The concept of synthetic lethality.** The loss or the inhibition of either of the protein products of gene A or B alone or the overexpression of gene A is viable (part **a**). Mutation (part **b**) or pharmacological inhibition (part **c**) of the protein product of gene B in cells with a mutation (parts **b**,**c**) or overexpression (part **d**) of gene A results in synthetic lethality. The thicker arrow denotes increased expression. The star shape denotes a mutation. The red crosses denote pharmacological inhibition. Viable cells are depicted as ovals, and inviable cells are depicted as random shapes.

In this Review, we highlight some of the advances and the challenges of exploiting synthetic lethality to identify new anticancer therapeutic targets, including the expansion of the synthetic lethality paradigm to include synthetic lethal interactions with gene overexpression and the implications of conditional synthetic lethality. We also discuss how large-scale synthetic lethality screening in model organisms can directly complement targeted synthetic lethality screening in human cell lines. Finally, we present recent innovations that are facilitating high-throughput synthetic lethality screening in human cell lines.

Expanding synthetic lethality

Synthetic lethality-based approaches could, in theory, be expanded beyond the targeting of loss-of-function or reduction-of-function mutations in tumours.

Synthetic dosage lethality. Cancer cells often exhibit gene overexpression, which can result either from somatic copy number alterations[13], one of the most common alterations observed in tumours, or from epigenetic changes that increase gene transcription. Regardless of origin, overexpressed genes in tumours can be targeted by identifying interaction partners that result in synthetic dosage lethality (SDL) (FIG. 1d). SDL is a genetic interaction in which the overexpression of one gene, combined with the reduction in function of a second gene, results in lethality. The SDL phenotype was first reported in yeast[14], and the concept was codified in a screen for genes that, when mutated, resulted in lethality when the gene *CTF13*, which encodes a centromere-binding protein, or the replication origin gene *ORC6* were overexpressed[15].

Three recent SDL screens in the budding yeast *Saccharomyces cerevisiae* using homologues of genes frequently overexpressed in tumours have identified SDL interactions that are conserved in human cancer cells. *MAD2*, which is overexpressed in several tumour types, including malignant lymphoma, liver cancer, lung cancer and colorectal carcinoma, exhibits SDL with the knockdown or inhibition of *PP2A*[16]. *CKS1B*, which is frequently overexpressed in breast, lung and liver tumours, shows SDL with the inhibition or knockdown of *PLK1* (REF. 17). Additionally, *TDP1*, which is overexpressed in rhabdomyosarcoma cell lines, displays SDL with the inhibition of histone deacetylases[18]. SDL has the potential to open new avenues of synthetic lethality-mediated cancer therapies by indirectly targeting these or other commonly overexpressed genes in tumours.

Conditional synthetic lethality. Tumour cells are heterogeneous and are located in diverse environments, both of which can affect genetic interactions, as many are condition-dependent genetic interactions (FIG. 2A). These conditions can be intrinsic, such as the genetic background and metabolic state of individual cells, or can be extrinsic, such as the cellular microenvironment and exposure to therapeutic agents. Therefore, certain genetic interactions may not be observed under standard laboratory conditions, and others may be dependent on specific experimental parameters. Conditional synthetic lethality could explain why some genetic interactions are specific to a cell line; these interactions are often referred to as 'context-specific' or 'private' synthetic lethal interactions, whereas interactions that are common to many cell lines are known as 'core' or 'pan' synthetic lethal interactions.

Genetic background can have both positive and negative effects on synthetic lethal interactions. Some genetic interactions require the disruption of three or more genes to generate a phenotype[19]. For example, triple-mutant analysis in *S. cerevisiae* uncovered trigenic interactions between the partially redundant histone chaperones ASF1 and CAC1 and the SWI/SNF translocase RDH54, the cyclins CLB5 and CLB6, and genes involved in chromosome segregation[20]. Conversely, background mutations can suppress synthetic lethal interactions, resulting in synthetic viability (FIG. 2B). For example, the loss of 53BP1 or the loss of components of the non-homologous end-joining pathway can suppress the synthetic lethal interaction between poly(ADP-ribose) polymerase 1 (PARP1) and the breast cancer susceptibility gene *BRCA1* (REFS 21,22). Therefore, the genetic background of a tumour, such as the loss of p53 or the activation of an oncogene, could either uncover or suppress synthetic lethal interactions.

Non-homologous end-joining
(NHEJ). The repair of double-strand DNA breaks by direct ligation without the use of a homologous template.

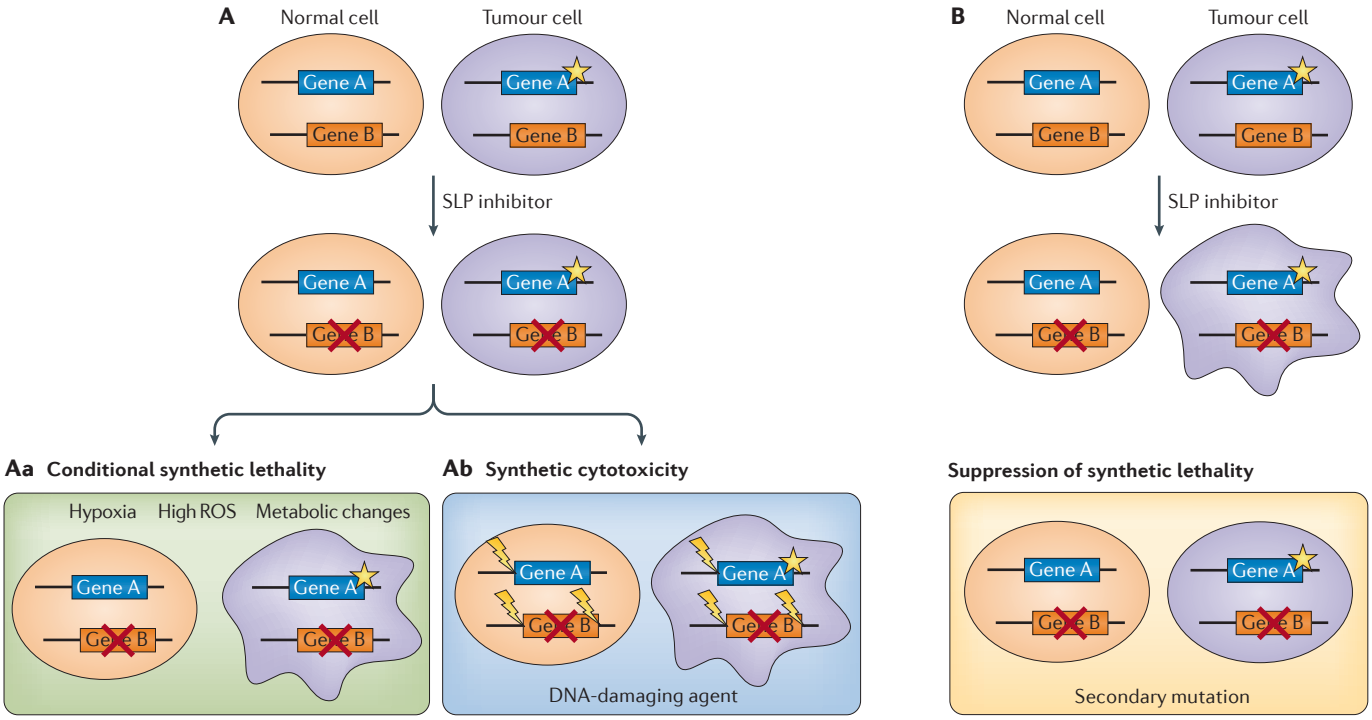


Figure 2 | **The concept of conditional synthetic lethality.** Synthetic lethal interactions may be dependent on certain intrinsic conditions such as genetic background, hypoxia or metabolic changes (part **Aa**), or extrinsic conditions such as treatment with DNA-damaging agents, which results in synthetic cytotoxicity (part **Ab**). Conversely, synthetic lethal interactions may be suppressed by conditions such as genetic background mutations (part **B**). The star shape denotes a mutation. The red crosses denote pharmacological inhibition. The lightning bolt denotes DNA damage. Viable cells are depicted as ovals, and inviable cells are depicted as random shapes. ROS, reactive oxygen species; SLP, synthetic lethal partner.

It may be possible to exploit conditional synthetic lethality to leverage tumour-associated conditions, such as hypoxia (FIG. 2Aa), increased mutational load, replicative stress, altered metabolism and exposure to standard-of-care antitumour therapeutics, to increase the range of synthetic lethal interactions. For example, hypoxia reduces the efficacy of homologous recombination[23], resulting in a DNA repair-compromised state that sensitizes cells to the inhibition of the repair protein PARP1 (REF. 24). Therefore, screening for synthetic lethal interactions in hypoxic or metabolic conditions that phenocopy the tumour microenvironment may reveal new conditional synthetic lethal interactions with an even greater specificity for tumour cell killing.

These conditional synthetic lethal interactions could be leveraged to increase the cytotoxicity of existing therapeutic agents resulting in synthetic cytotoxicity (FIG. 2Ab) and thereby increase the number of potential genetic interactions that can be therapeutically exploited in tumours. Many tumours are exposed to therapeutic agents, such as ionizing radiation or cytotoxic drugs, that change the tumour environment and that alter the genetic interaction network compared with untreated cells. Large-scale studies in *S. cerevisiae* have uncovered differential genetic interactions in response to exposure to DNA-damaging agents, demonstrating that genetic interaction networks can be dynamic and can 're-wire' in response to changing conditions[25,26]. A proof-of-principle synthetic cytotoxicity screen using a mutation in the *S. cerevisiae ATM* orthologue *TEL1*, which has few synthetic lethal partners, and a sub-lethal concentration of the topoisomerase inhibitor camptothecin increased the number of negative genetic interaction partners with the *TEL1* mutant[27]. PARP inhibitors have been used as a synthetic lethality-based monotherapy in homologous recombination-deficient tumours, but PARP inhibitors may also increase the sensitivity of tumour cells to DNA-damaging agents[28]; several ongoing clinical trials are assessing the efficacy of PARP inhibitors in combination with cytotoxic agents. For this approach to work, there must be a differential sensitivity between tumour cells and non-tumour cells to the combination of PARP inhibitors and the DNA-damaging agent. It is possible that tumour-specific mutations are the cause of the differential killing of tumour cells through the inhibition of PARP in the presence of DNA-damaging agents by a synthetic cytotoxicity mechanism. For example, in glioblastoma cell lines that contain mutations in the cohesin component *STAG2*, PARP inhibition resulted in synthetic cytotoxicity in response to the DNA-damaging chemotherapeutic temozolomide[29].

Synthetic lethality in model organisms

Given that there are hundreds of potential tumour query genes, thousands of potential synthetic lethal-partner genes and many different conditions, high-throughput approaches are needed to identify synthetic lethal interactions. Hartwell *et al.*[12] first proposed that model

Homologous recombination
The exchange of nucleotide sequences between identical (or near identical) DNA molecules. Homologous recombination is the most common form of homology-directed repair of double-strand DNA breaks.

Genetic interaction network
A genetic interaction occurs when the perturbation of one or more genes affects the phenotype of another gene alteration. A genetic interaction network defines the functional relationship between many genes.

Orthologue
Genes in different species that are originated from a single gene of the last common ancestor.

Modules
Groups of genes or proteins that act together in a common cellular function.

Synthetic sickness
A synthetic sickness interaction occurs between two genes when a perturbation (a mutation, RNA interference knockdown or inhibition) that affects either gene alone is viable but the disruption of both genes simultaneously results in a reduction of viability.

organisms, such as yeast, could enable the identification of synthetic lethality-based anticancer therapeutic targets[12]. Large-scale screens in model organisms can survey a much larger interaction space than is currently feasible in human cell lines and can identify potential cancer-relevant synthetic lethal interactions for direct testing in human cell lines, thereby reducing the number of genetic interaction pairs that need to be tested in human cell culture (FIG. 3). This can be achieved by mining existing model organism genetic interaction networks for synthetic lethal interactions with cancer-relevant homologues and by direct screening with mutations that affect homologues of cancer-associated genes.

The success of a cross-species synthetic lethality approach is predicated on the conservation of genetic interactions between model organisms and human cells. Although many of the genes, pathways and cellular processes that underlie the cancer phenotypes of genomic stability and proliferation control are evolutionarily conserved[30–32], it is difficult to predict whether a genetic interaction will be conserved, as the conservation of genetic interactions is gene and pathway dependent[33–36]. Although specific digenic interactions may not be conserved, genetic interaction networks often retain conserved interactions between biological processes[34,37,38], suggesting that, if direct orthologues of cancer-mutated genes are not present in a model organism, then conserved biological processes can be targeted by disrupting another gene that is involved in the same cellular process. For example, *S. cerevisiae* lacks orthologues of the tumour-associated homologous recombination genes *BRCA1* and *BRCA2* but instead contains other genes encoding components of the homologous recombination pathway, such as *RAD52* and *MRE11*. These genes can be mutated to disrupt homologous recombination, providing an opportunity to screen for synthetic lethal interactions in yeast cells that are deficient in homologous recombination. Similarly, genetic interaction networks can identify interactions between functional modules or processes from which synthetic lethal interactions could be inferred to certain genes within that process in human cells even if they are not conserved in the model organism. For example, yeast synthetic lethal interaction networks between cohesin subunits and replication fork mediators were used to predict synthetic lethality between mutations in cohesin and PARP inhibitors in human cell culture, even though the PARP family of genes is not conserved in yeast[29,39].

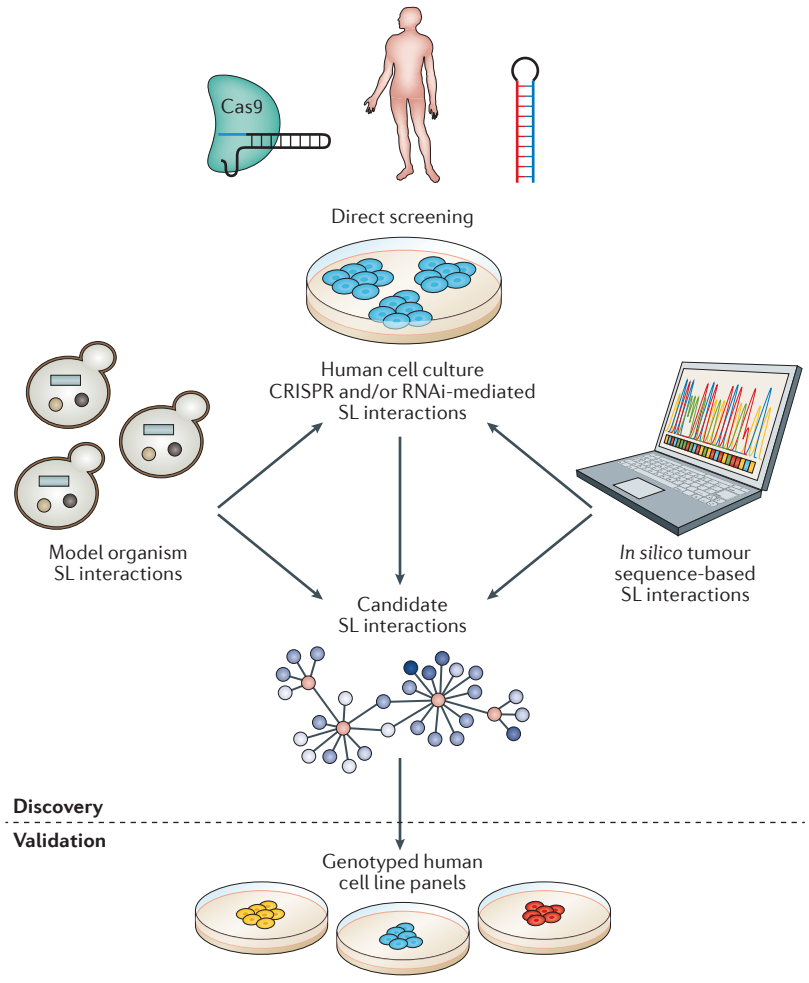


Figure 3 | **A cross-platform approach for discovering clinically relevant synthetic lethal interactions.** Synthetic lethal (SL) interaction data from model organisms, such as data from TheCellMap or BioGRID, and tumour genomic sequence can be mined to discover new candidate SL interactions for validation in human cell lines, or direct SL interaction screening can be undertaken in human cell lines. Candidate SL interactions can be validated in genotypically defined cell line panels to determine whether SL interactions are conserved across cell lines or are limited to specific cell lines. RNAi, RNA interference.

Large-scale synthetic lethality genetic networks in yeast. High-throughput mating methodologies in yeast, such as synthetic genetic array (SGA) analysis[40] and diploid synthetic lethality analysis with microarrays (dSLAM)[41], enable the large-scale construction of double mutants and quantification of genetic interactions but are not currently available for other model organisms. Recently, long-term efforts to map digenic interactions comprehensively in *S. cerevisiae* culminated in an interaction network map of more than 23 million double mutants, involving ~90% of all *S. cerevisiae* genes and covering most essential and non-essential gene combinations. This global interaction network identified more than 500,000 synthetic lethal and synthetic sickness interactions[42]. In addition to creating a global network of genetic interactions that can be mined for cancer-relevant synthetic lethal interactions, the principles of the comprehensive *S. cerevisiae* genetic interaction map provide a context for the elucidation of synthetic lethality genetic networks in human cancer cell lines. For example, although only ~1,000 genes in *S. cerevisiae* are individually essential for growth, hundreds of thousands of genetic interactions result in growth defects and ~10,000 digenic interactions between non-essential mutations result in synthetic lethality[42]. This suggests that the potential synthetic lethal interactions with tumour mutations that can be mined for drug targets is much larger than the space that can be targeted by exploiting the inhibition of oncogenes alone, increasing the probability of finding druggable targets.

Query-specific screens in yeast have been successful in identifying synthetic lethal interactions that are conserved in human cell culture[17,39,43]. The utility of synthetic lethal interaction screens in yeast for identifying candidates to be directly tested in human tumours was recently shown by a multi-query study[44]. Srivas *et al.* used 111 *S. cerevisiae* orthologues of human cancer driver mutations as query mutations with an array of 433 *S. cerevisiae* orthologues of potentially druggable genes. This screen identified 1,420 synthetic lethal interactions. The yeast synthetic lethal interactions were used to guide a tumour suppressor mutation–drug interaction screen in human cell culture of 21 drugs by 112 tumour suppressor gene RNAi knockdowns. The authors found that strong genetic interactions that were observed in *S. cerevisiae* were more likely to be conserved in human cells[44], validating the idea that screening in yeast (or another model organism) can narrow the number of candidate synthetic lethal interactions for testing in human cells.

Synthetic lethality-based screening in human cells

In the search for candidate synthetic lethal targets, unbiased synthetic lethality screens in any organism provide mechanistic and therapeutic insights that are not always intuitive. Compared with lower organisms such as yeast, the systematic interrogation of synthetic lethal interactions in human cells is still in its infancy. As human cells cannot be manipulated through genetic mating techniques, other technologies were required before high-throughput screens became feasible. RNAi-based gene targeting provided the first opportunity for easily scalable gene-specific manipulation in human cells, although it has since been viewed by some as a cautionary tale of the challenges of screening larger and more-complex genomes. Nevertheless, the goal of comprehensively mapping synthetic lethal interactions in human cells on a genome-wide scale is not insurmountable, especially as the discovery of CRISPR and its application to human synthetic lethality-based screening has transformed the field of human genetics.

RNAi and the evolution of the large-scale screen. Genome-wide studies in human cells would arguably not have been possible without the development of RNAi technology to specifically knockdown mRNAs through the introduction of exogenous small interfering RNA (siRNA) sequences[45–47]. Applied to large-scale loss-of-function screening, this technology launched the era of synthetic lethality-based high-throughput screening in human cells, with studies in transformed or immortalized human cell lines being the most common[48–51]. Although synthetic lethality screens using RNAi have made an important contribution to human functional genomics, the minimal overlap reported between independent RNAi screens has raised the issue of their reliability in annotating gene function[52]. Gene specificity of the RNAi library and the limitations of the human cell culture system in which the library is tested are two general areas of concern in human RNAi screening.

Considerable efforts have been made in recent years to improve the efficacy of RNAi libraries so that screens can be carried out with higher confidence. The problem of RNAi reagent specificity has been tackled by increasing the on-target robustness and reducing the off-target effects of RNAi sequences[53–56]. Library generation has also been improved through the incorporation of bioinformatics algorithms and tools[53,57,58]. Furthermore, the analysis of large-scale RNAi screens has emphasized the importance of reducing false positives by observing consistent phenotypes with multiple RNAi reagents that target the same gene[51,59,60].

Unfortunately, the generation of more-robust RNAi libraries does not address the problem of screening in cell lines. Regardless of whether a synthetic lethality screen is carried out in a cell line panel or in isogenic pairs (FIG. 4), cancer cell lines are still a model system. They often do not reflect the genotype or the heterogeneity of actual tumours, and they can be highly adaptable to the conditions and practices of cell culture in individual laboratories. Indeed, multiple screens that target the same cancer mutation have often reported highly variable, non-overlapping results[52,61]. These issues of irreproducibility are not limited to RNAi screening but are also a matter of debate for large-scale chemical screening[62–64] and underscore the need to confirm synthetic lethal interactions across multiple cell line contexts before proceeding with drug development.

Notably, the problems of both library specificity and cell line context still exist for genome-wide knockout screens using the CRISPR system. Nonetheless, RNAi screens have undoubtedly paved the way for screens using CRISPR technology and have brought to the forefront the challenges and limitations of human synthetic lethality screening regardless of the gene-targeting strategy used. Although CRISPR has become the method of choice for human genome-wide screening, RNAi remains a powerful tool that can be used as a complementary approach in screens or during hit validation. Moreover, this technology has the benefit of incomplete and transient knockdown phenotypes that might differ from CRISPR-generated complete knockout and may more closely parallel those that occur with chemical inhibition during therapeutic application.

Genome-wide screens using CRISPR–Cas9. In contrast to RNAi knockdown, the CRISPR–Cas9 system targets DNA (BOX 1), which is the basis for several differences between the technologies[65–67]. Unlike RNAi, single-guide RNAs (sgRNAs) can be designed for any DNA sequence in the nuclear genome, including intragenic DNA. Furthermore, CRISPR does not affect the endogenous RNAi pathway, although cell repair pathways are needed to repair the double-strand break (DSB). Finally, the knockout events that are produced by CRISPR are irreversible and do not depend on the long-term constitutive expression of transgenes in the cell, a property that can greatly facilitate the identification of synthetic lethal targets.

Even though the technology has only recently been discovered, researchers have learned from strategies that enhance RNAi library specificity and have made

Driver mutations
Mutations that confer a selective growth advantage to a cancer or a pre-cancerous cell.

Isogenic
Organisms or cell lines that contain identical or nearly identical genotypes.

Heterogeneity
In a cell line or tumour, the diversity of genotypes within the population.

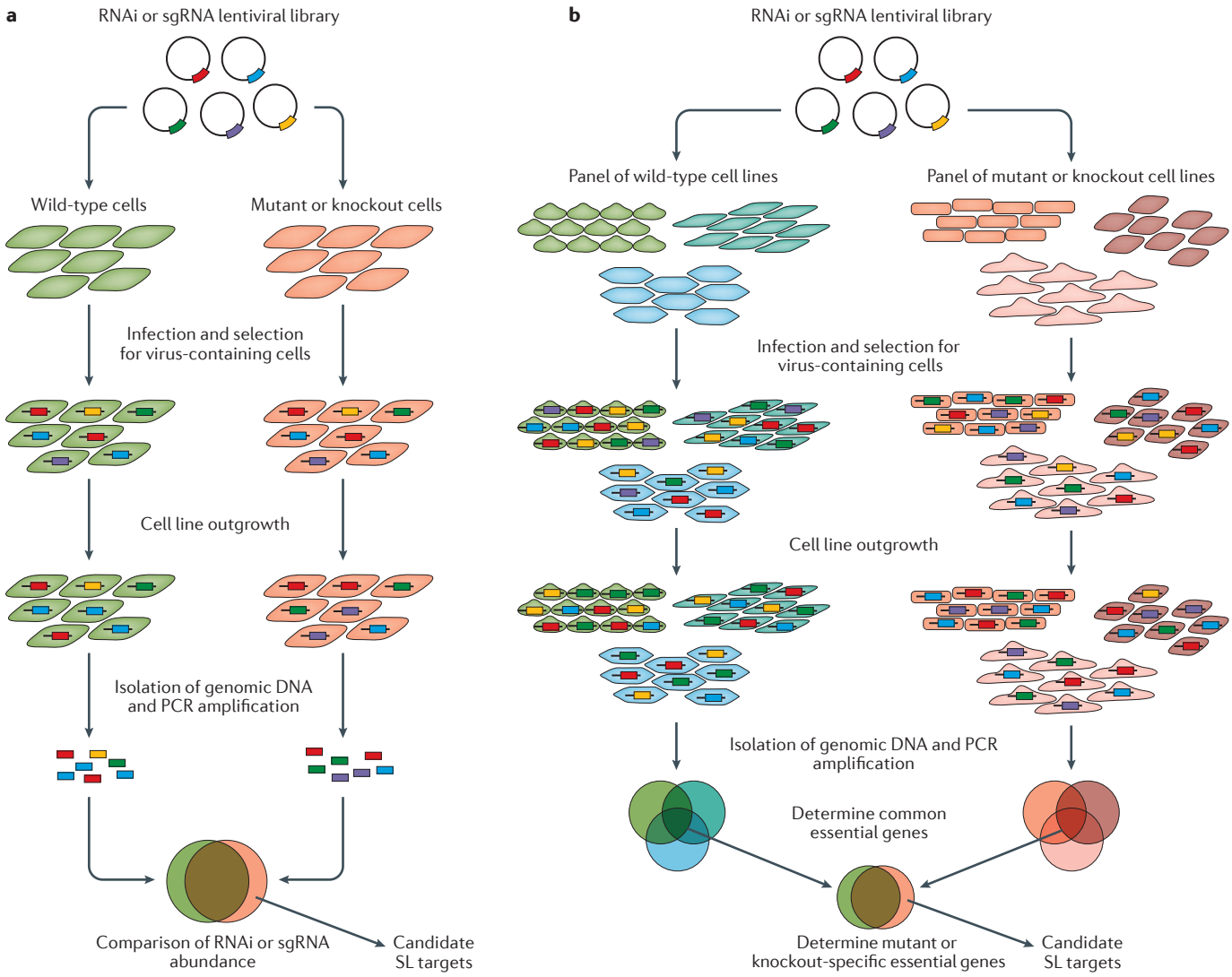


Figure 4 | **Strategy for large-scale synthetic lethality screens for a gene of interest in human cells.** Human synthetic lethality screens most commonly use either pairs of 'matched' or isogenic cell lines of the same background that have only the gene of interest mutated or inactivated (part **a**) or a panel of genetically diverse cell lines that are split into two groups depending on the status of the gene of interest (part **b**). After infection with a pool of lentivirus containing gene-specific small interfering RNAs (siRNAs) or single-guide RNAs (sgRNAs), cell populations are grown and next-generation sequencing technologies are used to identify sequences that are underrepresented (or 'drop out') solely in the cell line populations that are mutated or inactivated for the gene of interest. Genes targeted by multiple siRNAs or sgRNAs in this subset are candidate synthetic lethal (SL) partners for the gene of interest.

extensive efforts to improve sgRNA targeting. Although the refinement of the features of on-target robustness and an accounting of the full complement of off-target effects are still ongoing (for a review, see REF. 68), steps are currently being taken to incorporate these results into library design and have, so far, evolved a second generation of genome-wide CRISPR libraries that have exciting prospects for uncovering cancer-specific synthetic lethal targets[69–73].

Loss-of-function screens for essential genes using second-generation sgRNA libraries have consistently outperformed RNAi screens[70–72]. Furthermore, the expansion of these genome-wide screens across multiple cell lines has begun to uncover both core and cell line-specific fitness genes. Determining synthetic lethal targets using this method will probably involve extending these genome-wide screens to panels of cell lines in which cancer mutations of interest are known[74]. Alternatively, CRISPR–Cas9 synthetic lethality screens can be carried out on isogenic pairs, especially as the CRISPR technology itself can generate collections of cell knockouts either by targeting the same gene across multiple cell backgrounds or by producing robust genetic synthetic lethality networks for multiple genes that encode components of a protein complex or a signalling pathway of interest[75]. Such networks are likely to become more common as collections of cell line knockouts become more accessible.

Although the era of synthetic lethality screening using CRISPR is only just beginning and holds great promise for therapeutic discovery, this technology still has its limitations. First, as knockouts are generated independently by errors in DSB repair, individual cells within a population may contain the same sgRNA but have different mutations in the target sequence. This can result in a mix of phenotypes that can add more noise to large-scale screens, depending on the gene or the sgRNA region that is targeted[68]. Studies have also shown that CRISPR targeting and DSB generation are affected by chromatin structure, a feature that can vary between cell lines[73,76,77]. Similarly, amplified genomic regions also vary between cell lines, especially in those that are highly aneuploid, and recent experiments have demonstrated a high level of toxicity after genome editing in these regions[69,70]. This problem of high toxicity can be addressed by using multiple cell lines that cover a range of genomic contexts, which is already a necessity in human synthetic lethality screening. Alternatively, as the toxicity of CRISPR–Cas9 in amplified regions seems to be related to the DSBs generated by wild-type Cas9, CRISPR interference (CRISPRi) can be used to validate hits in these regions.

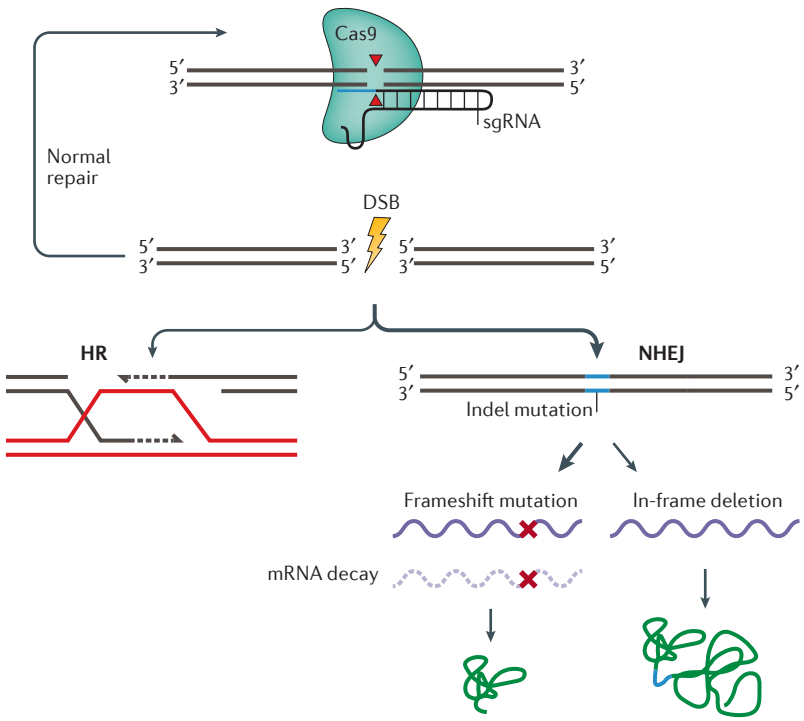
Exploring genetic networks with CRISPR. CRISPR technology has been adapted for the large-scale mapping of genetic networks in human cells. Both CRISPRi and CRISPR activation (CRISPRa) take advantage of epigenetic modifications and use a catalytically dead mutant of Cas9 (dCas9) fused to either a transcriptional repressor (CRISPRi) or a transcriptional activator (CRISPRa) domain[78,79]. When these Cas9–chromatin modifier fusion proteins are recruited by an sgRNA to bind near the promoter regions of target genes, they can regulate changes in gene expression levels. Improvements in sgRNA design and the addition of scaffolds to recruit more transcriptional modulators have made CRISPRi and CRISPRa reliable tools for high-throughput screening[73,80,81]. Unlike genome-editing screens, CRISPRi and CRISPRa screens are reversible and inducible, which allows the examination of the spatiotemporal dynamics of gene function. CRISPRi and CRISPRa screens are also complementary and, when used in conjunction, may generate a more robust data set that explores biochemical and cell signalling pathways[73,80].

As a method that investigates a larger genetic interaction space in a single experiment, combinatorial genetics *en masse* (CombiGEM)–CRISPR can generate global synthetic lethality networks with great efficiency[82]. CombiGEM–CRISPR takes advantage of the observation that sgRNAs can be multiplexed, with up to six independent sgRNAs being combined for co-editing events in one cell[83,84]. Barcoded CombiGEM pairwise libraries are generated through a two-step cloning process and consist of all possible pairwise combinations of sgRNAs. This pool can then be screened in a cell line for the pairs of sgRNAs that produce proliferation defects and analysed for negative genetic interactions[82]. Smaller, targeted pairwise synthetic lethality screens using a multiplex approach have already investigated interactions that are relevant to human cancers[85,86]. CombiGEM–CRISPR provides several other exciting opportunities for synthetic lethality screening that extend beyond pairwise genetic interaction screens. Subsequent cloning steps with CombiGEM–CRISPR can generate trigenic and higher-order libraries that could be useful for uncovering interactions that consist of multiple genes. Similarly, the screening of the same pairwise CombiGEM–CRISPR library under multiple conditions (for example, the presence or the absence of a drug) can also reveal context-dependent synthetic lethal interactions[87].

Box 1 | **CRISPR–Cas9 gene editing in synthetic lethality screening**

The CRISPR–Cas system was discovered as a form of immunity in bacteria and adapted for use in genome editing in other organisms ranging from yeast to human cells. As a genome-editing technique, CRISPR is relatively new, but it has rapidly become an important tool for synthetic lethality screening in mammalian cells. The CRISPR–Cas9 ribonucleoprotein complex is composed of a single-guide RNA (sgRNA) that enables targeting of the Cas9 endonuclease to specific sequences in the genome, where Cas9 introduces a blunt-ended double-strand break (DSB) that then needs to be repaired[65,67] (see the figure). Repair can occur through either the homologous recombination (HR) repair pathway or end-joining pathways such as non-homologous end-joining (NHEJ) and alternative end-joining (Alt-EJ). HR can be carried out using either the sister chromatid or exogenous donor DNA if it is available, although repair using donor DNA occurs at a very low rate and is not currently useful for screening.

End-joining pathways result in small insertions and/or deletions (indels). These small indels are normally selected for in CRISPR screens, as normal error-free repair will re-establish the endogenous sequence, which can then be re-cut by the sgRNA-guided Cas9 to restart the cycle. Libraries of sgRNAs for screening are designed to target the open reading frames (ORFs) of the genome[69–73]. Indels in the ORF can result in either a frameshift that creates a knockout of the allele through nonsense-mediated decay of the mRNA or truncation of the protein or an in-frame mutation that may or may not exert a phenotypic effect depending on the structural or functional importance of the altered region[70].



Until recently, synthetic lethality screens using CRISPR have only looked at the effects of fitness on cell populations. However, the method of Perturb-seq (also known as CRISP-seq and CRISPR droplet sequencing (CROP-seq)) combines the effects of CRISPR alterations with RNA-sequencing at the single-cell level[88–91]. This approach has the advantage of providing a transcriptome-wide view of the state of each cell after the knockout or the knockdown of a CRISPR target and avoids averaging phenotypic effects over the cell population. Perturb-seq can also look at the effects of combinations of sgRNAs, which is a useful technique for synthetic lethality research[89]. Furthermore, unlike traditional screening methods, Perturb-seq can go beyond measurements of cell fitness and can provide extensive molecular and pathway information on a single-cell basis, which could make the approach invaluable for the elucidation of synthetic lethal interaction mechanisms.

Screening using statistical genetics

The use of population statistics to predict genetic interactions is not a new technique, but current efforts have benefited from taking advantage of the rapidly accumulating cancer genome data that have become available from next-generation sequencing. Researchers can mine databases, such as The Cancer Genome Atlas (TCGA) or the Catalogue of Somatic Mutations in Cancer (COSMIC), to search for patterns of mutations that are mutually exclusive. These mutual exclusivity analyses have the advantage of using actual patient data, but they have a bias towards cancer mutations that have a higher frequency, an issue that has been addressed in more-recent statistical algorithms[92,93]. Furthermore, in computational analyses, the accuracy and the coverage of predicted networks improve as the size and the quality of the data improve. Efforts to improve mutual exclusivity analysis include using a hybrid model with knowledge of cell signalling pathways and analysing not only copy number alteration data but also gene mutation and expression data[92,94,95].

Whether mutual exclusivity occurs because the mutation of both members of the gene pair has no selective advantage or because there is a selective disadvantage is currently impossible to distinguish. In fact, it is possible that different gene pairs are mutually exclusive for different reasons depending on gene function[96]. Knowing the reason behind the mutual exclusivity of two genes is crucial for synthetic lethality prediction, as double mutation in a synthetic lethal gene pair should result in a selective disadvantage for cell growth.

Another approach to predict synthetic lethal interactions using bioinformatics is to combine population data with data from experimental studies. Jerby-Arnon *et al.*[97] developed DAISY, a computational pipeline that integrates three data sources: first, somatic copy number alterations and mutation data from clinical samples and cell lines; second, gene essentiality profiles from cell line RNAi screens; and third, cell line gene expression data. A combined interaction score using all three approaches predicts candidate synthetic lethality (as well as SDL) gene pairs. A subset of candidate synthetic lethal pairs predicted by the DAISY method has been validated in cells using RNAi and drugs, whereas a DAISY-generated genome-wide synthetic lethality network has shown promise in predicting clinical prognosis in breast cancer[97]. This integrative method suggests that successful *in silico* approaches will combine genomic and expression data from patient samples with experimental data from human cell lines. This study also highlights the role of statistical genetics in prioritizing target synthetic lethal pairs for experimental validation when a data set is too large to investigate practically.

Gene essentiality profiles
Sets of genes required for proliferation or viability in the context of a single cell line or tumour type.

Translating synthetic lethality into therapeutics

In theory, there are three benefits to using a synthetic lethality-based therapeutic strategy. First, synthetic lethality is selective for a cancer cell-specific genetic mutation, which should make it easy to identify patient responders. Second, when there is a large therapeutic window, chemotherapeutic treatment should have limited adverse effects, and lower drug doses should be efficacious. Third, the strategy can be applied to any type of cancer mutation, including tumour suppressors and those deemed undruggable. In practice, however, only one synthetic lethal interaction — that between PARP and BRCA1 and/or BRCA2 — has made the journey from discovery to clinical approval, although it has not done so without overcoming considerable obstacles (BOX 2). Synthetic lethality databases, such as SynLethDB[98], contain tens of thousands of interactions from the published literature; so why are there not more synthetic lethal interactions that share the success story of PARP?

It is difficult, if not impossible, for basic researchers to account for all the variables of a patient setting. However, clearly, additional parameters need to be determined for a synthetic lethal interaction before it can be considered for therapeutic application, even when a drug is available. With the recent publication of a comprehensive, single-condition yeast negative genetic interaction map and the addition of CRISPR screening approaches in human cells[42,69,72–74], the future is bright for synthetic lethality candidate screening, and perhaps now is the time to re-evaluate how we can best prioritize candidates for therapeutic testing.

Synthetic lethal candidates for cancer therapeutics are available from three sources: conservation from model organism genetic networks, human synthetic lethality screening and *in silico* predictions (FIG. 3). One straightforward method to increase the probability of finding true synthetic lethal interactions for therapeutics is to integrate data across these three platforms. In addition, to address the problems of irreproducibility in cell culture, synthetic lethal interactions need to be confirmed across multiple cell culture contexts and *in vivo*. Fortunately, cell culture techniques for cancer cells have advanced greatly in the past few years, with experiments using 3D cell cultures, patient-derived organoids, and larger cell line and biobank repositories becoming more common[99–101]. Notably, although the use of animals can limit the scale of the experiment, loss-of-function screens can also be carried out *in vivo*, with microenvironmental, immune and metabolic

Box 2 | **PARP inhibitors in synthetic lethality-based therapeutics: from discovery to the clinic**

Synthetic lethality is a useful approach to uncover biological mechanisms in both normal and cancerous cells, but the ultimate therapeutic goal is to add another weapon to the arsenal of personalized medicine. The synthetic lethal interaction of poly(ADP-ribose) polymerase (PARP) with the breast cancer susceptibility genes *BRCA1* and *BRCA2* was discovered by hypothesis-driven direct testing in 2005 (REFS 106,107). PARP inhibitors were first used in clinical trials as a synthetic lethal therapy in *BRCA1/2* germline-mutated tumours in 2009 (REF. 108) and currently represent the only synthetic lethality strategy that has been approved for use in patients with cancer. As such, it is useful to review its journey from discovery to the clinic and to examine the challenges that may need to be addressed for other synthetic lethal targets that enter the clinic.

Identification of an appropriate biomarker

Although early clinical trials focused on the efficacy of PARP inhibitors in germline-mutated *BRCA1/2* tumours[108], it was later discovered that the response was not necessarily limited to those with carrier mutations[109]. The sensitivity of *BRCA1/2*-mutated tumours to PARP inhibitors has been broadly linked to their defects in homologous recombination (HR) repair (see the figure). In an effort to increase the number of patients who might benefit from this synthetic lethality strategy, there have been efforts to identify other gene mutations in the HR pathway that are sensitive to PARP inhibitors[110,111]. However, not all mutations in a gene are equal in their effect on function. Similarly, the function of each gene in the pathway is unlikely to contribute equally to HR repair efficiency. This has led to current efforts to determine a 'HR repair defective' molecular signature using global profiling analyses that might be a better predictor of drug response[112].

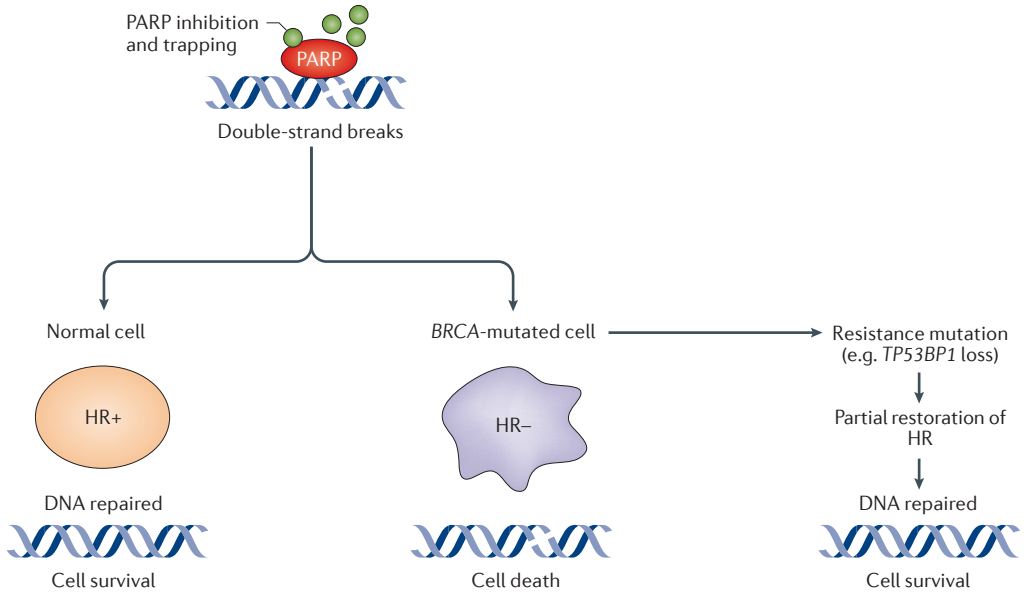
Investigation of resistance mechanisms

The possibility of resistance to PARP inhibitors was raised when it was discovered that not all *BRCA1/2*-mutated tumours were responsive to synthetic lethality intervention[108,109]. Known resistance mechanisms primarily involve the narrowing of the original synthetic lethality therapeutic window through the restoration of HR (see the figure). This can occur either genetically, through secondary mutations that partially restore the HR function of the *BRCA1* and/or *BRCA2* genes[113,114], or functionally, through mutation and the downregulation of compensatory repair pathways[22,115]. It is also important to consider how PARP inhibitors can indirectly modulate their toxicity. This includes their drug-specific off-target effects[116,117], as well as the downregulation of PARP-mediated non-DNA repair functions such as the regulation of transcription or cellular stress[118,119].

Elucidation of mechanism

Although PARP inhibitors have shown promise as a synthetic lethal therapeutic in the clinic, knowledge of the biological mechanism behind this lethality is still incomplete. One matter that remains under investigation is whether PARP inhibitors work mainly through catalytic inhibition or through the trapping of PARP proteins on the DNA[120,121]. Researchers are also more deeply pursuing the mechanism of lethality specific to HR-deficient cells, an issue that is partly complicated by the fact that both PARP and HR functions intersect at double-strand break repair and replication fork restart[112,122].

Ultimately, these three hurdles in the PARP synthetic lethality journey remind us that, although synthetic lethality-based cancer therapy is derived from a genetic interaction, the lethality still occurs at the phenotypic level. Therefore, the determination of the biological mechanism or mechanisms of synthetic lethality is not merely incidental but is fundamental to the understanding of therapeutic parameters such as dose, patient selection and the possibility of tumour resistance.



conditions aligning more closely with those found in tumours[102,103]. Finally, we propose extending preclinical studies to include several other criteria for the potential synthetic lethal therapeutic interaction, including the effect of standard chemotherapeutics, the prevalence and heterogeneity of the biomarker, possible resistance mechanisms and off-target factors, and a thorough elucidation of the biological mechanism. These practices can all help to determine the context dependency and therapeutic applicability of a synthetic lethal interaction.

It may also be necessary to evaluate potential synthetic lethal interactions beyond their potential to treat cancer in order to include those that can be applied to cancer prevention[104]. This could involve, for example, using PARP inhibitors to prevent breast cancer in women with *BRCA1* or *BRCA2* mutations before cancers become detectable. Evidence for this synthetic lethality-based chemopreventive strategy is still in its early stages and is mainly being gathered using animal models[104]. In theory, a synthetic lethality-based strategy may be more effective before pre-malignant lesions become highly heterogeneous. It also needs to be determined whether lower or intermittent drug doses will be effective for this strategy to avoid the toxicity that is associated with high continuous doses of chemotherapeutics[105].

Conclusions

Synthetic lethality is a simple genetic concept that continues to have a major impact on cancer research. Synthetic lethal interaction data from model organisms, bioinformatic predictions using tumour genome-sequencing data and direct screening of human cancer lines have identified potential drug targets. However, these data have also highlighted the mechanistic complexities underlying cancer phenotypes that will need to be unravelled to successfully identify the specific targets that can be used for the selective killing of tumour cells and to realize the full potential of personalized anticancer therapeutics.

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Competing interests statement
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DATABASES
Biological General Repository for Interaction Datasets (BioGRID): https://thebiogrid.org/
Catalogue of Somatic Mutations in Cancer: http://cancer.sanger.ac.uk/cosmic
SynLethDB: http://histone.sce.ntu.edu.sg/SynLethDB/
The Cancer Genome Atlas: https://cancergenome.nih.gov
TheCellMap: http://thecellmap.org/

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