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Searching for synthetic lethality in cancer

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The incentive to develop personalised therapy for cancer treatment is driven by the premise that it will increase therapeutic efficacy and reduce toxicity. Understanding the underlying cellular and molecular basis of the disease has been extremely important in the design of these novel therapies; however, identifying new drug targets for personalised therapies remains problematic. This review describes how the biological concept of synthetic lethality has been successfully implemented to identify new therapeutic approaches and targets in models from yeast through to human cells. We also discuss how recent technical advances combined with an increased understanding of the complexity of cellular networks may facilitate therapeutic advances in the future.

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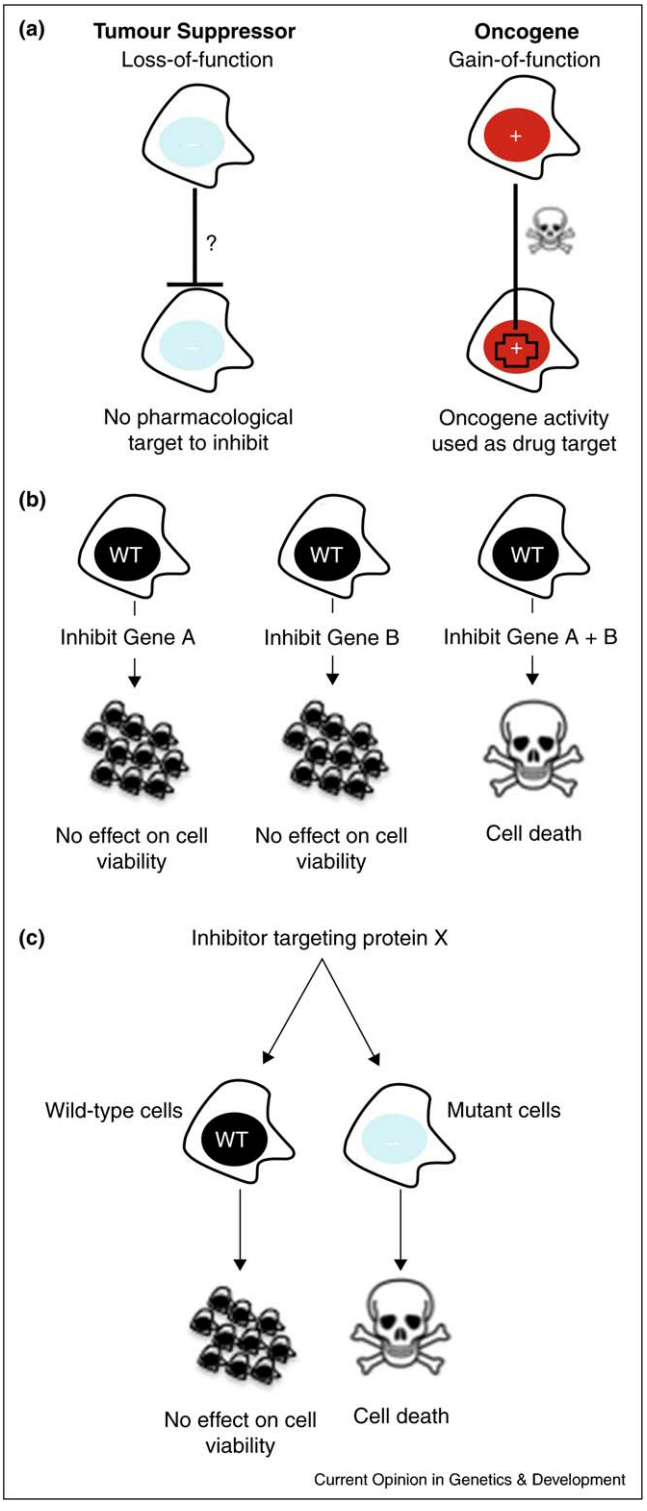
Introduction: targeting cancer with synthetic lethality

Cancer is fundamentally a genetic disease. During tumour development, in a multistep mutational process, cells acquire a set of genetic lesions that lead to the cancerous state. For example, gain of function mutations in oncogenes and loss of function mutations in tumour suppressor genes enable cells to acquire a series of characteristics ('hallmarks') that facilitate the tumorigenic process [1]. While these mutations promote the disease process they also offer therapeutic opportunities. This is perhaps best exemplified by the development of drugs that target gain of function oncogenic mutations such as the *ERBB2* amplification in breast cancer (with traztuzumab, lapatinib, *etc.*), the *BCR-ABL* translocation in chronic myeloid leukaemia (with imatinib, *etc.*) and more recently *BRAF* mutations in metastatic melanoma (with BRAF inhibitors) (reviewed in [2•]). However, despite these successes, developing drugs that selectively kill cancer cells without harming normal cells remains a considerable challenge. This process is particularly problematic when considering how to target a protein such as a dysfunctional tumour suppressor that is either largely inactive or even completely absent (Figure 1a). In this case, the concept of synthetic sickness/lethality (SSL) has been advanced as a potential approach [3,4•,5]. Two genes have a SSL relationship when inhibition or mutation of either gene alone does not cause loss of viability/sickness, but simultaneous inhibition of both genes results in reduced cell viability or an impairment of cellular health/fitness (Figure 1b). If one gene in an SSL relationship is a tumour suppressor gene, then its synthetic lethal partner becomes a candidate therapeutic target that could be used in tumour cells with a defined tumour suppressor gene dysfunction. SSL can occur between genes acting in the same biochemical pathway or in distinct but compensatory pathways [6] and components of the same pathway often share the same SSL partners [7,8]. The concept of SSL was first described by Bridges in 1922 (referenced in [9]), Dobzhansky in 1946 [10] and Sturtevant in 1956 [11] and was based primarily on studies in the fruit fly, *Drosophila melanogaster* (*D. melanogaster*). Since then numerous genetic screens have been carried out in additional model organisms to identify SSLs including studies in *Caenorhabditis elegans* (*C. elegans* [12,13]), *Saccharomyces cerevisiae* (*S. cerevisiae* [14,15,16•]) and *Escherichia coli* (*E. coli* [17]). New technology is now enabling the rapid identification of SSLs in human cells [18] that may allow the targeting of tumour suppressors or oncogenes that have limited pharmacological tractability (Figure 1c).

Lessons learned from synthetic lethality in model organisms

Lower eukaryotic model organisms are often used for SSL studies on the basis that they are relatively amenable to experimental manipulation; they have small genomes, can be easily mutated and mated to generate hybrid stains and are relatively easy to use with gene silencing techniques such as RNA interference (RNAi). SSLs in model organisms have been identified using both low-throughput approaches, where two different mutations are combined to assess SSL, or in larger screens where multiple mutations are combined. For example, Tong and colleagues developed a synthetic genetic array (SGA) assay in *S. cerevisiae*, where mutant yeast strains containing a single disrupted gene of interest were mated with an array of yeast strains harbouring ~4700 different deletions

Figure 1



Targeting cancer with synthetic lethality. **(a)** Left: illustrates the difficulty in developing an inhibitor targeting a cancer with a mutation in a tumour suppressor gene since there is little or no protein with which to inhibit. Right: portrays that the development of small molecule inhibitors targeting oncogenes is generally simpler since there is a product activity in which to target. **(b)** A schematic of the concept of synthetic lethality. Inhibition of either gene A or gene B alone has no effect on viability; however, when gene A and gene B are simultaneously inhibited it leads to cell death. **(c)** Highlights the advantages of using synthetic lethality when attempting to kill a cancer cell harbouring a 'non-drugable oncogene' or 'absent tumour suppressor'.

[16•]. Following haploid selection, SSL interactions were detected by the absence or poor growth of a particular mutant combination.

Ooi *et al.* [19••] refined this methodology by designing a SLAM (synthetic lethality analysed by microarray) approach. SLAM combines the SGA method with a unique bar code identification system, which utilises sequence 'tags' that flank each deletion. Parallel pools of 4700 mutant yeast strains were transformed with either a gene-targeting construct to disrupt the gene-of-interest or a control DNA fragment. Following selection, DNA was isolated from each yeast pool and PCR amplification was performed using primers recognising the 'tags' that flank each deletion in all 4700 strains. The PCR products were then hybridised to oligonucleotide microarrays to quantify the growth of each strain. Comparing the intensity of the control and the targeted pool hybridisations identified SSL combinations [19••]. This technology has more recently been extended to the yeast, *Schizosaccharomyces pombe* (*S. pombe*, [20]) and analogous approaches are being developed in *C. elegans* [13] and *E. coli* [17].

Although yeast SSL studies should provide a useful model for predicting fundamental biological interactions conserved in higher eukaryotes, in practice they are often not conserved in human cells. It appears that the 'wiring' of molecular networks from yeast to human cells may be so different that a mutation in orthologous alleles often predisposes cells from different organisms to different fates. Nevertheless, the development of methods to analyse high-throughput methods such as SGA and SLAM has provided invaluable lessons for the analysis of subsequent mammalian screens.

Synthetic lethality in human cells: from proof of concept to the clinic

Although SSL relationships were proposed more than a decade ago as having potential applications in cancer therapeutics [4•], it is only recently that such approaches have reached the clinic. We, and others, have demonstrated that dysfunction of either of the tumour suppressor genes, *BRCA1* or *BRCA2*, is synthetically lethal with inhibition of the DNA repair enzyme Poly(ADP-Ribose) Polymerase 1 (PARP1) [21•,22•]. BRCA1 or 2 deficient cells are not only inhibited by gene silencing of PARP1 but are also profoundly sensitive to potent small molecule PARP inhibitors, when compared to isogenic wild-type cells. These findings provided the rationale for clinical trials that are now assessing the potential of PARP inhibitors [23] and Phase 1 and 2 trial results suggest that PARP inhibitors can elicit sustained anti-tumour responses in

patients carrying *BRCA1* or *-2* mutations without causing many of the serious adverse side-effects associated with conventional chemotherapy [24•,25,26]. Subsequent 'hypothesis' driven approaches for identifying SSL interactions have involved predicting synthetic lethality based upon already proven associations, as components of the same pathway often share similar SSL partners. For example, we have recently demonstrated SSL between dysfunction of the *Phosphatase and Tensin Homolog* (*PTEN*) gene (OMIM 601728) and PARP inhibition [27]. This SSL relationship was predicted from the previous observation that *PTEN* mutant cells exhibit a similar DNA repair defect as in *BRCA1* or *BRCA2* mutant cells.

Recent examples of synthetic lethality by functional compensation

From the earliest studies of cell biology, it has been clear that cells readily adapt to a wide array of perturbations, such as gene mutation. Underlying these observations is a complex system of functional buffering, such that when one molecular function, gene or protein is lost, another can readily be used to mitigate any effects that could limit either cell survival or fitness. For example, from studies in *S. cerevisiae* and *C. elegans*, it is proposed that only 10–20% of genes are actually essential for viability [28–30], suggesting an extensive level of functional buffering.

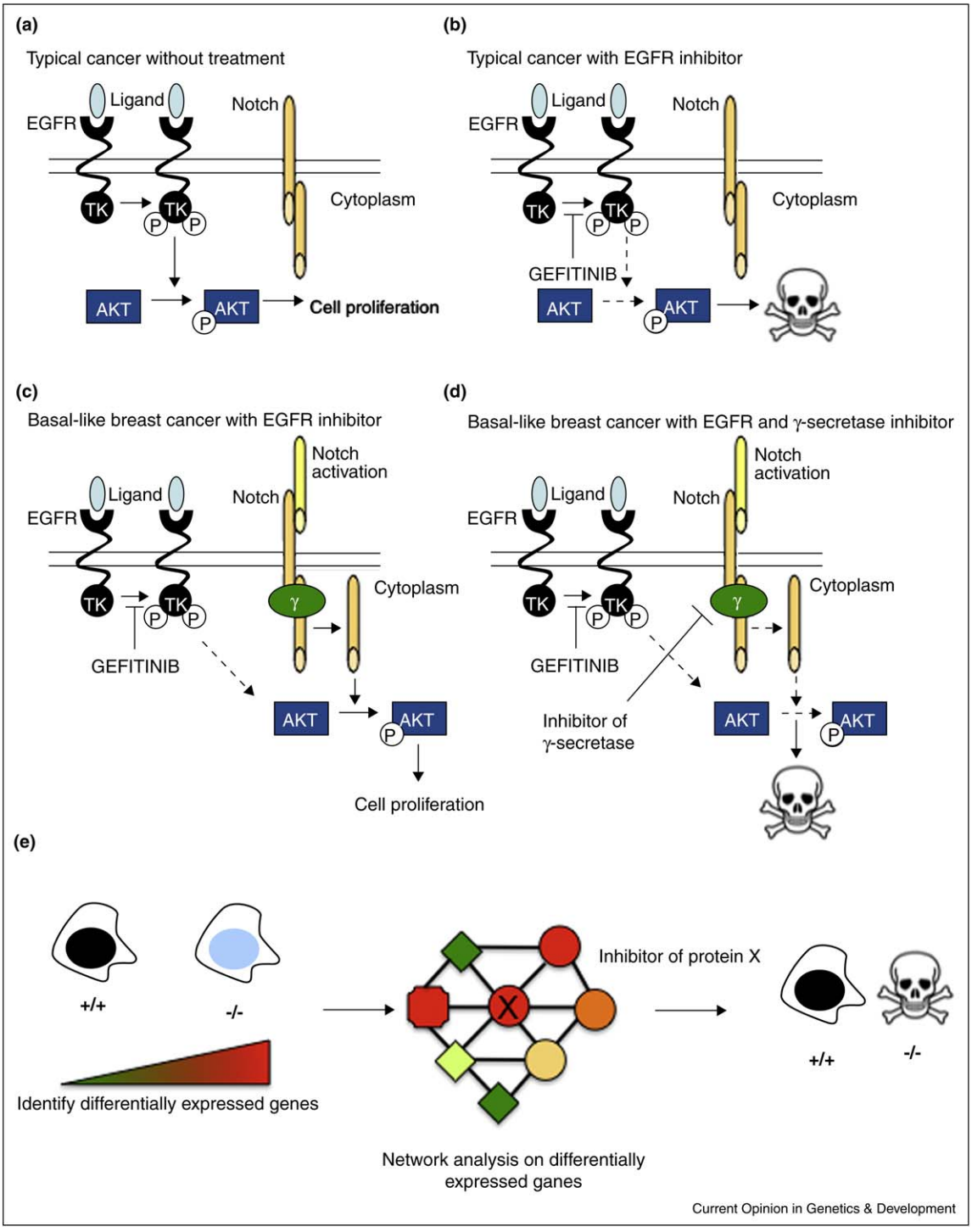
This concept of functional buffering provides us with a way to think about, and hopefully identify, synthetic lethal relationships [31••]. For example, it seems likely that some synthetic lethal partners could be elements of functional buffering networks. This seems to be the case with the BRCA/PARP SSL. DNA is constantly bombarded with damage, one of the most frequent forms being the formation of single strand DNA breaks (SSBs). These SSBs are normally repaired via a PARP-dependent mechanism and in the presence of PARP inhibitors SSBs accumulate and have the potential to limit cell viability if not repaired by another buffering process. In normal cells, DNA repair via BRCA1 and BRCA2 provides this functional buffering. However, in BRCA1 or -2 deficient cells, functional compensation is lost and SSBs ultimately compromise the viability of cells. In keeping with this functional buffering hypothesis, there is some evidence to suggest that in the absence of BRCA1 or 2 function, PARP1 is hyperactivated [32], possibly as a means to limit the impact of SSBs where a functional compensation mechanism is no longer present. Similarly, we have demonstrated a synthetic lethal interaction between DNA mismatch repair (MMR) proteins and particular proofreading DNA polymerases [33]. In this case, it seems likely that MMR proteins and their synthetic lethal DNA polymerase partners repair similar DNA lesions (oxidative DNA damage) but by differing mechanisms. This functional buffering is also represented by the observation that both MMR-deficient cell models and tumours express elevated expression of proofreading DNA polymerases [33]. Extending these concepts of functional buffering, mechanistic redundancy should allow us to predict additional SSL effects.

A variation of a compensatory SSL interaction was recently reported between the Epidermal Growth Factor Receptor (EGFR) and Notch signalling pathways in basal-like breast cancer (BLBC) (Figure 2a). EGFR promotes cell proliferation in a number of tumour types and small molecule inhibitors or antibodies targeting EGFR signalling have shown efficacy in lung, colon and head and neck cancers. The efficacy of anti-EGFR therapies may be due in part to the significant decrease in AKT activation that these agents elicit (Figure 2b). However, anti-EGFR inhibitors have demonstrated little clinical activity in breast cancer and it is believed that the hyperactivated Notch pathway seen in BLBC may be compensating for, or buffering, EGFR inhibition by maintaining levels of activated AKT (Figure 2c). Simultaneous inhibition of the EGFR and Notch signalling pathway, however, results in SSL, most likely by limiting the Notch-mediated escape mechanism and enabling AKT suppression (Figure 2d) [34••]. We envisage that the development of more integrative screening approaches will allow the prediction and validation of many more 'buffering' SSL interactions in the future (Figure 2e).

Techniques for the identification of new synthetic lethal interactions

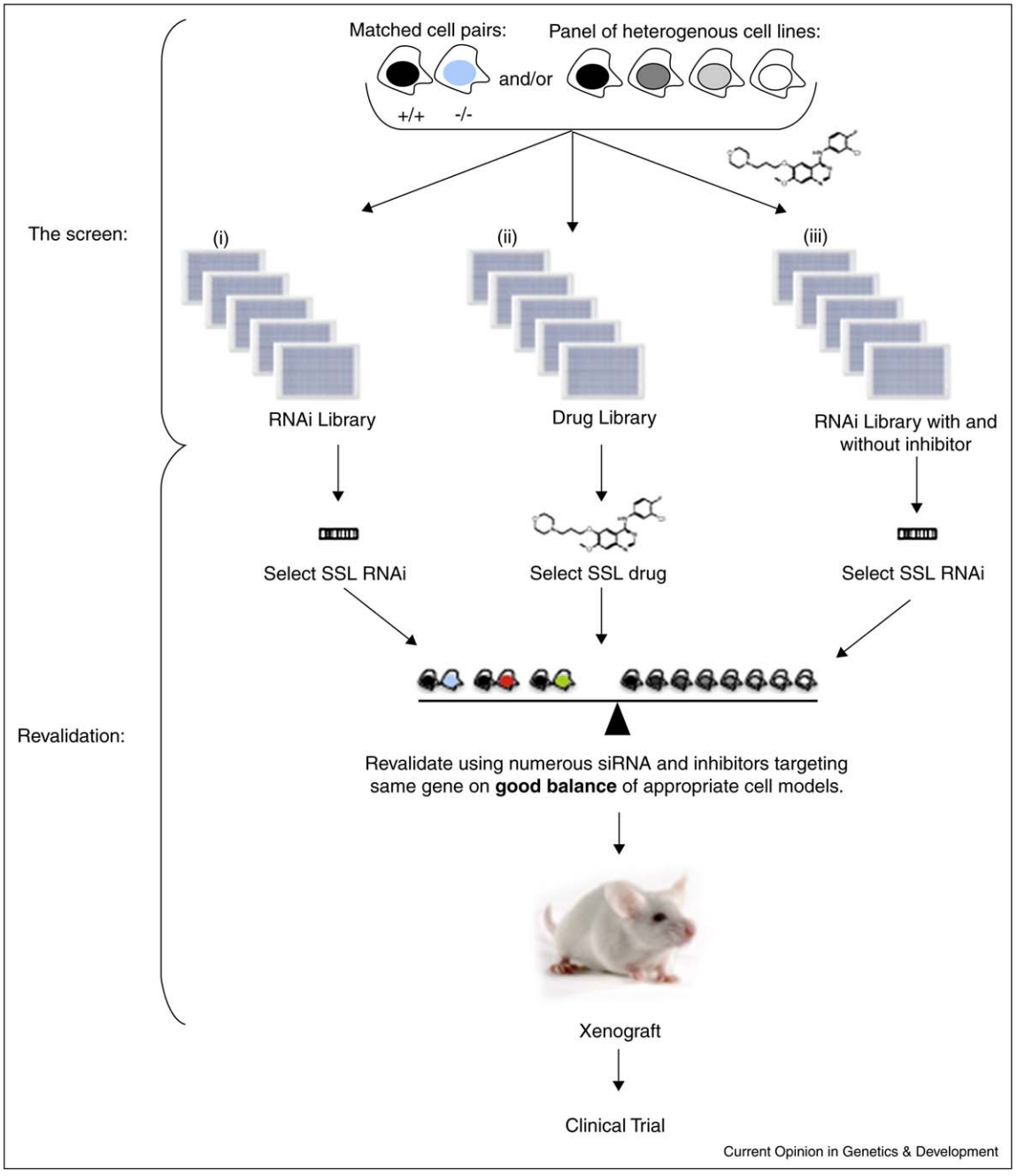
With the advent of new high throughput genetic screening technologies and the increased availability of genetically modified human cell lines, we have somewhat moved away from the 'hypothesis' driven methods towards an unbiased screening approach to human SSL identification. There are three common methods for identifying novel synthetic lethal interactions in human cells (Figure 3). These include: (i) RNAi screens using isogenic or functionally relevant cell models [18,35,36], (ii) high throughput screens with chemical libraries to identify compounds that kill cells in a genotype-specific manner [37] and (iii) synthetic lethal approaches using RNAi libraries in combination with chemical inhibitor [38–40]. Each of these approaches can present considerable technical challenges [18]. For example, the interpretation of both RNAi and chemical inhibitor screens is somewhat confounded by the considerable off-target effects observed using either approach [41]. This, in part, can be mitigated by validating both RNAi and chemical screen effects with multiple different RNAi reagents and/or inhibitors or even performing RNAi and chemical screens in parallel and integrating the resultant data [42]. However, the overlap between chemical inhibitor sensitivity and siRNA sensitivity requires careful interpretation [43] as many inhibitors

Figure 2



Synthetic lethality by functional compensation. **(a)** The EGFR pathway is often active in cancer. One of the downstream consequences of EGFR stimulation is the activation of AKT, which drives cellular proliferation. **(b)** EGFR inhibitor (gefitinib) sensitive cancers are unable to activate AKT and as a result die when treated with the inhibitor. **(c)** In basal-like breast cancer the notch pathway is hyperactivated. Notch activation compensates (or buffers) for EGFR inhibition by maintaining the levels of activated AKT. **(d)** Dual inhibition of EGFR and Notch, by inhibiting the essential Notch component γ-secretase, results in synthetic lethality since the levels of activated AKT are depleted. **(e)** A potential route for predicting further synthetic lethal interactions by functional compensation is illustrated. Firstly identifying genes that are up-regulated in mutant cell lines, when compared to the wild-type counterpart, with the premise that this difference in expression may be compensating for the effect of the mutant allele. Targeting the up-regulated protein product, or another in the same pathway, may be a potential route for therapy.

Figure 3

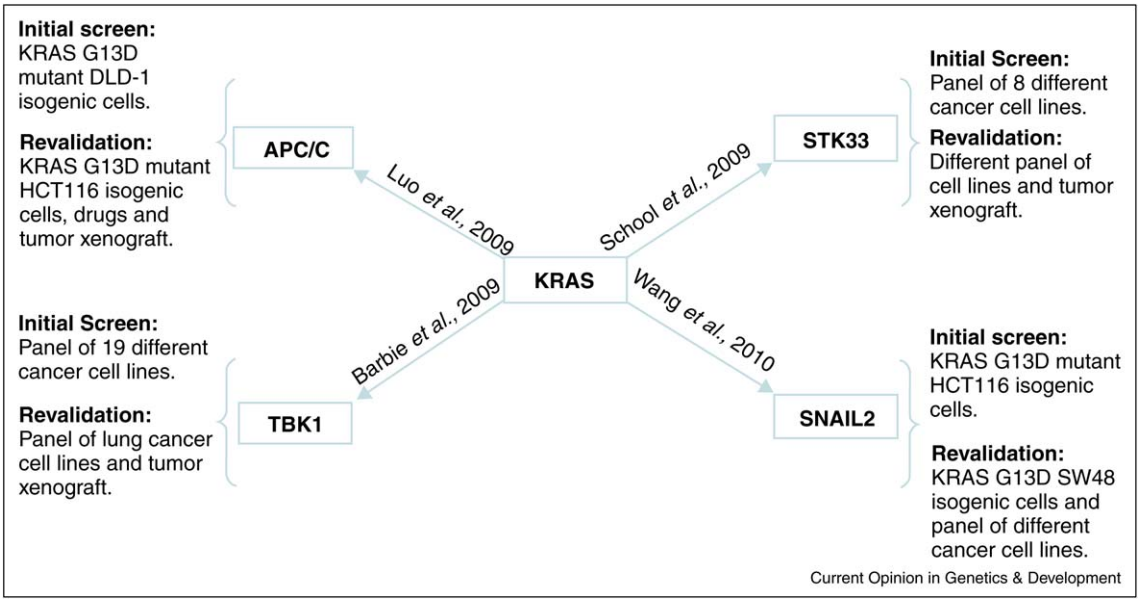


Techniques for the identification of further synthetic lethal interactions and revalidation models. Three common approaches for identifying synthetic lethal interactions in human cells are illustrated. These include (i) high-throughput RNAi screens on isogenic or functionally relevant cell models, (ii) high-throughput screens with chemical libraries to identify compounds that kill cells in a genotype-specific manner and (iii) synthetic lethal approaches using RNAi libraries in combination with single agent or dose of inhibitor. Following the screen and the identification of potential synthetic lethal interactions revalidation with numerous siRNA and inhibitors targeting the same gene on appropriate cell models. Preceding successful revalidation and xenograft studies could lead to a clinical trial.

target catalytic activities whereas RNAi approaches suppress target protein expression and thus inhibit both catalytic and non-catalytic, such as scaffolding, functions.

We recently combined genetic and chemical approaches in a high-throughput screen of a library of clinically used drugs to identify synthetic lethality effects with loss of the tumour suppressor gene *MSH2* [37]. Using isogenic cell

Figure 4



A summary of published KRAS RNAi screens is shown.

lines, with wild type MSH2 expression or MSH2 deficiency, we identified a number of compounds that were selectively lethal to the MSH2 deficient cells, including methotrexate. To establish the validity of the MSH2/methotrexate SSL, we used a panel of diverse RNAi reagents that silenced the methotrexate target, *DHFR* and also profiled multiple MSH2 models for methotrexate and *DHFR* siRNA sensitivity. Dissection of the mechanism indicated that an accumulation of oxidative DNA lesions might explain the MSH2/methotrexate SSL. Because our screen was of clinically used drugs, we were able to rapidly validate the screen effects in *in vivo* models, and then establish a phase 2, non-randomised clinical trial to assess the performance of methotrexate in *MSH2*-deficient metastatic colorectal cancer [37].

Recent SSL interaction studies highlight cancer cell heterogeneity

In addition to targeting tumour suppressor gene dysfunction, the concept of synthetic lethality has recently been extended to target oncogenes that in themselves have limited pharmacological tractability. For example, the *KRAS* oncogene is commonly mutated in colorectal, pancreatic and lung cancers but encodes a GTPase which has not yet proven to be 'druggable'. One approach to targeting KRAS may be to identify genes/proteins whose inhibition is synthetically lethal with *KRAS* mutation.

To date, four different *KRAS* SSL screens have been described. Luo and colleagues (2009) used a genome-wide RNAi screen in isogenic *KRAS* mutant and wild-type colorectal cell lines to identify synthetic lethality with inhibition of APC/C (anaphase-promoting complex/cyclosome), an effect that was reproduced in non-small cell lung cancer cell lines [44]. In an alternative approach, Scholl and colleagues [45] used an RNAi screen in a diverse panel of non-isogenic cancer cell lines to identify SSL between *KRAS* mutation and silencing of STK33. Similarly, Barbie and colleagues [46] used 19 tumour cells lines to identify *TBK1* as a synthetic lethal partner of oncogenic *KRAS*. Finally, Wang and colleagues screened a panel of isogenic colorectal cell lines to demonstrate SNAIL2 (zinc finger transcriptional repressor) SSL with *KRAS* mutation [47]. What is striking from a comparison of these papers (Figure 4) is the lack of *KRAS* synthetic lethal interactions that are common between studies and, more specifically, between genetically diverse tumour cell lines. Variations in experimental procedures in the differing studies (especially the use of differing RNAi libraries) will account for some of the differences in SSLs identified but it might also reflect the nature of *KRAS* synthetic lethality specifically and SSL relationships generally. It may be that some SSLs, such as those associated with *KRAS*, are much more affected by common modifier or genetic background loci than others.

Conclusions

Therapies derived from SSL interactions, such as that between the BRCA1/2 and PARP, are now showing success in the clinic. The application of genome-wide high-throughput screens (from yeast to humans) is likely

to lead to the discovery of many new SSLs. Whether these will prove to be clinically applicable will likely depend on the magnitude of the effect as well as the prevalence of genetic modifiers mediating resistance.

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