THE CONCEPT OF SYNTHETIC LETHALITY IN THE CONTEXT OF ANTICANCER THERAPY

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Abstract | Two genes are synthetic lethal if mutation of either alone is compatible with viability but mutation of both leads to death. So, targeting a gene that is synthetic lethal to a cancer-relevant mutation should kill only cancer cells and spare normal cells. Synthetic lethality therefore provides a conceptual framework for the development of cancer-specific cytotoxic agents. This paradigm has not been exploited in the past because there were no robust methods for systematically identifying synthetic lethal genes. This is changing as a result of the increased availability of chemical and genetic tools for perturbing gene function in somatic cells.

THERAPEUTIC INDEX
The therapeutic index for a drug is defined as the dose (concentration) required for toxic effects divided by the dose (concentration) required for therapeutic effects.

THERAPEUTIC WINDOW
The therapeutic window for a drug refers to the concentration range over which therapeutic effects can be expected.

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The bottleneck to the development of safe and effective anticancer drugs does not lie in an inability to identify chemicals that will kill cancer cells. In fact, thousands of compounds have been identified over the past 50 years that will accomplish this feat. Instead, the bottleneck lies in our inability to identify chemicals that will kill cancer cells at concentrations that do not harm patients. Most of the chemotherapeutic agents used today have remarkably low THERAPEUTIC INDICES and narrow THERAPEUTIC WINDOWS. The therapeutic window is influenced by a number of factors, including the shape of the curve that relates the intended biological effect of the drug to changes in the activity of its intended target ('on-target'), and the propensity of the drug to affect unintended targets ('off-targets') at higher doses. Off-target effects can cause toxicity and, in some cases, antagonize on-target biological effects. Most anticancer drugs in use today were discovered based on their ability to kill rapidly dividing cancer cells *in vitro*. Predictably, when administered to patients, many of these drugs also injure rapidly dividing normal cells, such as bone-marrow haematopoietic precursors and gastrointestinal mucosal epithelial cells. In addition, many of these drugs are toxic to normal cells that are not rapidly dividing. Examples include doxorubicin (toxic to the heart), bleomycin (toxic to the lung) and cytarabine (toxic to the cerebellum). These other forms of organ damage become particularly important (dose-limiting) in settings in which toxicity to rapidly dividing cells can be partially ameliorated through supportive-care measures (such as bone-marrow transplantation). For these reasons, it is imperative that anticancer drugs be developed that can kill cancer cells at clinically achievable concentrations, with therapeutic indices that are higher than those of classic cytotoxic agents.

Therapeutic index

Many factors influence the therapeutic index of a drug. Some relate to the quality of the drug itself — for example, its ability to distinguish between intended and unintended targets. Others relate to the nature of its target — for example, its distribution, its normal function(s), and the degree to which those functions must be altered to achieve the desired effect. Most antibacterial agents are remarkably safe because their targets are present in the organisms they are designed to kill but not in normal host cells. However, many other relatively 'safe' drugs — such as anti-hypertensives, anti-anxiety drugs and cholesterol-lowering agents — inhibit normal cellular proteins. These drugs are clinically useful because their effects are titratable (through changes in dose and schedule), and quantitative changes in the activities of their targets lead to the desired changes in host physiology.

Summary

- Many chemicals kill cancer cells but their toxicity to normal cells limits their usefulness as anticancer drugs.
- Epigenetic and genetic alterations within cancer cells, as well as changes in their microenvironment, might increase their requirement for a particular molecular target (or targets) relative to normal cells, creating an opportunity for selectivity.
- Two genes are synthetic lethal if mutation of either gene alone is compatible with viability but mutation of both leads to death. Inhibiting the products of genes that are synthetic lethal to cancer-causing mutations should, by definition, kill cells that harbour such mutations, while sparing normal cells.
- Most drugs induce a loss-of-function phenotype. High-throughput screens using matched cell-line pairs and chemical libraries allow the identification of chemicals that inhibit or kill cells in a genotype-specific manner. The challenge in this setting is to identify the relevant target (or targets) of compounds that score positively.
- Genome-wide RNA-interference screens can now be used to identify synthetic lethal interactions in cells that are derived from higher eukaryotes, including humans.
- Gene–gene interactions, including synthetic lethal interactions that are discovered in cell-culture experiments, will ultimately need to be validated *in vivo*. It seems likely that some gene–gene interactions will be highly robust, whereas others might be valid only in specific cells or under specific experimental conditions.

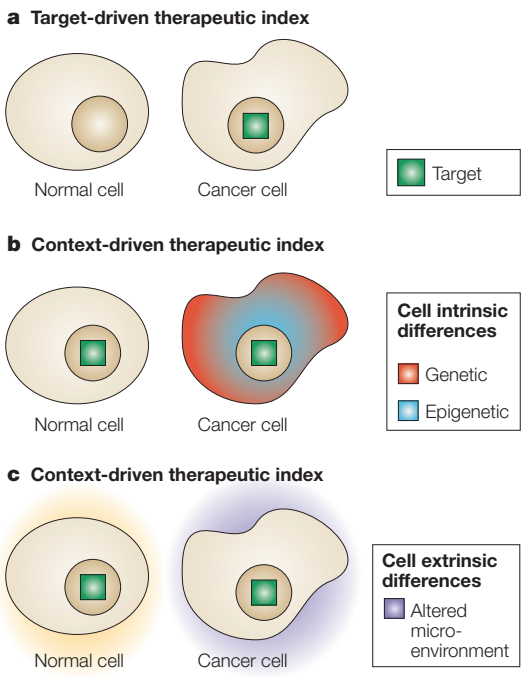


Figure 1 | **Framework for developing anticancer drugs with a high therapeutic index.** An anticancer drug might have a high therapeutic index because its target is uniquely present in cancer cells (**a**), or because the requirement for its target is quantitatively or qualitatively different in cancer cells than in normal cells (**b** and **c**). This differential requirement might be because of intrinsic differences in the cells (**b**), such as genetic (red) and epigenetic (blue) differences, or extrinsic differences in the cells (**c**), such as loss of survival signals provided by normal cell–cell and cell–matrix interactions. Modified with permission from REF. 2 © (2002) Elsevier Science.

Two paths can be envisioned to arrive at an anticancer drug that would selectively kill cancer cells. The first, which is modelled on the development of anti-infectious agents, would be to identify drug targets that are essential for the viability of cancer cells but are not present in normal cells (the so-called 'target-driven therapeutic index')[1,2] (FIG. 1). The fusion proteins generated by cancer-associated chromosomal translocations might, at first glance, seem to be ideal in this regard. However, this presumes that drugs can be developed that will discriminate between a particular protein (or functional subdomain) in its normal context and in its pathogenic, fused state. This might be difficult. For example, it is fallacious to argue that the efficacy and safety of imatinib mesylate (Glivec) for the treatment of chronic myelogenous leukaemia (CML) stems from the fact that its target, breakpoint cluster region (BCR)–Abelson murine leukaemia viral oncogene homologue (ABL), is unique to CML cells because imatinib mesylate inhibits the kinase activities of both BCR–ABL and ABL (in addition to several other cellular kinases)[3]. So, the relatively high therapeutic index of imatinib mesylate cannot be explained by the restriction of its target(s) to CML cells (see below for potential alternative explanations). Similarly, it might be difficult to develop drugs that directly inhibit oncoproteins that result from point mutations without affecting their normal counterparts.

A second way to achieve enhanced cancer-cell selectivity, however, would be to identify situations where the requirement for a particular target was enhanced in the context of a cancer cell compared with normal cells (the so-called 'context-driven therapeutic index')[1,2] (FIG. 1). The requirement for a particular target might be increased because of changes that are intrinsic to the cancer cell (for example, through epigenetic or genetic changes), extrinsic to the cancer cell (for example, as a result of microenvironmental changes leading to altered cell–matrix and cell–cell interactions), or both.

All of the anticancer drugs in use today affect targets that are shared between normal cells and cancer cells, including enzymes involved in fundamental processes such as DNA replication. The fact that their therapeutic indices, however small, exceed unity, coupled with the observation that they can, in certain settings, induce striking remissions and occasionally cures (for example, cisplatinum-based regimens for testicular cancer), indicates that contextual differences between normal cells and cancer cells are therapeutically exploitable. So, can our growing knowledge of cancer genetics, coupled with a more sophisticated understanding of gene–gene interactions, be used to identify drug targets that have enhanced therapeutic indices by virtue of such contextual differences? Studies of gene–gene interactions in model organisms have provided a conceptual framework for this task.

Synthetic lethality

Two genes ('*A*' and '*B*') are said to be 'synthetic lethal' if mutation of either gene alone is compatible with viability but simultaneous mutation of both genes

PARALOGUES
Paralogues are genes that share significant homology within a particular species. Such genes are paralogous to each other.

a Synthetic lethality

Gene *A*	Gene *B*	
A	*B*	Viable
A	*b*	Viable
a	*B*	Viable
a	*b*	Lethal

b Extragenic suppression

Gene *A*	Gene *B*	
A	*B*	Viable
A	*b*	Viable
a	*B*	Lethal
a	*b*	Viable

Figure 2 | **Gene–gene interactions: synthetic lethal and suppressive interactions for two genes.** Two genes ('*A*' and '*B*') are said to be 'synthetic lethal' if mutation of either gene alone is compatible with viability but simultaneous mutation of both genes causes death. *B* is an extragenic suppressor of *A* if mutation of *B* suppresses the phenotype observed when *A* is mutated. A lowercase letter denotes a mutant.

causes death[4–9] (FIG. 2). This concept can be extended to situations in which simultaneous mutation of two genes impairs cellular fitness more than mutation of either gene alone ('synthetic sick'). In either of these two situations, *A* buffers the effect of changes in *B* and vice-versa, but this buffering is lost when both *A* and *B* are mutated at the same time[4,6,10]. Synthetic lethal interactions have most commonly been described for loss-of-function alleles, but can also involve gain-of-function alleles. For example, gene *B* might become essential for survival when a particular gene *A* is overexpressed (known as synthetic dosage lethality)[11–13]. Approximately 20% of genes in the budding yeast *Saccharomyces cerevisiae* are individually essential, but genetic screens in this organism suggest that synthetic lethal interactions are common among the remaining 80% (perhaps on the order of 10 interactions per gene)[10,14,15].

Loss-of-function alleles having a synthetic lethal (or synthetic sick) relationship can often, but not always, be easily rationalized based on the functions of their protein products. They might, for example, be uniquely redundant with respect to an essential function (as occurs in some PARALOGUES), be two subunits of an essential multiprotein complex, be two interconnected components in an essential linear pathway (with each mutation decreasing the flux through the pathway), or participate in parallel pathways that are together essential for survival (for example, a crucial metabolic pathway and an alternative or salvage pathway). The concept of synthetic lethality can be further extended to embrace the situation where mutation of *A* is lethal only in combination with mutations that affect several non-essential genes *B*, *C*, *D* and so on[2,6].

It has been suggested that the concept of synthetic lethality could be used to choose anticancer drug targets[1,7,16]. In particular, the protein products of genes that are synthetic lethal to known cancer-causing mutations, if amenable to pharmacological attack (for example, if they encode an enzyme), should theoretically represent excellent targets for anticancer therapy. This approach simultaneously tackles two vexing problems in cancer pharmacology. The first relates to the fact that many cancer-associated mutations, like most drugs, induce a loss of function[1,2]. Therefore, it is not immediately obvious how to pharmacologically approach cancer cells in which, for example, a particular tumour-suppressor protein is crippled (or worse yet, absent). Targeting a protein that is synthetic lethal to such a lost or crippled protein provides an elegant solution to this problem. The second problem relates to whether it is possible to achieve selectivity by inhibiting proteins that are also important for cellular homestasis. If *A* and *B* are synthetic lethal (or synthetic sick), then inhibitors of *B* should selectively kill (or inhibit) cancer cells with mutant *A*. In the ideal situation, complete neutralization of *B*, genetically or pharmacologically, would have no effect on normal cells, and even partial inhibition of *B* in cancer cells would cause death (because of mutant *A*; FIG. 3, left panel). However, *B* inhibitors might display a significant therapeutic index even when these ideal conditions are not met. This would require that the *A* mutation shifts or alters the fitness dose–response curve of the *B* inhibitor such that keeping *B* activity below a certain threshold selectively impairs cells with mutant *A* (FIG. 3, middle and right panels).

It could be argued that some (and perhaps most) anticancer drugs in use today are, at least in hindsight, exploiting synthetic lethal, or synthetic sick, interactions. For example, synthetic lethal relationships between DNA-replication genes (such as certain DNA polymerases) and DNA-repair genes (such as mismatch-repair genes) are well documented in model organisms[7,16]. It seems likely that the efficacy of the many anticancer drugs that interfere with DNA synthesis is due, at least in some cases, to the presence of tumour-associated mutations that affect DNA repair or the response to DNA damage. Another example of synthetic interactions is provided by certain chemotherapeutic agents and mutations that directly or indirectly compromise the function of the retinoblastoma protein (pRB, encoded by the *RB1* gene) tumour suppressor. Inactivation of pRB has been documented in many cancers and leads to an increase in E2F activity, which, in turn, activates various genes involved in S-phase entry[17]. One of these, topoisomerase II, causes DNA strand breaks and apoptosis when bound to

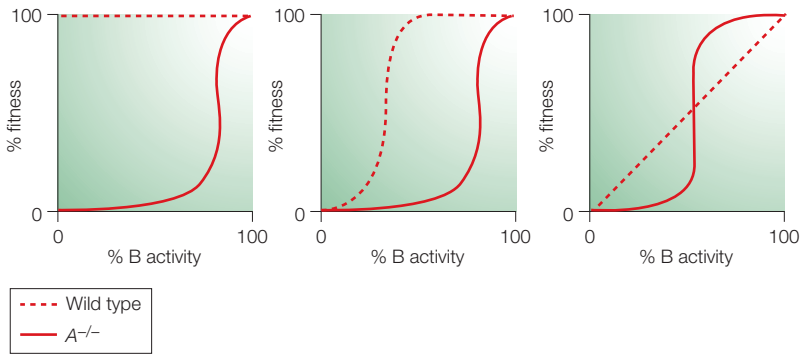


Figure 3 | **Theoretical fitness curves for wild-type and $A^{-/-}$ cells in response to a drug that inhibits the B gene product.** A reading of 0% fitness denotes death, whereas 100% fitness denotes the wild-type state (for simplicity, fitness >100% is not considered in these examples). In the middle panel, a therapeutic window is created by a shift in the fitness curve when gene *A* is absent. In the left and right panels the therapeutic window is created by changes in the shapes of the fitness curves when gene *A* is absent.

topoisomerase inhibitors such as etoposide. As would be predicted, pRB-pathway mutations sensitize cells to drugs that inhibit topoisomerase II (REFS 18–21). In addition, E2F1, like the oncoprotein MYC, increases the expression of many pro-apoptotic genes, including the p53 paralogue p73, which might sensitize pRB-defective cells to drugs that elicit additional apoptotic signals (such as DNA-damaging agents)[22–25].

Two newer anticancer agents also exploit contextual differences between cancer cells and normal cells. Studies in model organisms suggest that mutations affecting chaperones that are involved in protein folding can unmask the deleterious consequences of various mutations[26]. Preclinical data indicate that HSP90 (heat-shock protein of 90kDa) inhibitors have anticancer activity, and that certain mutant oncoproteins, such as mutant BRAF and mutant EGFR (epidermal growth factor receptor), have an increased requirement for HSP90 function[27–29]. One HSP90 inhibitor, 17AAG, has completed phase I testing and is entering phase II studies. The accumulation of mutated and/or misfolded proteins might also alter the requirement of a cell for proteasomal function[30]. The proteasomal inhibitor bortezomib is well tolerated in humans and was recently approved for the treatment of multiple myeloma[31].

Discovery of human synthetic lethal interactions

Our knowledge of the molecular networks that are established in normal cells and cancer cells is too rudimentary to allow reliable predictions of the genes that will be synthetic lethal to a given cancer gene. Nonetheless, a few ideas have been put forward for how synthetic lethal combinations might be achieved, based on first principles. Many oncoproteins, including E2F1 and MYC, represent a double-edged sword for cancer cells because they deliver both pro-mitogenic and pro-apoptotic signals. A counter-intuitive approach to treating cancer cells that have hyperactive oncoproteins such as these would be treating them with drugs that enhance their action further, in the hope of crossing an apoptotic threshold. For example, E2F1 is negatively regulated by both pRB and cyclin A[32–35] . Loss of the pRB pathway establishes a positive-feedback loop in which E2F1 activates its own promoter[36], and blocking the remaining interaction of cyclin A with E2F1 kills transformed cells but not their normal counterparts[37–39]. Unfortunately, inhibiting the activity of the cyclin-A partner CDK2 (cyclin-dependent kinase 2) does not have the same effect[40], possibly because another catalytic partner can substitute for CDK2 in its absence[41,42]. Synthetic lethal interactions might also be predicted based on the loss of particular cell-cycle checkpoints[16]. For example, S-phase cells, in contrast to G1 cells, can be induced to undergo premature chromosomal condensation under certain conditions, such as treatment with caffeine at doses that inhibit ATR (ataxia telangiectasia and RAD3-related protein)[43–46]. Cells that lack p53, which has a role in G1 control, are more susceptible to caffeine than their wild-type counterparts[47].

There are now multiple examples of cancers that seem to be dependent on or 'addicted' to certain activated oncogenes (gene-replacement experiments suggest that tumour cells can also become addicted to the inactivation of tumour-suppressor genes). Oncogene addiction might underlie the success of the kinase inhibitor imatinib mesylate for CML (in which the oncogene is *BCR–ABL*) and gastrointestinal stromal tumours (in which the oncogene is *KIT*)[3] and of the EGFR inhibitor gefitinib for EGFR-mutated non-small-cell lung cancer[48–51]. Bernard Weinstein, who coined the term 'oncogene addiction', initially envisioned that this phenomena was related to the ability of such oncogenes, which can be viewed as nodes in complex molecular networks, to simultaneously deliver proliferative and antiproliferative signals[52] (FIG. 4a). As long as the oncogene signal is sustained, the proliferative signal — which might promote mitogenesis, survival, or both — would dominate. However, if the oncogene is acutely silenced, the antiproliferative signal dominates, leading to cessation of growth or cell death (in this scenario it must be invoked that the antiproliferative signal 'decays' more slowly than the proliferative signal when the oncogene is inhibited)[2].

Superimposed on the network abnormalities that are induced by activated oncogenes are network abnormalities that are induced by mutations at other loci. The resulting abnormalities in molecular circuitry create additional opportunities for oncogene addiction[1,2,53,54], including those that arise as a result of gene–gene interactions, such as synthetic lethality and extragenic suppression. Cancers arise through sequential genetic changes that ultimately convert a normal cell to a fully transformed one. These mutations are under selective pressure to be adaptive or neutral, from the point of view of the cancer, in the context of the mutations that preceded them (FIG. 4b). It seems likely, *a priori*, that some of the mutations that occur late in the evolution of a cancer cell might only be advantageous, or indeed even tolerated, because of the mutations that preceded them (or put another way, these mutations would be deleterious if not for the mutations that had preceded them). In the extreme case, an early *A* mutation might be an extragenic suppressor of the lethality that would otherwise be caused by a late *B* mutation (FIG. 2, right panel). If this is true, correcting the *A* mutation should cause death because of the acquisition of the *B* mutation. For example, *RB1* inactivation, as described above, leads to increased E2F activity, which can stimulate S-phase entry but can also promote p53-dependent apoptosis[55,56]. So, a tumour in which *TP53* was already mutated might derive an additional benefit from mutating *RB1* but at the price of becoming addicted to p53 loss (in the sense that restoring p53 function would lead to apoptosis).

Similarly, Mills and colleagues have suggested that oncogene addiction might arise because of the loss of collateral signalling pathways. This is due to genomic instability coupled with the loss of selection pressure to maintain the collateral signalling pathways[57], a process referred to as 'genetic streamlining'[58] (FIG. 4c). Collectively, these ideas suggest that the pathways that

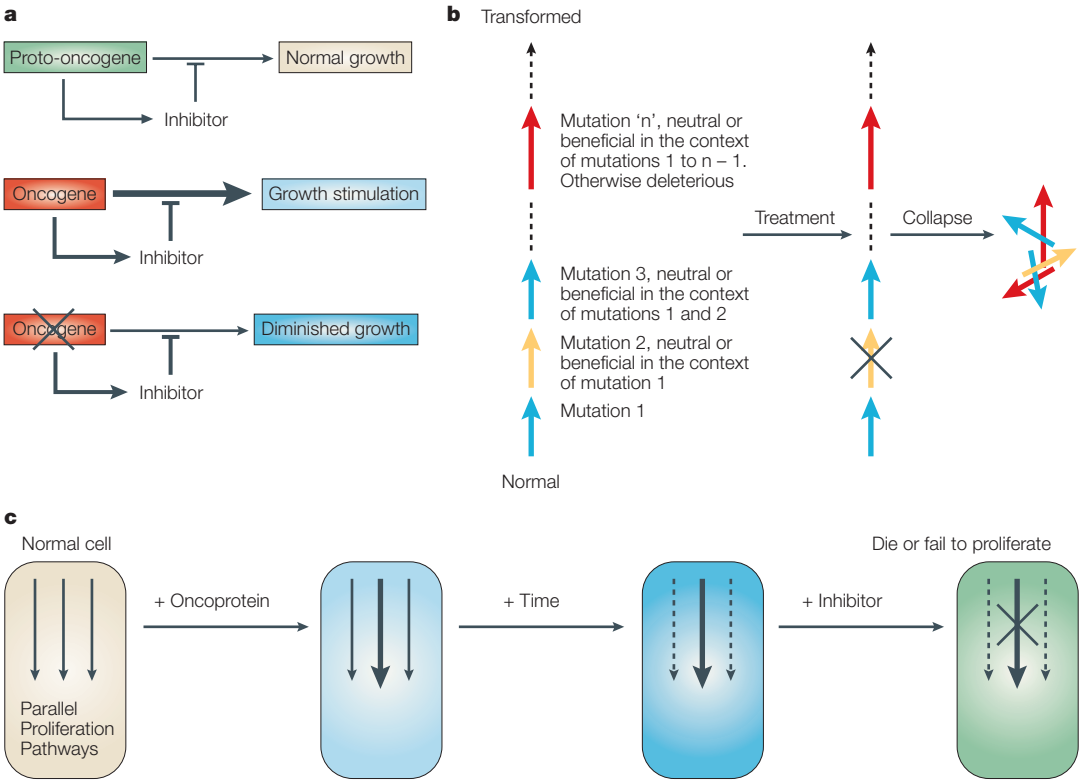


Figure 4 | **Models of oncogene addiction. a** | Many oncogenes paradoxically induce pro-mitogenic signals as well as anti-mitogenic (or pro-apoptotic) signals. Growth stimulation results from oncogene activation presumably because the former is dominant to the latter. However, acute inactivation of the oncogene might cause growth cessation or death if the anti-mitogenic/pro-apoptotic signals decay more slowly than the mitogenic signals (for example, because of differences in mRNA and protein half-life). Adapted from REF. 53. **b** | Oncogene dependency due to gene–gene interactions. Cancer cells accumulate mutations (arrows) over time that cumulatively lead to a transformed phenotype. Selection favours acquisition of mutations that are neutral or beneficial (adaptive) in the context of the mutations that preceded them. However, some of these changes might be deleterious (red arrow) were it not for the changes that preceded them. If true, correcting early genetic changes (yellow arrow) will unmask these deleterious effects. In this model, cancer cells behave like a molecular 'house of cards'. **c** | Activation (indicated by bold arrow) of an oncogenic pathway diminishes selection pressure to maintain collateral signalling pathways. Silencing of these collateral pathways over time, because of genetic or epigenetic changes, leads to oncogene dependency. Adapted from REF. 57.

are activated early in the course of tumour progression (owing to oncogene activation or tumour-suppressor-gene inactivation) are likely to be excellent therapeutic targets because of synthetic interactions with the mutational changes that followed them. Silencing these pathways should reveal the deleterious consequences of these subsequent changes, whether these changes did or did not contribute to tumour progression. The potential interrelationship between oncogene addiction and synthetic lethality is illustrated by the phosphatase and tensin homologue (PTEN) tumour-suppressor protein, which negatively regulates the phosphatidylinositol 3-kinase (PI3K) pathway, and mTOR (mammalian target of rapamycin). *PTEN*$^{-/-}$ cells are reported be more sensitive to the antiproliferative effects of mTOR inhibitors than their wild-type counterparts[59]. This observation indicates that *PTEN*$^{-/-}$ cells are 'addicted' to PI3K–mTOR signalling, and that PTEN and mTOR have a synthetic sick relationship.

Chromosomal deletions in cancer cells lead to the loss of one or both copies of many genes. Frei suggested that cancer-cell vulnerabilities to pharmacological attack might also be gleaned by examining the functions of contiguous genes that are homozygously deleted along with tumour-suppressor genes[60]. For example, the gene encoding methylthioadenosine phosphorylase (MTAP) — which has a role in a salvage pathway for adenosine biosynthesis — is often co-deleted with the adjacent *CDKN2A* locus, which encodes the tumour-suppressor proteins INK4A and ARF on 9p21 (REF. 61). As would be predicted, cells that lack MTAP have increased sensitivity to L-alanosine — a potent inhibitor of *de novo* AMP synthesis — and to an inhibitor of *de novo* purine-nucleotide synthesis, 6-methylmercaptopurine riboside (MMPR)[62].

Kamb suggested that expression databases be mined for paralogous genes in which one or more members were underexpressed in cancer cells relative to normal cells (for example, as a result of haploinsufficiency or homozygous deletion)[58]. A drug that inhibited the remaining paralogue(s), but not the differentially expressed paralogue, would, theoretically, be cancer-cell selective. This approach, however, presumes that it is possible to develop drugs that can discriminate between

BROMODEOXYURIDINE (BRDU) INCORPORATION
Incorporation of the thymidine nucleotide analogue bromodeoxyuridine into DNA can be used to measure the rate of DNA synthesis.

ISOGENIC
Two cell lines are isogenic if they are derived from the same parental cell line, or from one another such that they are genetically identical.

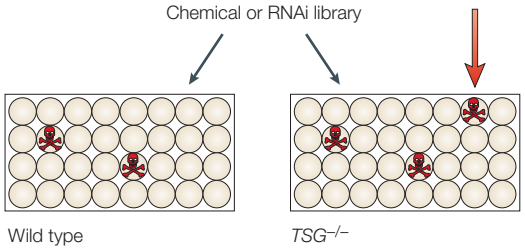


Figure 5 | **Synthetic lethal screening with chemical or interfering RNA libraries.** Isogenic cell-line pairs that do or do not harbour a cancer-relevant mutation (in the case illustrated, the cell-line pair differs only with respect to a particular tumour-suppressor gene (*TSG*)) are grown in multiwell plates to which different chemical or genetic (short interfering RNAs, short hairpin RNAs or other interfering RNAs) perturbants are added. In time, such assays might be carried out using microarrays spotted with chemicals or siRNA species[104,105]. A 'hit' is a perturbant that is cytostatic or cytotoxic to the cell with the cancer-relevant mutation (arrow). It should be noted that the interpretation of such assays needs to consider potentially confounding effects, such as differences in proliferation rate and cell-cycle distribution.

paralogous proteins. Moreover, synthetic lethal screens in yeast indicate that paralogous pairs represent a minority of the potential synthetic lethal combinations in a cell[10,15,63]. Therefore, unbiased chemical and genetic screens are likely to be the most fruitful methods for identifying novel synthetic lethal relationships on which to base new cancer treatments.

Screens for synthetic lethal interactors

The example of topisomerase II inhibitors, as cited above, demonstrates that proteins bound to drugs might have effects that are very different from those predicted by true null mutations, or by techniques such as RNA interference (RNAi) that cause quantitative reductions in protein abundance. For example, a drug might interfere with one function of a multifunctional protein, or cause a protein to act in a dominant-negative or dominant-positive manner. For this reason, screens for synthetic lethality that are carried out using libraries of chemical compounds are likely to be complementary to screens that are carried out using genetic tools (such as RNAi or short interfering RNA; siRNA).

Chemical screens. Hartwell and Friend pioneered the idea of screening for drug-like chemicals that specifically kill yeast deletion mutants with defects in cell-cycle checkpoints or DNA repair[16,64]. This paradigm can be extended to human cells. A number of groups have identified chemicals from collections of pure compounds, or that are present in complex mixtures (for example, extracts or broths), that selectively inhibit cells with cancer-relevant genetic alterations using isogenic human cell-line pairs grown in multiwell plates (FIG. 5). Schreiber and co-workers identified marine sponge extracts that preferentially inhibited the proliferation of *Trp53*$^{-/-}$ mouse embryonic fibroblasts, as determined by BROMODEOXYURIDINE (BRDU) INCORPORATION, relative to wild-type mouse embryonic fibroblasts[65]. However, the chemical entities responsible for these effects were not identified. Kinzler and co-workers co-cultured *KRAS*-mutated colon cancer cells (engineered to produce blue fluorescent protein) with a subclone in which the mutant *KRAS* allele was eliminated by homologous recombination (and engineered to produce yellow fluorescent protein), and monitored differential killing using the ratio of blue/yellow fluorescence[66] (FIG. 6a). Several chemical entities, including a novel cytidine nucleoside, were found that selectively killed cells containing mutant *KRAS*. A fluorescence-based mammalian synthetic lethal assay, which was modelled after earlier yeast assays[67], was also developed by Canaani and colleagues[68,69] (FIG. 6b). Leder and co-workers discovered a small molecule called F16, which selectively kills ERBB2 (also known as HER2/NEU)-overexpressing mammary epithelial cells, compared with their normal counterparts[70,71]. The toxicity of F16 correlates with its selective uptake in, and disruption of, mitochondria of cells that are transformed with ERBB2. Stockwell and co-workers identified a number of compounds that preferentially killed primary human cells that were transformed *in vitro* with human telomerase reverse transcriptase (TERT), RAS, and oncoproteins that affect pRB, p53 and/or protein phosphatase 2A (PP2A)[21]. Included among these were clinically useful inhibitors of topoisomerase I and II. In a focused screen of pro-apoptotic agents Quon and colleagues discovered that human cells overexpressing MYC displayed increased sensitivity to the death receptor DR5 agonist tumour-necrosis-factor-related apoptosis-inducing ligand (TRAIL) *in vitro* and *in vivo*, and linked this to p53-independent induction of DR5 by MYC[72]. Recent studies suggest that it is possible to screen pairwise combinations of drugs against ISOGENIC cell lines to uncover novel drug–gene and drug–drug interactions[73,74].

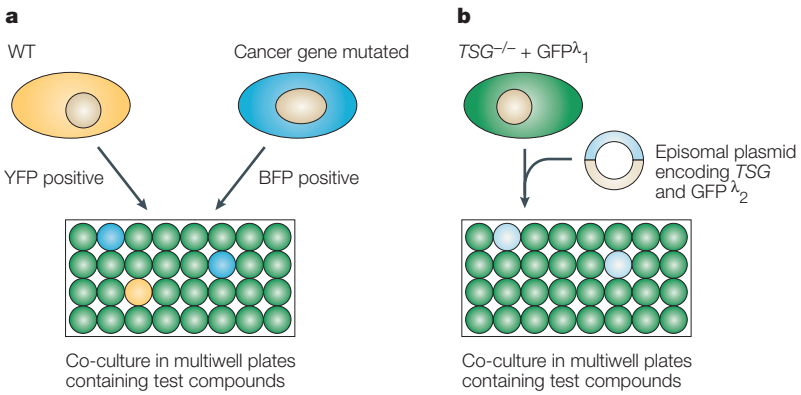


Figure 6 | **Fluorescence-based mammalian synthetic lethal assay. a** | The Kinzler method[66]. Isogenic cell-line pairs that do/do not harbour a cancer-relevant mutation are engineered to produce blue fluorescent protein (BFP) and yellow fluorescent protein (YFP), respectively, and are co-cultured in multiwell plates to which different chemicals are added. Selective killing of blue cells is indicative of a synthetic lethal interaction (yellow well). **b** | The Canaani method[68,69]. Cells lacking a tumour-suppressor gene (*TSG*) are engineered to stably produce a green fluorescent protein (GFP) with an emission wavelength of '1'. These cells are transfected with an unstable episomal plasmid encoding the *TSG* along with a GFP that has a different emission wavelength ('2'). Retention of the episomal plasmid after exposure to chemical or genetic perturbants is indicative of a synthetic lethal relationship. WT, wild type.

The use of isogenic cell-line pairs to identify compounds that selectively kill cancer cells as a result of synthetic interactions is a powerful approach for the following reason. It is not uncommon for ~1% of the compounds in a chemical library to inhibit the growth of human cancer cells at the concentrations used in typical high-throughput screens. This translates into thousands of potential anticancer drugs from a screen conducted with 10^5 to 10^6 compounds (such as might be found at a large pharmaceutical company or public consortium). Without the use of a filter, such as differential killing in a genotype-specific manner, there are too many 'hits' to pursue. In the past, this has led to 'hits' being prioritized on the basis of factors such as ease of synthesis, potency, intellectual-property issues and the likelihood of having desirable absorption, distribution, metabolism and excretion (ADME) properties based on accepted criteria such as 'LIPINSKI'S RULES'[75,76]. Although they are important, none of these latter considerations address selectivity. Furthermore, these factors can sometimes be addressed by modifying the chemical structure of the initial compound (medicinal chemistry). It would be ironic if chemicals that can selectively kill cancer cells through synthetic lethal interactions were present but missed for this reason during the countless cytotoxic screens that have been conducted since the mid-twentieth century.

A generic problem for cell-based screening of libraries of chemical compounds relates to successful target identification. In some cases, it is possible to use a chemical entity identified in such a screen to capture its protein target by affinity chromatography[77,78]. For chemicals that induce a phenotype in yeast, mutants that display increased or decreased resistance (fitness) can be sought[79,80]. Such mutants often provide clues as to the pathways that are affected by a compound, and therefore its potential target (or targets). A conceptually attractive approach to target identification would be to generate compendia of molecular signatures (for example, gene-expression profiles) for various loss-of-function mutations in a suitable host (for example, yeast or human cells)[81]. The signature generated by the compound of interest could then be compared *in silico* to the compendium, with the rationale that the compound signature and target-disruption signature should be near(est) neighbours in an ideal situation. The search for targets of chemicals identified in cell-based synthetic lethal screens should also be expedited by a knowledge of the genes that score as synthetic lethal in genetic screens carried out in model organisms and human cells, as described below.

LIPINSKI'S RULES
Lipinski noted that the following properties predict that a chemical will have poor bioavailability after oral administration: molecular mass greater than 500 Da, high lipophilicity (calculated LogP greater than 5, where LogP indicates solubility in octanol relative to water), more than 5 hydrogen-bond donors and more than 10 hydrogen-bond acceptors.

Genetic screens. In the past, genetic screens for synthetic lethal interactors have been largely relegated to model organisms such as yeast, the fruitfly *Drosophila melanogaster* and the worm *Caenorhabditis elegans* that are amenable to forward-genetic approaches. Typically, these approaches have combined random mutagenesis with phenotypic screens, reflecting the retention of the query gene linked to a suitable reporter. Synthetic lethal screens in yeast have been invaluable for elucidating certain principles surrounding synthetic lethal interactions. Unfortunately, many tumour-suppressor genes and oncogenes do not have clear yeast orthologues. Although forward-genetic screens are more cumbersome in fruitflies and worms than in yeast, they offer the advantage that their genomes do contain orthologues of most human cancer genes. In worms the *RB1* orthologue, *lin-35*, has been well studied in the context of vulvar development[82]. Fay and co-workers reported that a gene encoding a ubiquitin-conjugating enzyme related to human UBCH7 is synthetic lethal to *lin-35* (REF. 83), as is the worm homologue of *CDH1* (REF. 84). Using a fruitfly-based screen in which the fruitfly *RB1*-like gene *Rbf1* was conditionally inactivated in the eye, Belvin and co-workers discovered that RBF1 is synthetic lethal to a novel prolyl isomerase[85]. It is not yet known whether these synthetic lethal interactions will hold true in all cell types, nor whether they will hold true across species.

However, forward-genetic approaches such as these are now giving way to genome-wide reverse-genetic approaches. Successful studies have been carried out in yeast (BOX 1) but, for the reasons cited above, metazoan models are usually more appropriate than yeast for synthetic lethal screens for human cancer genes.

RNAi is a powerful method for silencing genes in worms and fruitflies, and collections of interfering RNAs have been created to facilitate high-throughput genome-wide screens in these organisms[86–89] (for an excellent review, see REF. 90). RNAi can be conveniently achieved in wild-type or mutant worms by growing them on lawns of *Escherichia coli* carrying a plasmid that produces the interfering RNAs of interest, which are then ingested. Alternatively, interfering RNAs can be delivered to worms by soaking them in a solution that contains the appropriate molecules. An interfering RNA that exacerbated the mutant phenotype without affecting wild-type animals would indicate a synthetic lethal, or synthetic sick, interaction. High-throughput screens have also been conducted to identify interfering RNAs that inhibit the proliferation of fruitfly cells grown in multiwell plates[88]. Such screens could easily be adapted to carry out synthetic lethal screens. In this scenario, the identification of interfering RNAs that do not affect wild-type fruitfly cells but kill fruitfly cells in which the gene of interest was mutated or silenced would be desired. If required, silencing could be accomplished by simultaneously administering two interfering RNAs (one corresponding to the query gene and one corresponding to the gene of interest).

Many cancer-relevant genes are linked to specific types of cancer despite being ubiquitously expressed and performing functions that are thought to be generic rather than tissue specific. In addition, there are now many examples where different phenotypes have been observed following heterozygous inactivation of a particular tumour-suppressor gene in both mice and humans. These observations indicate that context, with respect to cell-type and species, is important. As a corollary,

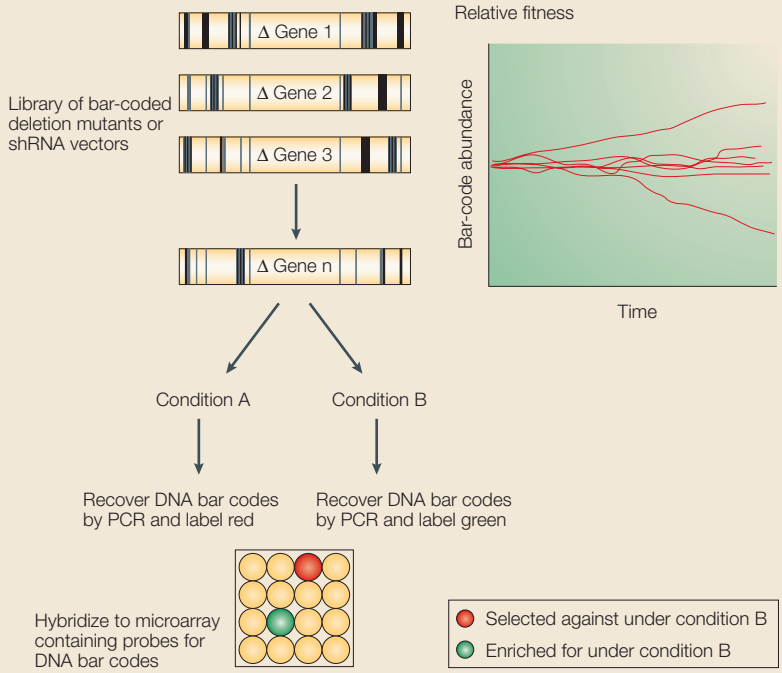
Box 1 | **DNA bar code screens for genes that alter fitness**

Screens in yeast

In yeast, synthetic lethal interactions have been identified by systematically creating double mutants (by mating or transformation) and studying their fitness, either by robotically spotting them in arrays[14,15] or following their behaviour in pools with the use of DNA 'bar codes'[106]. Deletion mutants have been generated for almost every gene, with each strain carrying two unique DNA sequences or bar codes — one immediately upstream and one immediately downstream of the gene deletion — that serve as strain identifiers[106–109]. The upstream and downstream bar codes are each flanked by invariant sequences that can be used to PCR amplify either all possible upstream bar codes or all possible downstream bar codes. Pools of yeast representing the various deletion mutants can be grown under different sets of conditions. The abundance, and therefore the 'fitness', of individual deletion strains can be monitored by recovering the bar codes using PCR and hybridizing them to oligonucleotide arrays where each feature is complementary to, and therefore interrogates, a different bar code[106–108]. Similar assays were reported earlier using *Escherichia coli* that had been mutagenized with transposons bearing unique DNA sequence tags[110]. This approach could be used to look for yeast deletion mutants that become 'unfit' (synthetic sick or synthetic lethal) in the context of a mutation of interest (for example, a DNA-repair enzyme that is deleted in human cancers).

Screens in mammalian cells

Cells are created where inactivation of a particular gene is linked to the introduction of a unique DNA sequence that serves as a molecular identifier or bar code. Inactivation can be accomplished by homologous recombination (as has been done in yeast) or with short hairpin RNA (shRNA) vectors. In the example shown, two bar codes, one 'upstream' and one 'downstream' have been used per gene. Pools of bar-coded cells are grown under two sets of experimental conditions, such as presence or absence of a chemotherapeutic agent or of a query gene. The bar codes are then recovered by PCR (using primers that recognize invariant sequences flanking either the upstream or downstream bar codes), fluorescently labelled (red for one condition and green for the other), and hybridized to microarrays containing probes complementary to the individual bar codes. Selection for or against a particular deletion strain under the test conditions relative to the control conditions gives rise to changes in the red/green ratio (bottom). The abundance of particular clones over time in response to a particular condition, as determined by normalized fluorescence intensity, can also be used to monitor fitness (right).



they indicate that synthetic lethal relationships ultimately need to be discovered or validated in relevant human cells, and that caution needs to be exercised when extrapolating cell-culture results to intact organisms. In the past, the use of RNAi in mammalian cells was problematic because double-stranded RNA elicits an antiviral response on entry into mammalian cells. In 2001, however, Tuschl and co-workers showed that siRNAs can be used to silence genes in mammalian cells without triggering a nonspecific host response[91]. Soon thereafter several groups showed that the actions of siRNAs in cells can be mimicked with short hairpin RNAs (shRNAs) encoded by plasmid or viral vectors[92–96]. siRNA libraries and shRNA vector libraries are being created, and proof-of-concept experiments indicate that these libraries can be used to carry out genome-wide phenotypic screens in mammalian cells (including human cells)[97–100]. In theory, these libraries could be used to carry out synthetic lethal screens using isogenic cell-line pairs, scoring for siRNA (or shRNA) species that specifically kill cells with a cancer-relevant mutation in a one well/one siRNA (or shRNA) species format (FIG. 5). Alternatively, several groups are incorporating DNA 'bar codes' (BOX 1) into shRNA vectors, modelled after the use of DNA bar codes in yeast and *E. coli* (or have used the shRNA sequence itself as a bar code)[97,98]. If successful, it should be possible to infect isogenic cell-line pairs with pools of vectors encoding different shRNAs, and then identify those shRNAs that cause a fitness defect specifically in those cells that harbour the cancer-relevant mutation under investigation.

Combination therapy

Random mutations that lead to gene inactivation should theoretically decrease the genetic buffering capacity of an individual cancer cell. As outlined above, therapies predicated on synthetic lethal relationships are one way to exploit this. At the same time, random mutations and genome plasticity, viewed at the level of a tumour, markedly increase the likelihood that rare therapy-resistant subclones will emerge. Decades of clinical experience, including recent examples of imatinib mesylate resistance[101,102], as well as tumour models incorporating the use of conditionally expressed oncogenes[103], support this view. A 1-cm^3 tumour already contains >10^9 cells. So, the likelihood of clinical success will increase with early diagnosis (to minimize the number of cells in the pool from which resistant cells might arise) and the use of effective drug combinations. The use of drug combinations to minimize chemotherapeutic resistance is a well-established pharmaceutical principle. It is based on the knowledge that the probability of a given cell being simultaneously resistant to a combination of non-cross-resistant drugs varies as the product of the probabilities of becoming resistant to each of the individual components. The choice of which drugs to combine might be based on a knowledge of cancer molecular biology (for example, by simultaneously targeting two or more cancer-relevant mutations), empirical testing (for example, by systematically testing combinations of active agents for additive or synergistic effects) or both.

Implications and future directions

Over the decades, the medical therapy of metastatic cancer has, with a few notable exceptions, been a frustrating and often futile exercise. This has contributed to the view that each mutation within a cancer cell is another plate of armour that serves as a barrier to successful therapy. However, our empirical knowledge of the susceptibility of cancer cells to drugs in humans stems from an armamentarium that was largely discovered and developed using the same paradigm. Moreover, there is every reason to believe that certain genetic changes within cancer cells will create liabilities under the appropriate conditions. There are now tools to systematically search for mutated oncogenes that encode molecules, such as kinases, that can be targeted by drugs, as well as the tools to reveal vulnerabilities created by synthetic lethal interactions. Understanding how the phenotypes created by cancer genotypes (for example, tumour type and resistance to therapy), as well as synthetic lethal relationships, are influenced by contextual differences (for example, cell type and species) remains a formidable task. Nonetheless, we are clearly poised to move away from empirically discovered cytotoxics and towards new agents that are based on a knowledge of cancer genetics and a more sophisticated view of gene–gene interactions.

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Competing interests statement

The authors declare no competing financial interests.

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