Synthetic Lethality in Anticancer Drug Discovery and Target Identification

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Abstract: Background: The concept of synthetic lethality (which arises when simultaneous mutations in two or more genes lead to cell death but a mutation in only one of the genes does not) has been exploited to develop new genotype-selective anticancer agents, identify novel therapeutic targets and characterize genes associated with treatment responses. This review discusses recent advances in anticancer drug discovery and target identification with synthetic lethal approaches. Results: We first discuss about the concept and mechanisms of synthetic lethality to facilitate the understanding of using this concept as a research platform in various areas of anticancer studies. We then focused on recent advance in the discovery of novel anticancer agents, identification of genetic lethal partners of oncoproteins and characterization of genes associated with treatment responses based on the principle of synthetic lethality. Conclusion: Information obtained about synthetic lethal interactions among genes and/or between genes and therapeutic agents provides insights into the molecular mechanisms of some anticancer agents and biological processes and has potential implications for targeted therapy, personalized therapy and the rational design of combinatorial treatment for cancers.

Key words: Lethal genes, neoplasms, drug discovery, genetic interaction, ras genes, mutation

INTRODUCTION

Functional deregulation of several key signaling pathways as a result of genetic and epigenetic alterations is believed to be the driving force behind carcinogenesis and progression of cancers (Ding et al., 2008; Wood et al., 2007). This functional deregulation provides an opportunity for targeted cancer therapies. Small molecules and antibodies that directly inhibit critical nodes in oncogenic signaling networks such as trastuzumab against human epidermal growth factor receptor 2 (HER2) (Leyland-Jones, 2002), erlotinib, gefitinib and cetuximab against epidermal growth factor receptor (EGFR) (Lynch et al., 2004; Adams and Weiner, 2005), imatinib against the BCR-ABL fusion protein (Druker et al., 1996) and sorafenib against Raf kinase and vascular endothelial growth factor receptor (Wilhelm et al., 2004) have already been used to treat various cancers in humans. Nevertheless, many critical nodes in oncogenic signaling networks may not be targeted by small molecules. For example, functional loss in tumor suppressor genes caused by gene deletions may not be restored through small molecules. Moreover, the functions of some oncogene products, such as Ras and c-Myc, have been found to be difficult to modulate directly through small molecules (Hartwell et al., 1997).

The concept of synthetic lethality may provide a new platform for anticancer drug development and an opportunity to eliminate malignant cells by indirectly targeting cancer-driving molecules that otherwise cannot be targeted by small molecules (Hartwell et al., 1997; Kaelin, 2005; Chan and Giaccia, 2011). This synthetic lethality strategy should yield agents with high selectivity against cancer cells with the altered genotype and is expected to minimize treatment-induced toxicity to normal cells, thereby improving the safety of therapeutic agents.

THE CONCEPT OF SYNTHETIC LETHALITY

The term "synthetic lethality" was originally used to refer to a lethal phenotype caused by mutations in two genes; two genes are synthetic lethal when cells or living organisms with mutations in only one of the two genes are viable but the combination of both mutations is lethal (Dobzhansky, 1946). By evaluating the effects of homozygous mutations on viability in Drosophila, Dobzhansky noticed that some homozygous mutations resulted in organisms with normal viability when they...
signal pathways lead to the dysfunction of E and thus to cell death. In this case, A1 is synthetic lethal with B1, B2 or B3 but not with A2 or A3 and vice versa.

Studies in yeast revealed that synthetic lethal interactions occurred significantly more frequently between genes with the same mutant phenotype, between genes encoding proteins with the same subcellular localization and between genes involved in similar biological processes or bridging bioprocesses (Tong et al., 2001; Tong et al., 2004; Ye et al., 2005; Costanzo et al., 2010). On average, each gene might have more than 30 synthetic lethal interactions (Tong et al., 2004; Pan et al., 2006; Lin et al., 2008). Moreover, synthetic lethality may also occur as a result of gain-of-function mutations and is then called synthetic dosage lethality (Kroll et al., 1996; Measday et al., 2005). Indeed, RAS oncogene can induce either cell transformation or apoptosis, depending on cell type and context (Tanaka et al., 1994; Serrano et al., 1997). Expression of oncogenic RAS in primary human or rodent cells often results in apoptosis or senescence, whereas expression of oncogenic RAS in immortal cells or cells with inactivation of p53, p16 or the transcriptional activator interferon regulatory factor 1 leads to transformation and tumorigenesis (Tanaka et al., 1994; Serrano et al., 1997). Oncogene-induced apoptosis and/or senescence were also observed for MYC (Hoffman and Liebermann, 2008, Herrmann et al., 2005), STAT5 and E2F1 (Mallette et al., 2007), suggesting that normal cells have barriers to safeguard against malignant transformation. When cancer cells become dependent on elevated activity of an oncogene for survival, oncogene addiction occurs (Ehrenreiter et al., 2009; Wise et al., 2008). Then, inhibiting downstream molecules in a single linear pathway of the oncogene to which the cells are addicted may also induce synthetic lethality.

ANTICANCER DRUG DISCOVERY AND DEVELOPMENT

The selective killing of BRAF mutant cancer cells by Mitogen-activated Protein (MAP) kinase kinase (MEK) inhibitors (Solit et al., 2006; Corcoran et al., 2010) could be explained as a synthetic lethality caused by inhibition of the downstream component in a single linear essential pathway because of oncogene addiction. Various constitutively active mutations of the BRAF gene have been identified in human cancers (Davies et al., 2002). The exquisite dependence of BRAF mutant or other RAF isoform active tumors on MEK activity may provide a personalized therapeutic strategy for patients with this type of cancer (Solit et al., 2006; Villanueva et al., 2010).
On the other hand, knowledge of parallel molecular pathways can also facilitate the development of anticancer drugs based on synthetic lethality. An example is selective killing of BRCA1 and BRCA2 mutant cancer cells by poly(ADP-ribose) polymerase 1 (PARP1) inhibitors. BRCA1 and BRCA2 are tumor suppressor genes important for DNA Double-Strand Break (DSB) repair by homologous recombination, possibly by interacting with and recruiting RAD51 to the DNA DSBs (Chen et al., 1998; Patel et al., 1998). Loss-of-function mutations in these genes predispose carriers to breast, ovarian and other types of cancers (Antonou et al., 2003; Thompson et al., 2002). In contrast, PARP1 is required for the assembly or stability of nuclear foci of the Single-Strand Break (SSB) repair protein XRCC1 (El-Khamisy et al., 2003) and to facilitate repair of DNA SSBs. PARP1 may not be directly involved in DSB repair and homologous recombination as PARP1−/− embryonic stem cells and embryonic fibroblasts exhibited normal repair of DNA DSBs and RAD51 foci formation (Yang et al., 2004). Nevertheless, PARP1−/− mice have defective DNA SSB repair and increased homologous recombination, sister chromatid exchange and chromosome instability (Yang et al., 2004; De Murea et al., 1997).

Two groups simultaneously reported that defects in BRCA1 and BRCA2 genes in some breast and ovarian cancer cells make them highly sensitive to small-molecule PARP inhibitors (Bryant et al., 2005; Farmer et al., 2005). BRCA4 mutant cells are 1000 times more sensitive to PARP1 than are BRCA4 wild-type cells (Farmer et al., 2005). Moreover, nanoparticle mediated delivery of PARP1 specific siRNA resulted in induction of apoptosis in breast-deficient ovarian tumor cells both in vitro and in vivo and prolonged the survival of mice bearing tumors derived from Brca1-deficient ovarian cancer cells but not from Brca1 wild-type cells (Goldberg et al., 2011). This proof-of-concept result led to phase I clinical trials of an orally active PARP1 inhibitor, olaparib (AZD2281), in cancer patients with or without BRCA1 or BRCA2 mutations (Fong et al., 2009, 2010). The results showed that durable objective antitumor activity was observed only in confirmed carriers of BRCA1 or BRCA2 mutations. The follow-up multicenter phase II clinical trials in patients with BRCA1 or BRCA2 mutations and advanced breast cancer or recurrent ovarian cancer also showed promising results (Tutt et al., 2010).

Nevertheless, genetic interactions remain unknown for most cancer related genes. Thus, synthetic lethality screening is an approach to identify cytotoxic agents targeted to cancer cells with mutations in a particular oncogene or tumor suppressor gene. Using the human colon cancer cell line DLD-1 which contains the mutant K-Ras allele and its isogenic derivative in which the mutant K-Ras allele has been deleted by homologous recombination, Torrance et al. (2001) performed synthetic lethality screening on 30,000 compounds and identified 2 compounds, triphenyl tetrazolium and a sulfynil cytidine derivative which demonstrated approximately sixfold selectivity for cell lines containing mutant KRAS (Torrance et al., 2001). Similarly, using immortalized human fibroblasts with or without the mutant HRAS gene, Stockwell's group performed synthetic lethality screening on 23, 550 compounds and found that camptothecin and a new compound named erastin were more effective in killing HRAS-expressing cells than their isogenic counterparts (Dolma et al., 2003). Subsequently, erastin was found to exhibit lethal selectivity in human tumor cells harboring mutations in the HRAS, KRAS or BRAF oncoproteins by acting on mitochondrial voltage-dependent anion channels and inducing oxidative cell death (Yagoda et al., 2007). STF-62247, a small molecule that is synthetic lethal for the von Hippel-Lindau tumor suppressor gene (VHL), was identified by Turcotte et al. (2008) after screening 64, 000 compounds on renal cancer cells with mutant or wild-type VHL. STF-62247 selectively suppresses VHL mutant tumor cell growth through autophagy induction, possibly by acting on Golgi trafficking pathways (Turcotte et al., 2008).

We used immortalized human ovarian epithelial cells (designated T29) and their tumorigenic derivatives transformed with either mutant HRAS (T29H1) or mutant KRAS (T29K1) (Liu et al., 2004) to screen a chemical library consisting of 10,000 compounds. We identified a compound, designated oncrasini-1 that was not toxic to T29 or T29H1 cells at any of the doses tested but induced apoptosis in T29K1 cells at a wide range of doses (Guo et al., 2008). Oncrasin-1 was also effective against several human lung cancer cells that harbor KRAS mutations (Guo et al., 2008). Molecular characterization revealed that oncrasini-1 suppressed phosphorylation of the C-terminal domain (CTD) of the largest subunit of RNA polymerase II (RNAP II) and induced co-aggregation of protein kinase C iota (PKC ε) and splicing factors into megaspliosomes in sensitive cells (Guo et al., 2009). Interestingly, a Ras-dependent pathway that regulates CTD phosphorylation or function was reported in cardiac myocytes (Abdelali et al., 1998) and in yeast (Chang et al., 2004). Mutations compromising the function of the CTD were synthetic lethal in yeast with elevated levels of Ras activity (Howard et al., 2002). Evidence also indicates that oncogene-transformed cells, such as c-Myc-transformed cells, were more sensitive than their normal counterparts to RNAP II inhibitors,
suggesting that RNAP II may serve as a therapeutic target for anticancer therapy (Koumenis and Giaccia, 1997).

Compound optimization through synthesis and analysis of analogues led to the identification of several compounds that are more effective than oncaspin-1 in inducing apoptosis in a subset of cancer cells. One of these, NSC-741909, was found to suppress the growth of a subset of NCI-60 cancer cell lines, including those with mutations in KRAS and PIK3CA. Mechanistic studies by reverse-phase protein microarray revealed that NSC-741909 treatment led to sustained activation of c-Jun N-terminal Kinase (JNK) by suppressing their dephosphorylation, possibly by inducing oxidative stress and inactivating MAP kinase phosphatases (Wei et al., 2009; Wei et al., 2010). It is also interesting that cancer cells with increased Akt (Nogueira et al., 2008) or Ras (Trachootham et al., 2006) oncoprotein activity can be selectively killed through oxidative apoptosis.

TARGET IDENTIFICATION

Using the human colon cancer KRAS mutant cell line DLD-1 and its isogenic derivative with the mutant KRAS gene disrupted, Elledge’s group screened a library of about 75,000 retroviral shRNAs targeting 32,293 unique human transcripts and identified 368 KRAS synthetic lethal candidate genes with a stringent cutoff and 1613 genes with relaxed statistical criteria (Luo et al., 2009). Genes involved in the regulation of several biological processes or pathways, including nucleic acid metabolism, ribosome biogenesis, protein neddylation or sumoylation, RNA splicing, the cell cycle, mitosis and proteasome complexes, were found to be required as additional support to maintain the Ras oncopgenic state (Luo et al., 2009). In particular, KRAS mutant cells are hypersensitive to inhibition of PLK1, a serine/threonine protein kinase that has important functions throughout the M phase of the cell cycle, including the regulation of centrosome maturation, spindle assembly, mitotic exit and cytokinesis (Petronczki et al., 2008). Small-molecule inhibitors that disrupt mitosis, including paclitaxel and the PLK1 inhibitor BI-2536 (Steegmaier et al., 2007), were found to be synthetic lethal in Ras mutant cells (Luo et al., 2009).

Using the murine K-ras-induced lung cancer cell lines LKR10 and LKR13 (Johnson et al., 2001), Vicent et al. (2010) performed an in vitro proliferation screen and an in vivo tumorigenesis screen on a shRNA library containing genes associated with the KRAS gene expression signature (Sweet-Cordero et al., 2004), genes previously implicated as K-Ras effectors and potential transcriptional regulators and they identified 23 genes required for K-ras-mediated tumorigenesis (Vicent et al., 2010). The transcription factor Wilms’ tumor 1 (WT1), one of the genes identified, was found to be a critical regulator of senescence and proliferation in cells expressing oncogenic K-ras. Silencing of WT1 in cells expressing activated K-ras triggered senescence in vitro and suppressed tumor growth in vivo (Vicent et al., 2010). In K-ras mutant mouse tumor cells, senescence was also induced by ablation of CDK4 but not by ablation of CDK2 or CDK6 (Puyol et al., 2010).

Scholl et al. (2009) performed synthetic lethality screening with shRNA constructs targeting 1011 human genes, including most known and putative protein kinase genes and a selection of protein phosphatase genes and known cancer-related genes, on 8 human cancer cell lines with mutant or wild-type KRAS and on normal human fibroblasts and immortalized human mammary epithelial cells. The results showed that STK33 which encodes a putative member of the calcium/calmodulin-dependent protein kinase subfamily of serine/threonine protein kinases, is required for the survival of several KRAS mutant and KRAS-dependent (i.e., with impaired viability after KRAS knockdown) cancer cell lines. The viability of KRAS wild-type, NRAS mutant or KRAS mutant but KRAS-independent (i.e., KRAS knockdown did not impair viability) cancer cells was not affected by STK33 knockdown (Scholl et al., 2009). Mechanistic characterization showed that the catalytic activity of STK33 is essential for maintaining S6K1 activity in mutant KRAS-dependent cells (Scholl et al., 2009). S6K1 suppresses mitochondrial apoptosis by phosphorylation and inactivation of the BH3-only pro-apoptotic protein BAD (Harada et al., 2001; Zha et al., 1996). Suppression of STK33 decreased BAD phosphorylation and promoted mitochondria-mediated apoptosis (Scholl et al., 2009).

TBK1, a noncanonical IκB kinase that regulates the stability of IκB (Chien et al., 2006), was another synthetic lethal partner for mutant KRAS identified by shRNA screening (Barbie et al., 2009). Using a similar shRNA library to that used by Scholl et al. (2009), Barbie et al. (2009) screened 19 cell lines with or without mutant KRAS allele and identified 45 synthetic lethal partners for mutant KRAS, one of which was TBK1. Suppression of TBK1 induced apoptosis in KRAS-dependent cancer cells but not in KRAS-independent cancer cells (Barbie et al., 2009). Analysis of expression profiles of human lung adenocarcinomas revealed that most KRAS mutant tumors showed RAS signature activation and co-expression of the NF-κB signature. Interestingly, 30 of 109 KRAS wild-type tumors also showed RAS and NF-κB signature co-activation (Barbie et al., 2009). In vitro study revealed
that KRAS wild-type and KRAS-dependent (KRAS si RNA susceptible) cancer cells were also susceptible to TBK1 inhibition, suggesting that a subset of KRAS wild-type tumors depend on TBK1 and NF-κB signaling for survival. Suppression of TBK1 in KRAS mutant cancer cells restored cytoplasmic levels of IκB, reduced the total and nuclear c-Rel levels and downregulated BCL-2L (Barbie et al., 2009).

Indeed, a study of susceptibility to siRNA-mediated KRAS depletion in lung and pancreatic cancer cell lines showed that cancer cell lines harboring KRAS mutations can be broadly classified into KRAS-dependent and KRAS-independent groups (Singh et al., 2009). Gene expression profiling analysis revealed that a 46-gene signature could be used to segregate the two groups. Levels of genes encoding Syk tyrosine kinase (SYK), integrin b6 subunit (ITGB6) and the RON receptor tyrosine kinase (MST1R) and a gene named ANKRD22, with unknown function, were relatively high in KRAS-dependent lung and pancreatic cancer cell lines (Singh et al., 2009). Knockdown of those four genes induced growth inhibition in KRAS-dependent cell lines but not in KRAS-independent cell lines. Moreover, KRAS-dependent cell lines showed substantially greater sensitivity to a small-molecule inhibitor of Syk (R406) (Braselmann et al., 2006) than did KRAS-independent cell lines.

GENES ASSOCIATED WITH TREATMENT RESPONSE

The tumor suppressor gene p53 is inactivated in about 50% of human cancers because of genetic mutations (Vogelstein et al., 2000). A recent study showed that Ataxia Telangiectasia Mutated (ATM) kinase directly modulated p53-mediated apoptosis or cell cycle arrest (Jiang et al., 2009). In cells and tumors that lacked a functional p53 pathway, inactivation of ATM or its downstream molecule CHK2 was sufficient to globally sensitize the cells to genotoxic chemotherapy with cisplatin or doxorubicin (Jiang et al., 2009). In contrast, in p53 wild-type cells, inhibition of ATM or CHK2 resulted in a substantial survival benefit, suggesting that a combination of cisplatin and doxorubicin with inhibitors of ATM and CHK2 could benefit patients with p53 mutant tumors. Several clinical trials of CHK1/CHK2 inhibitors in combination with genotoxic agents for cancer treatment are currently under way (Bolderson et al., 2009).

A study to investigate TRAIL-induced apoptosis in HeLa cells after knockdown of 510 genes encoding known and predicted kinases, proteins with known functions in TRAIL-mediated signaling pathways or proteins with unknown functions led to the identification of several genes whose knockdown either enhanced or inhibited TRAIL-mediated apoptosis (Aza-Blanc et al., 2003). siRNA against PAK1 and AKT1 strongly enhanced TRAIL activity, whereas siRNA against MYC or the WNT transducer TCF4 inhibited TRAIL-induced apoptosis, suggesting that PAK1 and AKT1 overexpression may cause TRAIL resistance and that the MYC and WNT pathways are required for TRAIL-mediated apoptosis (Aza-Blanc et al., 2003). RNAi screening also identified topoisomerase levels as critical factors in determining response to doxorubicin or camptothecin treatment in vitro and in vivo (Burgess et al., 2008).

Whitehurst et al. (2007) performed synthetic lethality screening for gene targets that specifically reduce cell viability in the presence of an otherwise sublethal dose of paclitaxel in the human non-small-cell lung cancer line NCI-H1155. They used a library of more than 84,000 chemically synthesized siRNAs targeting 21,127 unique human genes and applied highly stringent statistical criteria to identify a set of 87 candidate genes whose knockdown sensitizes cells to paclitaxel. Several of those targets sensitized lung cancer cells to paclitaxel more than 1000 fold. The candidates included multiple genes encoding core components of the proteasome and proteins involved in the function of microtubules, posttranslational modification, cell adhesion and cancer/testis antigens (Whitehurst et al., 2007). This observation indicates the possible benefit of combinatorial therapeutic regimens of paclitaxel plus the proteasome inhibitor bortezomib and the possible resistance of tumors with high levels of cancer/testis antigens to paclitaxel treatment. Nevertheless, clinical trials of paclitaxel plus bortezomib showed that such a combination may also increase toxicity (Croghan et al., 2010; Cresta et al., 2008).

Astsaturov et al., 2010 used a siRNA library targeting 638 genes encoding proteins with evidence of functional interaction with the EGFR signaling network, including those transcriptionally responsive to inhibition or stimulation of EGFR, to screen for genes associated with response to EGFR inhibitors; their study identified 61 genes whose knockdown sensitized the A431 cervical adenocarcinoma cell line to the EGFR inhibitors erlotinib or cetuximab (Astsaturov et al., 2010). Most of those genes encode proteins connected in a physically interacting network, including kinases and phosphatases. Nevertheless, when 45 of those genes were tested in 7 other cell lines for sensitization to erlotinib or cetuximab, none of them sensitized all tested cell lines, although several of them sensitized 3-5 of the cell lines (Astsaturov et al., 2010), suggesting that synthetic lethality is highly dependent on cell context.
CHALLENGES AND PERSPECTIVES

The biological functions of compounds identified through cell-based synthetic lethality screening are likely to be unknown. Identifying the cellular targets of these compounds is challenging and time-consuming. To identify targets of those compounds, some chemical biology approaches may be used, including conjugating compounds to biotin or resin beads and performing affinity-based target precipitation and subsequent protein identification by mass spectrometry analysis (Godl et al., 2003; Yagoda et al., 2007; Bantscheff et al., 2007). However, conjugating and immobilizing a small compound may change its biological function and protein binding specificity. Alternatively, target proteins may be enriched by protecting the targets from protease-mediated degradation through binding of unmodified compounds to their targets (Lomerick et al., 2009). The results may depend on the efficiency and specificity of the protection.

Note that synthetic lethal interactions may be highly dependent on cell context. Cell lines harboring the same mutant oncogene or tumor suppressor gene may not have the same synthetic lethal partner, as exemplified by KRAS mutant cancer cell lines which have KRAS-dependent and KRAS-independent subgroups (Singh et al., 2009). Different genetic and epigenetic alterations in other gene loci may account for this variation. Moreover, some KRAS wild-type cancer cells may be susceptible to a synthetic lethal partner of mutant KRAS as about 30% of KRAS wild-type tumors may have a molecular signature similar to that of KRAS-mutant tumors (Barbie et al., 2009). A possible explanation is that, even in the absence of a Ras mutation, increased Ras activity in human cancers frequently results from gene amplification (Hoa et al., 2002; Filmas and Buick, 1985); overexpression (Coleman et al., 1994); an increase in upstream signals from tyrosine kinase growth factor receptors, such as HER2 (Ehrhardt et al., 2004); overexpression of other oncogenes, such as c-Myc (Bild et al., 2006); or a decrease in microRNA, such as let-7 miRNA family members (Johnson et al., 2005). Therefore, once a synthetic lethal agent or gene partner is identified through screening, further characterization in more cell lines is warranted to delineate mechanisms and other factors that may contribute to the observed synthetic lethality.

CONCLUSION

Synthetic lethality has been employed as research tools to identify genetic interaction among genes in yeast. In combination with siRNA technology, synthetic lethality is a useful research platform for identification of lethal partners of oncogenes and tumor suppressor genes in mammalian cells. Such synthetic lethal partners may serve as potential therapeutic targets for future drug development. Moreover, synthetic lethality screen is becoming a useful approach in identifying genes that sensitize cells to chemotherapy or radiotherapy or small molecules that selectively induce cell death in a subset of mutant cancer cells. The development of genotype-selective anticancer agents and advances of our knowledge about networks of genetic interactions is expected to impact on personalized anticancer therapy and facilitate the rational design of combinatorial therapy to enhance therapeutic efficacy.

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