Review

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Building high-resolution synthetic lethal networks: a 'Google map' of the cancer cell

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The most commonly used therapies for cancer involve delivering high doses of radiation or toxic chemicals to the patient that also cause substantial damage to normal tissue. To overcome this, researchers have recently resorted to a basic biological concept called 'synthetic lethality' (SL) that takes advantage of interactions between gene pairs. The identification of SL interactions is of considerable therapeutic interest because if a particular gene is SL with a tumor-causing mutation, then the targeting that gene carries therapeutic advantages. Mapping these interactions in the context of human cancer cells could hold the key to effective, targeted cancer treatments. In this review, we cover the recent advances that aim to identify these SL interactions using unbiased genetic screens.

Challenges in identifying efficient therapeutic targets in malignant tumors

Unlike single gene-associated diseases, such as Tay–Sachs syndrome or Duchenne's muscular dystrophy, malignancies are diseases of dynamically evolving defective genomes. The continued changes in DNA sequences result in specific genetic dependencies (see Glossary) of individual clones within a single tumor or within different metastatic tumors carried by the same individual. Extensive sequencing of tumor samples continues to provide the blueprint of the cancer genome, allowing for the design of effective targeted therapies against driver mutations [1]. Indeed, targeted therapies, such as imatinib for suppressing activity of the BCR-Abl fusion protein in chronic myeloid leukemia [2], and trastuzumab for human epidermal growth factor receptor 2 (HER2) inhibition in breast cancer [3], represent important milestones in the genotype-directed treatment approaches. Despite the fact that targeting cancer-initiating mutations is relatively straightforward, this strategy has been applied with limited success. For example, no effective targeted therapies have been found for *KRAS* mutation-driven tumors, although >20% of cancers harbor a mutation in this gene [4]. This is likely because KRAS activates multiple critical effectors, such as the mitogen-activated protein kinase–extracellular signaling kinase signaling pathway (MEK–ERK), the phosphoinositide-3-kinase–protein kinase B intracellular signaling pathway (PI3K–AKT), and nuclear factor kappaB (NF-κB) pathways [5]. The situation becomes even more challenging when cancer aggressiveness is driven by the loss of a tumor suppressor such as *TP53*, *Rb*, *PTEN*, or *EPHB6*, because these mutations provide no obvious targets for treatment [6].

Although many advances have been made in recent years in developing new anticancer drugs, success is still seriously hindered by the limited available information on

Glossary

Arrayed screen: the use of individual siRNA, shRNA, or sgRNA (currently not available) to query the genome on a gene-by-gene basis within a cell line to study the effect on cell fitness in microtiter plates is referred to as arrayed screen.
Chemical genetics: the utilization of small molecules to investigate genetic relationships between gene pairs by temporarily altering a gene's functionality in a dose-dependent manner.
Differential essentiality: analogous to differential expression, the idea of defining lethality only to cancer cells, but not to normal cells, is referred to as differential essentiality.
Epistatic-Mini Array Profiling (E-MAP): E-MAP is a strategy adopted by yeast biologists to study the functions of functionally related genes in order to generate high-density interaction networks.
Genetic dependency: the property of the cells to depend on specific cellular pathways for their survival. Shutting this pathway essentially leads to cell death.
Genetic interaction: the observation of an unexpected phenotype between any two genes when both are genetically altered.
Induced essentiality: the property to induce lethality not because of the functional relation between two genes, but due to genetic dependency of one mutation on another is referred to as induced lethality.
Interactome: the complete network of interactions between gene pairs (genetic interactome) or proteins (physical interactome) within a cell. Alternatively, the complete interaction of components within a given biological process can also be referred to as an interactome.
Negative genetic interaction (NGI): NGI refers to the relationship between two genes that leads to a lethal phenotype when they are genetically altered. Synthetic lethality and synthetic dosage lethality are two major types of NGIs.
Pooled screen: the use of a pool of viral particles, each expressing a distinct shRNA or sgRNA to knockdown or knockout genes, respectively, within a cell line to study the effect on cell fitness as a pool.

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potentially druggable targets. Indeed, many developing drugs are repeatedly directed towards a narrow scope of molecules [7], and a broader method of assessment of the molecular machinery of cancer cells is urgently needed to increase the current range of targets and eventually improve treatment efficiency. In response to this demand, scientists are now beginning to recover information from systematic genome-scale approaches that identify novel therapeutic targets by taking advantage of altered tumor genetics [8–11].

In this review, we discuss in detail one of these strategies, based on negative genetic interactions (NGIs), and show how it can be used to discover novel targetable vulnerabilities of cancer cells. We then explain how this knowledge should support the development of a network-based approach to build a 'Google map' of the cancer cell in designing effective genotype-targeted cancer therapies.

Exploiting tumor genetics

In an effort to facilitate the development of anticancer drugs directed at specific molecules, many researchers have turned to exploiting the interactions between gene pairs, which can reveal their phenotypic relationships [12]. These genetic interactions can be broadly classified as NGIs that cause a fitness defect, and positive genetic interactions that lead to growth and survival advantages. Since most of the phenotypic traits of cancer are caused by genetic alterations, such as gain-of-function (amplification or overactivation of proto-oncogenes) and/or loss-of-function (deletion or epigenetic silencing of tumor suppressors), the negative genetic interactions (NGIs) that lead to a fitness defect can be exploited for targeted therapy by two approaches, referred to as 'synthetic lethality' (SL) and 'synthetic dosage lethality' (SDL) (Figure 1A). Two genes are said to cause SL if inactivating mutations in either gene alone leaves the cell viable, but together, they cause death [12]. By contrast, two genes are said to exhibit SDL interaction when an overactivation of a gene is lethal only when another, nonlethal mutation is present [13]. From the genetic principles, we now understand that the interacting genes are often functionally coherent and perhaps work in parallel pathways. However, in cancer cells, due to the plasticity of biological networks, genetic dependency may arise from an unexpected pathway, which might also lead to lethality. These types of NGIs may be referred to as 'induced essentiality' [14].

Conceptually, NGIs can also be modeled by inhibiting products of one or both of the genes using small molecule inhibitors, an approach referred to as chemical genetics (Figure 1A). Identification of such a lethal relationship between any potential gene of interest and an oncogene or tumor suppressor would represent a valuable strategy for developing new therapies in cancer treatment [15]. A prominent example of exploiting SL for cancer therapeutics is the NGI observed between the breast cancer genes *BRCA1* or *BRCA2* and the poly(ADP-ribose) polymerase gene *PARP1* [16,17]. Although the phase III clinical trial of the PARP inhibitor, iniparib (Sanofi-Aventis) has reached a dead end, it is likely that its specificity and the dependency of only 10–20% of the triple negative breast cancer patients on 'BRCAness' are responsible for its failure [18]. This is also in line with the highly contextual character of the translational potential of discovery science, illustrated by another example. Patients with *BRAF* (V600E) mutant melanoma responded to a small-molecule inhibitor of the BRAF kinase [19], while *BRAF* (V600E) mutant colon tumors were almost invariably resistant to the same BRAF inhibitor that had proven to be successful in treating melanomas with the identical *BRAF* mutations [20]. Understanding the contextual role of the epidermal growth factor receptor gene (*EGFR*) has now indicated that combined inhibition of both *EGFR* and the proto-oncogene serine/threonine-protein kinase *RAF* is essential for improved efficacy in colon cancer [21].

Overall, a potential way to overcome complex malignancy-dependent mechanisms and tumor heterogeneity-associated challenges is to identify genetic dependencies of tumor cells and target the associated pathways rather than individual driver mutations. NGIs (both SL and SDL) reveal the genetic principles that underlie the evolution of cancer genomes and demonstrate how combinations of molecular events contribute to cancer phenotypes. Building a systematic network of NGIs will provide comprehensive knowledge of how various molecular pathways synergize with one another to sustain a malignant tumor. This is analogous to building the 'Google map' for a cancer cell. Since the key strategy in cancer therapy is to block all the alternate pathways, it is critical to know how things are re-routed within a cancer cell. Because genes function in multiple biological processes, it only makes sense to understand their functions in the context of a cellular circuit. As Marc Kirschner correctly pointed out, 'it becomes imperative to build a quantitative map that will enable researchers to better predict how drugs will act' [22]. When Google started building street maps, their propaganda was, 'with Google maps, we are attempting to make finding your way around the world effortless and enjoyable' (Willem Van Lancker, Visual Designer for Google maps, http://www.core77.com). NGIs precisely fall into the science of developing these maps that, in theory, should make it relatively easy to find biologically relevant targets. Below, we describe key strategies currently adopted to generate these maps.

Strategic approaches for identifying NGIs as potential targets for cancer therapy

Translating NGIs from model systems to define potential therapeutic targets

Information collected from genetically tractable organisms, particularly the yeast *Saccharomyces cerevisiae*, can be used to identify cancer targets, provided that the genes and their functions are conserved [23]. Despite the evolutionary distance between human cells and those of other eukaryotic organisms, some inferences can be successfully made by comparing genetic interactions in model systems to determine therapeutic targets against human cancers (Figure 1B) [24–30]. For instance, comparative and cross-species genomic approaches have allowed the prediction of conserved SL interactions, such as the ones observed between *RAD54B* and *FEN1* [25]. Similarly, SL interactions between *RAD54B* and *SOD1* have been mapped from yeast to humans, with the inhibition of

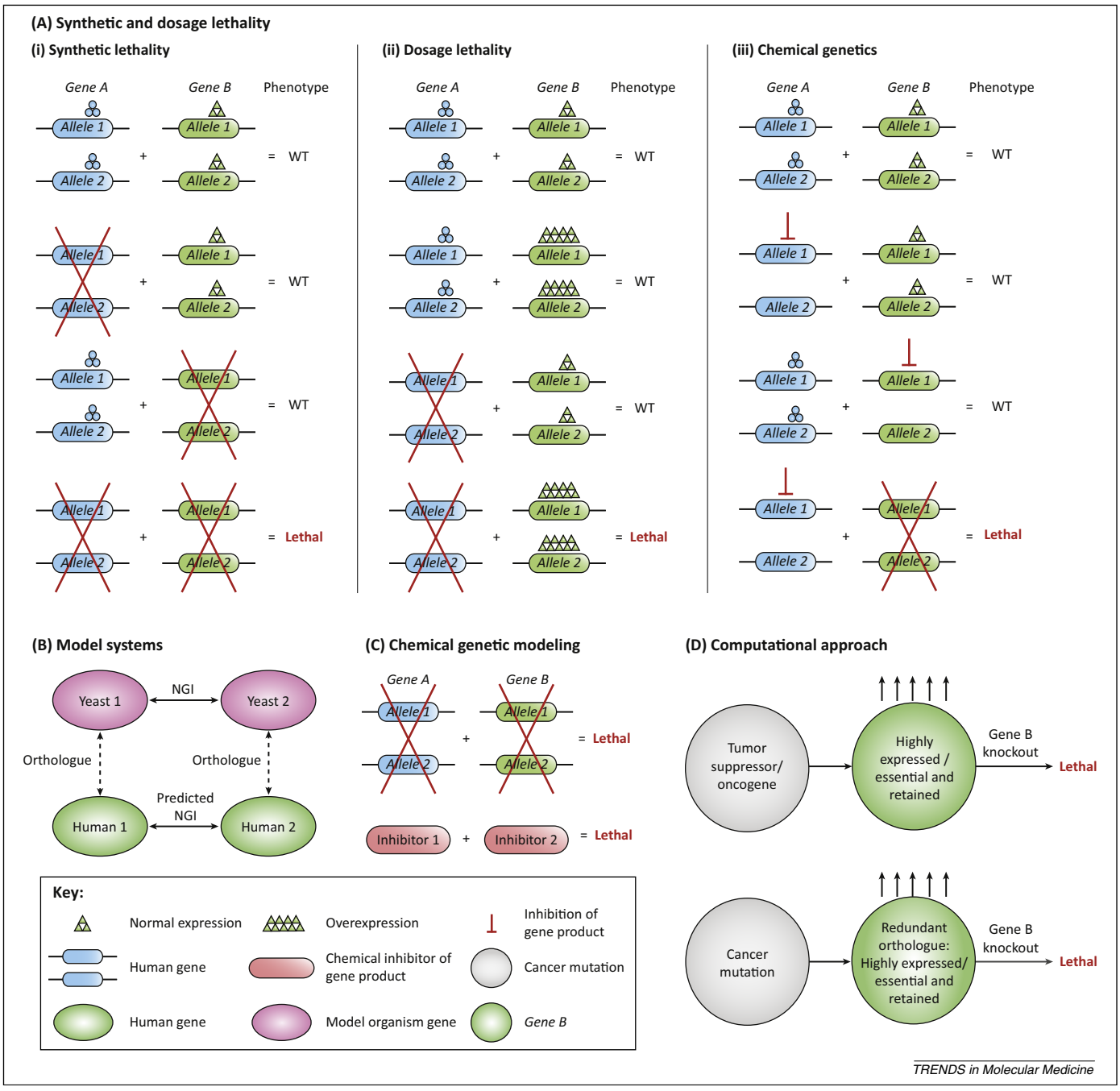

Figure 1. Current strategies for defining negative genetic interactions (NGIs) for cancer target identification. **(A)** A schematic representation of (i) synthetic lethality, (ii) dosage lethality, and (iii) chemical genetic strategies. **(B)** Mapping interactions in human cells using information from model systems. The figure illustrates that if two genes in any species are found to interact, then it is possible to predict that their orthologous human genes may interact as well. **(C)** In the case of small molecule inhibitors, a list of NGI hits can be mapped to chemical inhibitors from Drugbank v3; Chembl v11; and GVKBio databases that can model the interaction. **(D)** A schematic of how computational analysis can be used to predict synthetic lethality (SL) interactions. Gene B is found to share either a SL or synthetic dosage lethality (SDL) relationship with a cancer mutation, provided Gene B becomes highly expressed and essential within the cell. This knowledge can be applied to any redundant, essential orthologs of Gene B, implying that a similar cancerous mutation would lead to high expression and essentiality of such an ortholog, with its knockout similarly resulting in cellular lethality.

SOD1 leading to apoptosis in RAD54B-deficient colorectal cancer cells [30]. Also, systematic orthology mapping of yeast interactions has identified a SL relation between the tumor suppressor gene *SMARCB1* and *PSMA4*, and another between alveolar soft-part sarcoma-associated *ASPSCR1* and *PSMC2* [31]. By translating yeast interaction data, PP2A has been identified as a potential target due to its SDL relation with the spindle checkpoint protein MAD2, which is often overexpressed in cancer [27].

Unfortunately, comparative genomics may not reveal all interactions that could be translated for therapeutics. This is because genetic interactions observed in lower eukaryotes are not always truly conserved over large evolutionary distances [14,32,33]. In fact, the conservation of genetic interactions, even between two yeast species, is estimated to be only ~20% [34,35]. Thus, the therapeutically translatable genetic interactions for cancer cells may not be in congruence with interactions defined in normal

yeast or other model systems, or could be restricted to a specific type of malignancy. It could be predicted that the biological process in question might dictate the level of conservation and indeed, a higher percentage of genetic interactions (~43%) were found to overlap with the highly conserved mitotic spindle assembly pathway between two distant model systems, *S. cerevisiae* and *Caenorhabditis elegans* [33]. In addition, effects on embryonic lethality versus life span, fitness, or proliferation rate may generate differences in estimating the levels of interactions and their conservations in various biological models.

Above all, the largest available NGI datasets in yeast cover only the nonessential genes and, therefore, screening of the more conserved essential genes using temperature-sensitive mutants [36] may reveal a higher level of conservation. Although stringent orthology mapping systems, such as Inparanoid (http://inparanoid.sbc.su.se/cgi-bin/index.cgi), may cover a maximum of only 3000–5000 human genes, with respect to the yeast genome, there are an estimated ~22 000 genes in the human genome, and defining SL–SDL interactions from model systems alone cannot be expected to be sufficient. Perhaps mapping systems that use orthologous clustering mechanisms, such as eggNOG (http://eggnog.embl.de), may help to relate the more distant orthologs and facilitate drug target identification using evolutionarily distant models.

Knowledge-based validation of NGIs

Years of experimental knowledge have enabled the research community to predict potential NGIs that could be translated for therapeutics [37]. One of the most explored areas of SL–SDL interactions that preferentially kill cancer cells by exploiting dependencies that are not shared by normal tissue are those involved in the DNA damage response or checkpoint control pathways. In 2001, Gurley and Kemp were first to demonstrate synthetic lethality in mice among genes that are frequently mutated in cancers, the *ATM* and *DNA-PK* genes [38]. Similarly, in nontumor cells the p38/MK2 pathway is not required for checkpoint functions, whereas in tumor cells lacking p53, the p38/MK2 pathway becomes crucial for arresting the cell cycle [39]. Therefore, in p53-deficient tumor cells, the MK2 pathway is the prime candidate for inducing a SL response. Another example of targeting the DNA damage pathway includes induction of SL in tumors possessing *BRCA* mutations. In healthy cells, DNA double-strand breaks (DSB) are fixed via homologous recombination repair, a process that depends on *BRCA1/2*, with *RAD51/52* acting as a backup. However, in cases of *BRCA* mutation, *RAD51* becomes a prime target for SL, whereby disruption of phenylalanine 79 in the RAD52-DNA binding domain leads to a buildup of DSB and subsequent lethality [40].

Apart from targeting genome maintenance systems by SL, there are other instances where NGIs have resulted in potential therapeutic avenues. For example, EGFR is often highly expressed in basal-like breast cancers [41], which are highly aggressive and commonly display the triple-negative phenotype: missing the estrogen and progesterone receptors and not overexpressing *HER2*. EGFR expression is not the only important factor in this malignancy, because Notch receptor signaling is also often overactivated [42]. In such cases, inhibition of either EGFR or Notch signaling alone is not enough to suppress the proliferation of basal-like breast cancer cells. However, coinhibition of both pathways reveals a potential SL interaction with positive results for the suppression of tumor growth [43]. These observations have triggered the current development of specific γ-secretase inhibitors with therapeutic properties [43]. It is expected that, with a combined treatment targeting both the EGFR and Notch pathways, patients suffering from the triple-negative basal-like breast cancer would have a more positive outcome [43]. Although these are excellent examples, the target identification strategy is not exhaustive and is based purely on past experience and educated guesses.

Chemical genetic modeling of NGIs

Small molecules that selectively kill cancer cells, but not normal cells, have also been applied to identify cancer targets by chemical genetic modeling (Figure 1C). A classical example of this is the SL interaction of a rapamycin derivative, CCI-779, with the loss of the tumor suppressor *PTEN* [44,45]. In this case, the goal was to inhibit downstream components of the PI3-K/AKT signaling pathway, negatively controlled by the PTEN phosphatase, by the use of CCI-779. Because loss of *PTEN* leads to constitutive activation of Akt, providing growth advantage, inhibiting the Akt downstream target, the mammalian target of rapamycin (mTOR) kinase, with CCI-779 blocks the uncontrolled growth driven by Akt [44–46]. In a similar manner, the reliance of cancer cells that harbor DNA damage lesions on *ATM* or *ATM*- and *RAD3*-related (ATR) pathways for their survival, have led researchers to anticipate their SL relation. Accordingly, an ATR inhibitor, VE-821, has recently been shown to be synthetically lethal in the ATM-p53 pathway in cells treated with a DNA-damaging drug, cisplatin [47]. The aurora kinase inhibitor, VX-680, has also been found to exhibit SDL with the proto-oncogene *c-Myc* in retinal epithelial cells, where c-MYC is overexpressed, and this lethality is attributed to the temporal coupling of DNA synthesis with a functional role for the chromosome passenger protein complex in mitosis [48]. Similarly, dissecting the molecular mechanism of a SL relation between B cell receptor (BCR) signaling and the pro-survival NF-κB signaling pathway using their specific inhibitors ibrutinib and lenalidomide, respectively, suggested a therapeutic potential for their coinhibition in the activated B cell-like subtype of diffuse large B cell lymphoma [49]. Therefore, rather than screening a large chemical library, where the off-target effects of the chemicals need to be evaluated, modeling SL interactions by chemical genetics provides a good translatable strategy.

While screening thousands of compounds across a compendium of genetic mutations is extremely challenging and laborious, it is noteworthy to highlight an excellent study by Muellner *et al.* in this context [50]. The group systematically analyzed gene–drug interactions by pooling over 80 different genetically-altered, barcoded isogenic population of cells with mutations in breast cancer-relevant genes (*BRCA*, *c-MYC*, *NOTCH*, *PTEN*) all derived from MCF10A

breast cancer cells. They treated them with 87 small molecules enriched for kinase inhibitors and clinically relevant compounds at different concentrations [50]. This work led to the identification of several SL interactions, in addition to discovering a new mechanism of resistance to PI3K inhibitors with direct clinical implications [50]. With recent developments in mammalian genetics, reverse chemical genetic approaches are also now feasible and are discussed below (Box 1).

Recent genome-wide approaches that aim to identify NGIs directly in human cancer cells

Genomic tools of genetic manipulation

The advent of RNAi [mediated by siRNA and short hairpin RNA (shRNA), and the recent class of RNA guided endonucleases single guide RNA (sgRNA)] has provided the specificity needed to perform systematic functional genomics screens in human cancer cells [11,51–58]. While the siRNA and shRNA reagents function at the mRNA level, sgRNAs function at the genome level to manipulate gene functionality (Figure 2). These reagents typically query every single gene in the human genome to identify NGIs. The most popular RNAi reagents are from the Netherland Cancer Institute (NKI) library [59], the RNAi consortium (TRC) library [51], the Hannon–Elledge library [60] and the Ultra-complex shRNA library [56]. They differ in their size, design, and coverage, but perform the same function of gene silencing. However, a potentially serious pitfall is that RNAi may induce off-target effects and these could confound SL–SDL identities. Moreover, ineffective RNAi agents can lead to false negatives. The latest sgRNA library uses the clustered, regularly interspaced short palindromic repeats (CRISPR) that function as an adaptive immune system in bacteria [54,55,61–63].

The CRISPR-Cas9 system is an antiviral, defense mechanism adopted by bacterial cells where the guide sequences correspond to an attacking phage virus. However, replacing these guide sequences with any target sequence of interest and co-expressing the bacterial Cas9 nuclease results in DSB of the targeted DNA. In the absence of a donor sequence that enables DSB repair by homologous recombination, the indel-forming Nonhomologous End Joining DNA repair pathway comes into play. This leads to frame shifts and/or premature stop codons, effectively disrupting the open reading frame of a targeted region in the genome.

Due to the nature of short guide sequences, researchers have taken advantage of the large-scale oligonucleotide synthesis and generated pooled libraries currently available only in this format. The most popular sgRNA libraries include those that were developed by the Sabatini–Lander and Zhang labs. The Sabatini library consists of 73 151 sgRNA plasmids that cover a total of 7114 human genes, and the Zhang lab (GeCKO) library consists of 64 751 unique guide sequences targeting 18 080 human genes [54,55]. More recently, the Yusa lab constructed 87 897 unique sgRNA plasmids, targeting 19 150 murine protein-coding regions [64]. Importantly, these reagents provide the unique opportunity to directly query the cancer genome. If one truly appreciates that studies in model systems could lead to potential drug targets, the direct study of human cancer cells has the potential to define targets of even higher precision.

Systematic genome-wide screening of NGIs in the pooled format

The original idea of a pooled screen emerged from the strategy to define genetic or chemical genetic interactions in the yeast deletion collection, where a pool of yeast mutants were evaluated for their growth defects based on the barcodes incorporated into them [65]. Because the transduction of cells with pooled lentiviral particles, which express unique sequences of shRNA or sgRNA that get integrated into the genome, can be quantitated like a barcode, researchers extended this negative selection screen to identify those gene knockdowns (shRNA) or knockouts (sgRNA) that caused lethality [11,54–56,66]. An ideal way to define specific SL–SDL interactions is to use isogenic pairs of cell lines where genes essential to the survival of a derived line that harbors a cancer-specific mutation, but not essential to the well being of a parental line, will be considered SL–SDL [11]. Considering the genetic drift associated with human cancer cells, isogenic cell lines offer the unique opportunity to capture specific interactions associated with a given molecular alteration. Identifying SL–SDL interactions, after the selective growth of transduced cells each incorporated with shRNA or sgRNA, can be quantified by

Box 1. Computational prediction of cancer targets

In many cases, it is simply too costly or too time consuming to carry out SL–SDL screens for all genes of interest across multiple cell types. Indeed, most experimental screens are unable to encompass the large spectrum of cancer types and to define potential targets. To resolve these shortcomings, computational genomics has risen to the forefront as a useful technique for predicting NGIs. Computational genomics employs the use of statistical analysis to determine biological interactions using genomic sequences and other functionally relevant data [93,94]. There are a number of traditional studies that used gene expression signatures to either classify different cancer subtypes or predict biomarkers and potential cancer targets. Recently, using differential gene expression, the proto-oncogene *ECT2* was found to exhibit a SL relation with the retinoblastoma (RB)/E2F pathway [95]. By following this approach, a systematic examination of gene expression profiles from the NCI-60 panel and the RNA-seq data from The Cancer Genome Atlas (TCGA) project recently reported a number of genes that are SL with loss-of-function mutations of *TP53* [96]. In fact, with the availability of gene expression, copy number variation and most importantly large-scale gene-essentiality datasets, one can predict the NGIs for any given oncogene and tumor suppressor (see Figure 1D in main text). For example, the SL interactions of the tumor suppressor *PTEN* should be essential and must be retained with high expression or copy-number in cells when *PTEN* is lost. Accordingly, the Copy Number Variation (CNV) and gene expression datasets from TCGA (http://cancergenome.nih.gov) or the Cancer Cell Line Encyclopedia database (http://www.broadinstitute.org/ccle/home) may reveal a correlation between the loss of *PTEN* and any specific gene retention.

The DePinho laboratory that identified the metabolic enzyme ENO2 as a potential target, adopted a slight modification of this approach [97]. Expression and CNV analyses revealed that *ENO2* is retained with high expression by glioblastoma cells that have lost its paralog *ENO1*. Further validation studies have revealed that ENO2 inhibition induces SL in *ENO1*-deleted glioblastoma cells [97]. Similar efforts to target functionally redundant paralogs of mutated cancer genes are becoming increasingly popular [98]. While computational predictions are perhaps unbiased and may lead to many unexpected interactions, they require rigorous validation in experimental systems.

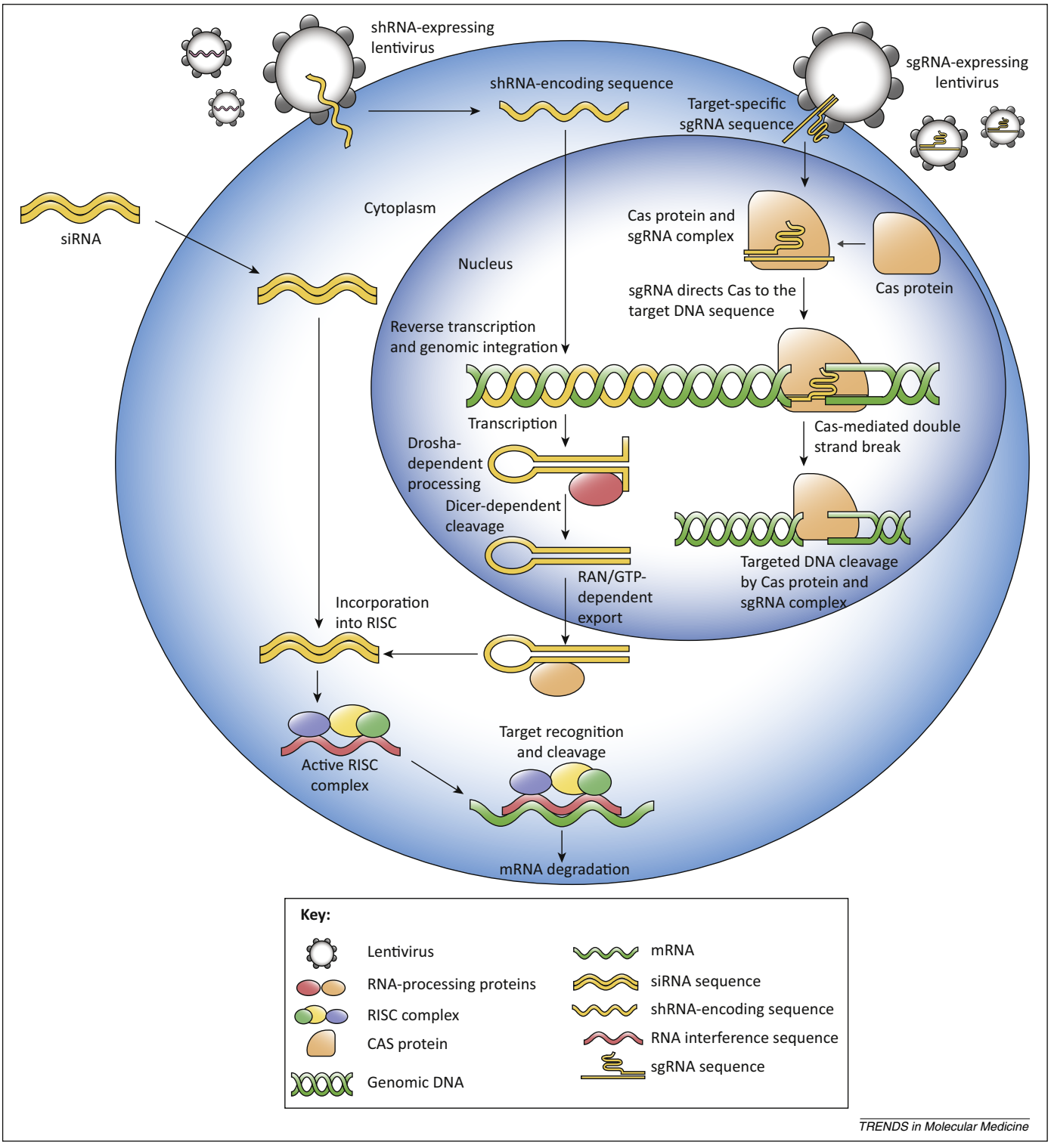

Figure 2. Schematic representation of siRNA, short hairpin RNA (shRNA), and single guide RNA (sgRNA) processing. (siRNA) After transfection in the cell, the two siRNA strands are unpaired by a RNA-induced silencing complex (RISC), with one strand being used to target a complementary mRNA strand. (shRNA) A shRNA-encoding sequence enters the cell via a viral vector and is integrated into the genome through reverse transcription. After being expressed in the nucleus, the shRNA is processed by Drosha and Dicer and exported out of the nucleus. The shRNA is then processed in the same way as a siRNA, leading to knockdown of the mRNA targeted by its sequence. (sgRNA) A sgRNA sequence is inserted into the cell via a viral vector and directed to the genome where it forms a complex with a cellular apoptosis susceptibility (Cas) protein. As Cas is a bacterial protein, it must either be inserted into the cell along with the sgRNA sequence or else it must have been previously cloned into the cell for stable or inducible expression. Once the complex is formed, the sgRNA directs Cas to the target gene where Cas mediates a double strand break and knockdown of the gene at the genomic level. Abbreviations: RAN, Ras-related nuclear protein.

amplifying the barcode sequences from a single mixture of genomic DNA using universal primers. Deep sequencing or barcode microarrays can be used to measure the abundance of each sequence in the isogenic cell population (Figure 3A). The loss of specific sequences within the mutated cell population, as compared to the parental control, identifies which shRNA or sgRNA targets are synthetically lethal.

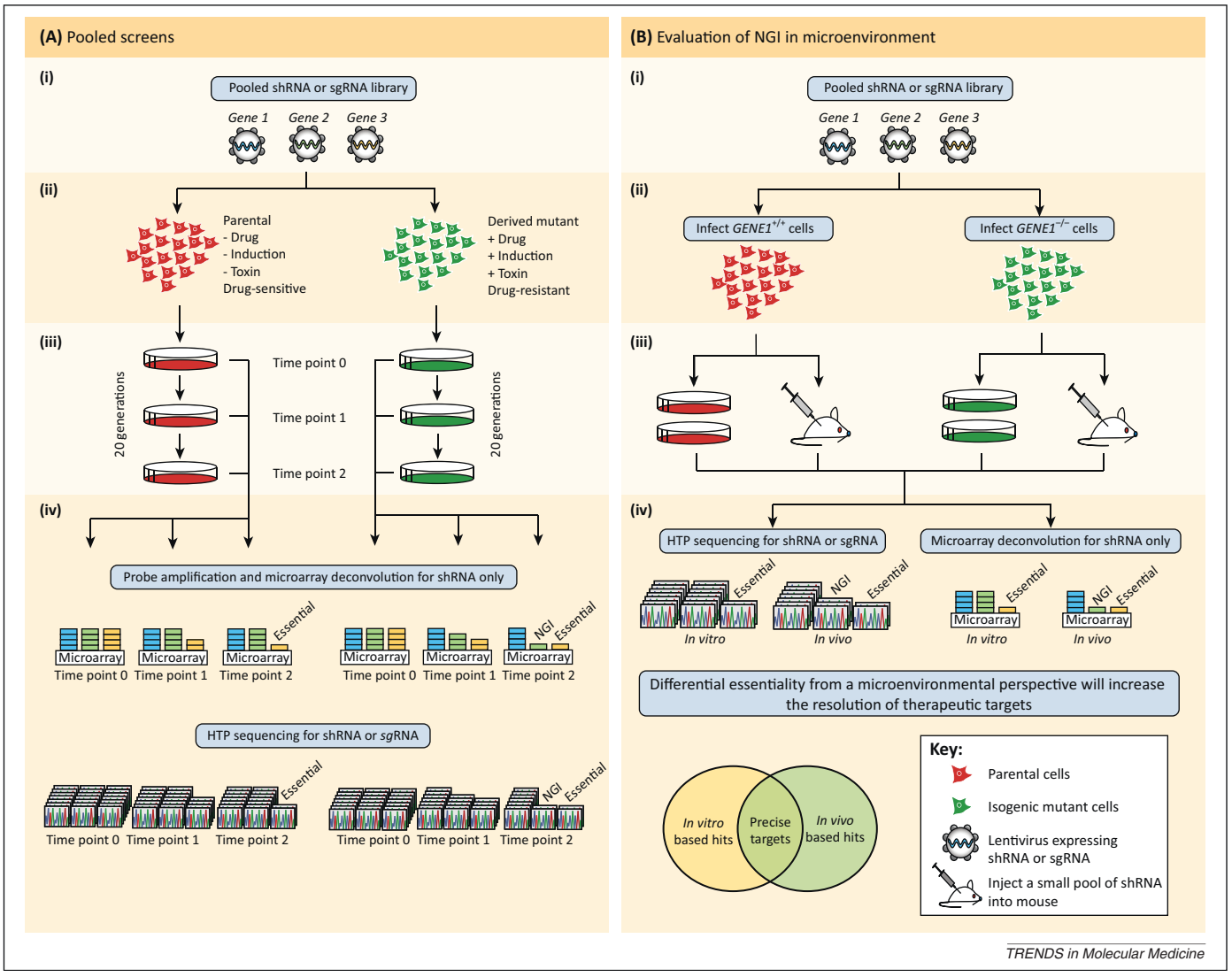

Figure 3. Schematic representation of pooled screens. **(A)** Pooled screens allow for direct comparisons between a parental cell line lacking a cancerous mutation or treatment, and one, which is considered to be cancerous. (i) A lentiviral-based library containing short-hairpin RNAs (shRNAs) or single guide RNAs (sgRNAs) targeting all genes in the genome is used to infect an entire cell population, such that each cell harbors individual knockdown or knockout. (ii) Large scale transduction of the viral pool can be done in isogenic populations. (iii) An outgrowth assay is done by passing the cells for a minimum of 20 generations to allow for the interfering RNAs to have an effect on the population. (iv) Microarray deconvolution or high throughput (HTP) sequencing strategies can then be applied to detect which shRNA or sgRNA is synthetically lethal with the mutation or treatment of interest. **(B)** Schematic representation of the pooled screen in a tumor microenvironment. (i), (ii), and (iv) are as described in Figure 3A. (iii) Transduced cell lines are propagated *in vitro* and *in vivo*. However, the amount of shRNAs or sgRNAs must be reduced to a few hundred for feasibility. Note that sgRNA sub-pools will need to be synthesized and cloned for this purpose.

The Elledge laboratory was the first to identify SDL interactions for the oncogenic *KRAS* mutation, where *PLK1* was shown to cause selective lethality to *KRAS* mutant cells [10]. A similar study identified *STK33* as a potential target for mutant *KRAS* [67]. However, subsequent studies to validate either the loss of *STK33* or small molecule inhibitors designed to inhibit the kinase activity of STK33 did not show selective killing of the cancer cells [68–71]. While it is important to identify robustly validated targets, working with cancer cells poses the challenge of dealing with genetic drift and the progressively changing genetic landscape of oncogenic addictions. This plasticity in cellular wiring might be one of the reasons for the discrepancy within these studies. Nevertheless, the pooled screening approach has recently been scaled up to identify ~200 NGIs and build the first interaction network in human cancer cells [11]. Importantly, this study also showed that NGI profiles could determine the genetic dependency of cancer cells [11]. For example, the SL interaction profile of *PTEN*$^{-/-}$ cells derived from isogenic screens was found to be significantly enriched in cell lines known to be *PTEN*/*PI3K* mutant compared to cell lines harboring wild type *PTEN*/*PI3K* genes.

Nowadays more focused pooled screens targeting specific subsets of the genome such as the kinome or epigenome are also becoming popular [72,73]. Although the pooled screening strategy offers key advantages in that it is scalable and a genome-wide screen can be performed in a span of 3–4 weeks (depending on the doubling time of the cells) in an unbiased fashion, a major shortcoming of this approach is the considerable rate of false positives associated with the screening format. However, pooled screening using sgRNA is a promising strategy due to the higher specificity offered by these reagents in generating stable

mutants [54,55]. Moreover, unlike the pooled shRNA approach that targets only transcribed genes, pooled sgRNA screens increase screening coverage due to their genome-editing capabilities. Although it is likely that pooled sgRNA screens will be sought after, pooled shRNA screens still offer a unique opportunity to query functions of relevant genes as they cause hypomorphic expression, and not a complete loss of function, mimicking the action of most clinically used drugs. Another potential strategy to at least partially eliminate false positive hits will be to filter out the hits through gene expression analysis [11]. Optimizing the screening procedures by sampling at different time points to identify the shRNA dropouts will greatly reduce the noise as opposed to an end point assay. In addition, scoring of multiple hairpins for the same gene is extremely important as relying on a single hairpin has shown to be particularly noisy in de-convolving the data due to a dicer-independent, cell type-dependent alternate targeting sequence generator [74,75].

An extended application of the pooled screening format in cancer target identification is its application in chemical genetics. Predicting which combination of drugs will work successfully in therapy remains a major challenge. Such trials are expensive, time consuming, and often not rationally designed. How then, do we predict which current or new drugs will act to more effectively kill cancer cells? To circumvent these limitations, pooled screens offer the unprecedented advantage to systematically search for targets that would act synergistically with a clinically approved drug [76]. For example, a clinically approved EGFR inhibitor, cetuximab, efficiently treats patients with cancer who harbor activating mutations in *EGFR*. However, patients carrying activating mutations in both *EGFR* and *KRAS* will not respond to cetuximab treatment alone due to the EGFR-independent activation of KRAS effector pathways. Identification of the cell surface receptor CD83 to exhibit NGI with both KRAS and cetuximab treatment offers a new therapeutic opportunity [11]. Such targets can be examined with a much higher degree of confidence for a positive response.

Similarly, genes that contribute to drug resistance can be easily identified using the unbiased pooled screening platform by assessing their essentiality specifically in drug-treated resistant populations, because the gain-of-function is easily selected (Figure 3A). However, screening in the arrayed format (described below) is the preferred method of identifying drug synergism, because it is a one-on-one analysis. Although the *in vitro* pooled screen can reveal many insights related to potential therapeutic cancer targets, the option to identify targets *in vivo* is a valuable asset (Figure 3B) [77,78]. Recently, to identify regulatory switches in controlling T-cell function in immunosuppressive tumors, an *in vivo* pooled shRNA screen identified a PP2A subunit as a key regulator of immune

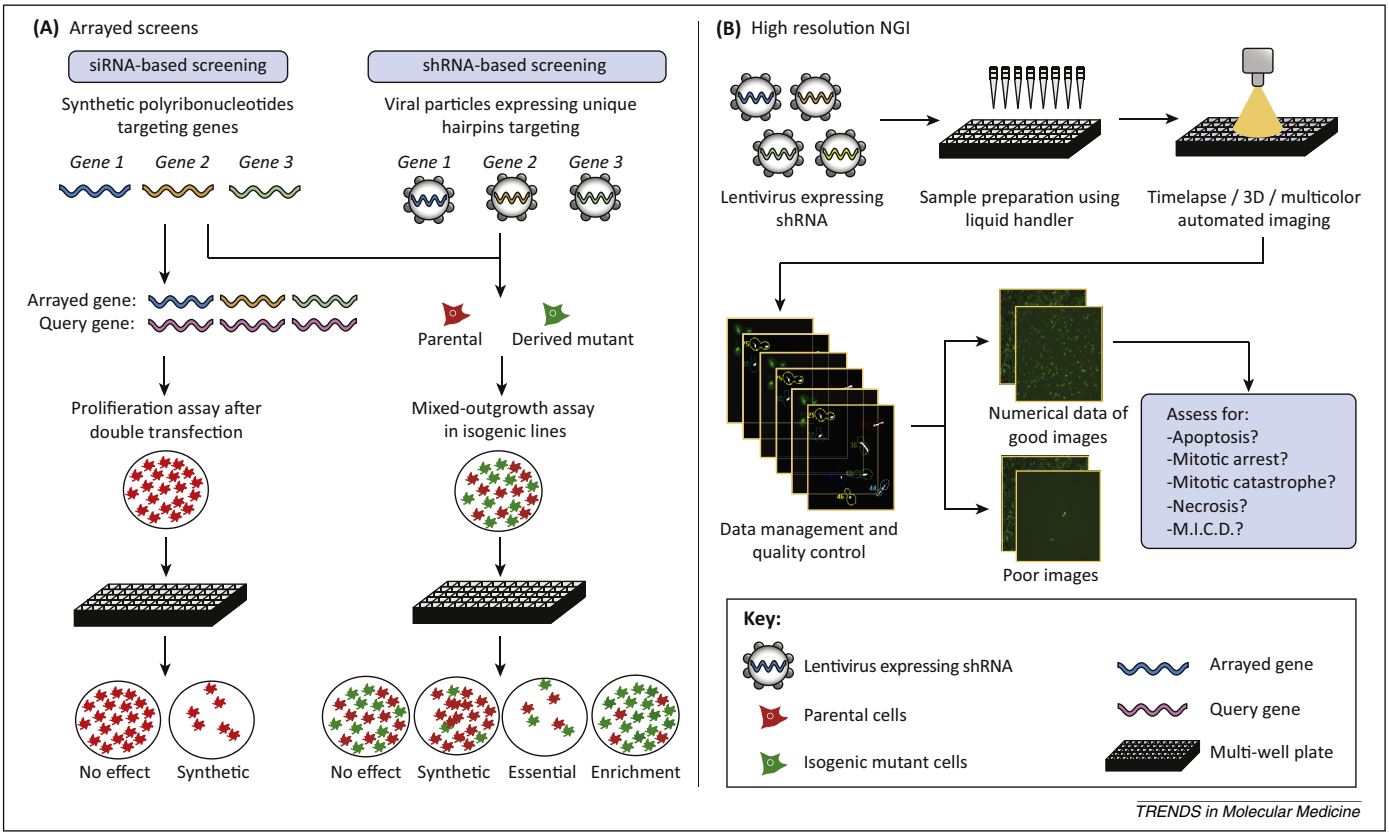

Figure 4. Schematic representation of arrayed screen and high-resolution synthetic lethality (SL) screens. **(A)** Arrayed screens are designed to identify interactions on a gene-by-gene basis. The scheme on the left describes the pipeline using siRNAs to assess synthetic lethal effects on an individual population of cells. The scheme on the right shows how siRNAs and short hairpin RNAs (shRNAs) can be used to assess a mixed population of cells, including parental and isogenically derived cell lines. Briefly, both the pipelines rely on imaging and counting the number of objects. **(B)** Schematic representation depicting the pipeline involved in automated imaging and automated image analysis of cellular phenotypes that cause the lethal phenotype. High-resolution screening can be used to assess the cause of lethality, such as apoptosis, mitotic arrest, necrosis, or other factors using specific fluorescent reporters. Such determinations are made based on the ability to observe many cellular features over time, including cells per well, fluorescence intensity of the nuclei, mean nuclear area, or other features. Abbreviation: MICD, mitosis independent cell death.

function in the tumor microenvironment [77]. Unlike the *in vitro* pooled screen format, in the *in vivo* format, a good representation of hairpins with decent reproducibility can be obtained only when the pool size is limited in the range of a few hundred hairpins. Perhaps the *in vivo* pooled screening format could be most effectively used as a validation strategy to evaluate the top hits from the *in vitro* screen. Subsequently selected hits will need to be evaluated for off-target effects using shRNA-resistant rescue constructs [11].

High-resolution mapping of NGIs in the arrayed format

While the pooled screening format is a quick assay, a more sensitive way to query the genome is to assay individual knockdowns on a gene-by-gene basis (Figure 4A). However, extending this approach in a genome-wide fashion in human cells is expensive and requires extensive automation. Perhaps, the simplest way to overcome this was undertaken by the Krogan lab. They used an endonuclease-prepared siRNA (esiRNA) to generate libraries of siRNA sequences targeting ~130 genes involved in chromatin remodeling. This approach of querying functionally related genes is referred to as epistatic mini array profile (E-MAP) [79]. The E-MAP approach, performed in mouse fibroblasts, to assay 11 000 pairs of genes is particularly more efficient than other methods in defining functional connections between gene pairs because it directly examines functionally coherent genes [80]. Similarly, focused studies using druggable targets for the identification of *MYC* synthetic lethal genes were done in human foreskin fibroblasts, primary cells which are notoriously difficult to transfect with plasmids, but feasible with siRNAs. This study opened the possibility of carrying out screens directly in primary tumor cultures [81,82]. However, due to the transient effect of siRNAs, the NGIs cannot be monitored over a number of generations and the killing efficiency of potential targets (as fast killers, slow killers, and continuous killers [66]) is somewhat difficult to assess. To partially overcome this, a co-culture

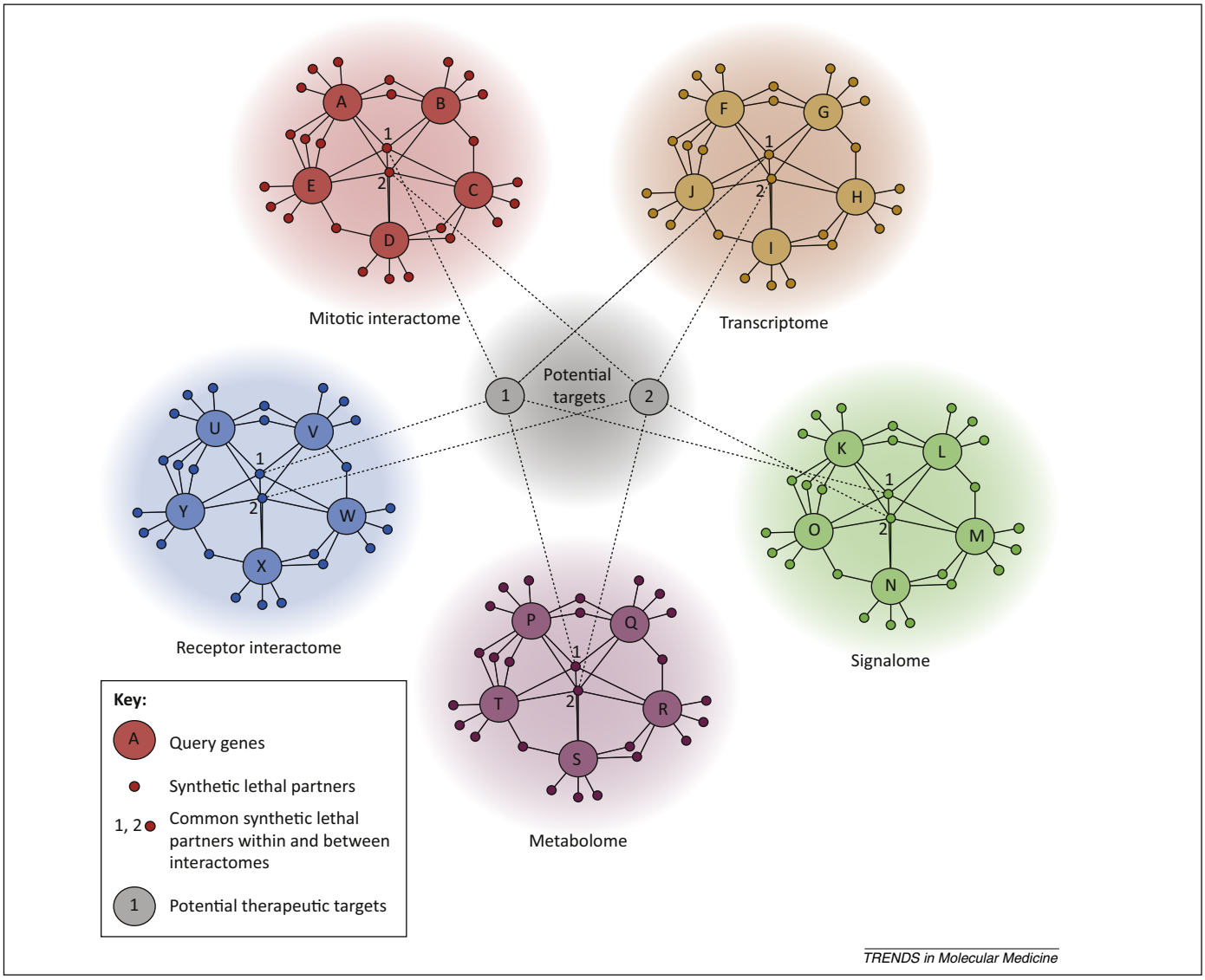

Figure 5. Building the Google map of the cancer cell. As genetic interactions are confirmed between various genes in a given biological process, sub-interaction networks begin to emerge. Such interactomes, or E-MAPs (epistatic mini array profiles), are networks of query genes (nodes represented with alphabets) that relate to particular cellular systems, such as metabolism, signaling, transcription, translation, or others. Within each E-MAP, potential gene targets (synthetic lethal partners – depicted as tiny nodes) are determined to be synthetic lethal partners. As more of these E-MAPs are generated, common synthetic lethal partners from each can be found (nodes represented in numericals), thereby pointing to therapeutic targets for cancer treatment on a whole-cell level.

assay was recently developed, where both parental and the isogenic derived cells are transfected together, minimizing the variability in toxicity-related issues and systematically quantifying differential proliferation rates [11]. This system is easily amenable to the lentivirus-based shRNA knockdown to query individual interactions for more generations (Figure 4A). Importantly, the lentivirus-based system provides a unique opportunity to directly query the human genome even in cells that are difficult to transfect. Currently, the sgRNA system is not amenable to this approach, because these libraries were generated by large-scale oligonucleotide synthesis. Future developments of individual sgRNA libraries will enable the perturbation of individual genes and identify potential targets.

While gene-by-gene analysis is a powerful way to identify targets, this approach does not reveal the mechanism of the SL–SDL interactions. To circumvent this, the Boone lab took a genome-wide approach to define phenotype-based, SL interactions in *S. cerevisiae* [83,84]. The ability to define a SL relationship based on cellular phenotype is referred to as 'high-resolution mapping of NGIs' (Figure 4B). This study demonstrated that morphological features linked SL interactions with molecular clues that underlie the lethal phenotype in the simple eukaryote yeast. The Boutros lab applied this approach in *Drosophila schneider* cells [85], and subsequently, in human cancer cells to detect therapeutically relevant interactions [86]. The high content imaging-based approach generated a number of comparative features, including cells per well, mean nuclear area, intensity of the nuclei, and was followed up by analysis of the cells for any morphological or population changes. In addition, specific fluorescent reporters of cell death, for example, monitoring caspase activation, may reveal if these cells undergo apoptosis or other types of cell death, providing clues as to which pathways are activated for lethality.

Concluding remarks and future perspectives

The different strategies described here, which are used to distinguish targetable vulnerabilities in cancer cells, do not rely on any general aspect of tissue specificity. Neither do they rely on any specific cellular mechanisms that maintain genome instability or cancer cell signaling or metabolic pathways. Instead, these targeting strategies depend on genetically determined differences between normal and cancerous tissue. However, these differences embodied by tumor genetics are not due to routine genetic redundancies. The situation in which cancer cells continue to undergo mutations as a part of their malignant evolution forces them to genetically depend on compensatory alternative pathways, resulting in a deviation from the standard redundancies. Therefore, it is essential to develop a blueprint of the cellular circuitry in order to understand the genetic dependencies of various cancer cells. However, will the identification of NGIs for key cancer genes be sufficient? As indicated above, the differential response of BRAF inhibitors to colon and melanoma are highly context dependent, and therefore, mapping the NGIs for key cancer genes alone is not going to be sufficient [87]. However, building an interaction network of functionally related genes or mapping the interactions of cancer genes that result in co-occurring mutations may reveal factors that affect contextual dependency. While we finally have the tools to map the system, the question is where to start?

Yeast biologists adopted an E-MAP approach, in which they focused on functionally related genes rather than taking a whole genome approach [79]. Such an approach results in the construction of high-density maps for a given biological process revealing specific molecular interactions [52,56]. Although focusing on selected gene sets may be criticized for bias, mapping the complete interactome to attack the human genome, similar to what has been performed in yeast over the past 10 years, is a daunting task [88]. In addition, unlike the interactions identified in yeast [88], interactions identified in human cancer cells demand extensive validation across multiple cell types of different tissue and cancer origin. Recent methods to map interactions in a hierarchical fashion that provide directionality to interactions are just beginning [89], and building the 'Google map' of the cancer genome requires an E-MAP style concerted effort from the cancer genomics community (Figure 5). In deed, a large-scale data driven study has recently demonstrated that synthetic lethal networks can predict gene essentiality, clinical prognosis of patient survival and drug response [99].

The future of cancer therapeutics relies upon a systems-level understanding of the functional roles of relevant genes, and the genetic dependencies they attribute to tumor cells. For many years, cancer has been classified based on the part of the body where it has developed.

Box 2. Outstanding questions

- Can synthetic lethality really deliver targeted therapy? Despite several years of knowledge in SL–SDL there is no valuable therapeutic strategy yet in place. This is because the challenge lies at two independent levels.
- First, while sequencing of the tumor may reveal an inventory of mutations, what is the genetic dependency of a given individual tumor? And will blocking this genetic dependency lead to selective killing of the tumor cells alone? This requires personalized medicine. As the genotype-targeted therapy strongly relies on the genetic dependencies of the tumors, a complete mechanism to re-classify patients based on the genetic lesions they harbor ('KRAS Clinic' rather than 'Colon Cancer Clinic') will be required. The current classification of cancer based on the body part affected will not suit the personalized medicine that will evolve from these approaches. Efforts to set up sequencing facilities within the clinics and methods to run synthetic lethal screens on primary tissues will drastically change the way we address these issues.
- Second, which SL–SDL interaction will be applicable to block a given genetic dependency of a tumor? Cell-based assays have not generated any convincing targeted therapies. Whether a single target validated by cell-based assays will be able to stand the test of mouse models, followed by clinical trials, is still a major question. Approaches that use chemical genetic modeling of the identified interactions or combination therapy to attain synergism in both cell-based assays and *in vivo* models are currently the standard procedures. However, due to tumor heterogeneity and the contextual nature of the synthetic lethal interactions, there is a compelling requirement for a biomarker that must indicate which of the SL–SDL interactions will be applicable to a given genetic dependency of a tumor. Significant evaluation of this biomarker in the context of cellular plasticity must be in place before a robust validation.

However, tumor sequencing has shown that there is only a limited set of genes that are typically altered in the majority of tumors [90,91]. This means that a detailed classification of different malignancies based on their genetic causes, as well as generating the complete interactome of these genetic lesions, could yield a much more accurate understanding and identification of therapeutic targets. Synthetic lethality offers to reveal the vulnerable interactions of the cancer-causing genetic lesions. The role of SL in the selective killing of premalignant cells by short-term intermittent therapy (SITEP) is another emerging idea that may help to initiate an entirely new approach to cancer prevention [92]. Overall, these strategies depend on building the 'Google map' of cancer cells, which holds promise to personalized medicine (Box 2).

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