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REVIEW ARTICLE

Synthetic lethal interactions for the development of cancer therapeutics: biological and methodological advancements

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Abstract Synthetic lethal interaction is defined as a combination of two mutations that is lethal when present in the same cell; each individual mutation is non-lethal. Synthetic lethal interactions attract attention in cancer research fields since the discovery of synthetic lethal genes with either oncogenes or tumor suppressor genes (TSGs) provides novel cancer therapeutic targets. Due to the selective lethal effect on cancer cells harboring specific genetic alterations, it is expected that targeting synthetic lethal genes would provide wider therapeutic windows compared with cytotoxic chemotherapeutics. Here, we review the current status of the application of synthetic lethal screening in cancer research fields from biological and methodological viewpoints. Very recent studies seeking to identify synthetic lethal genes with K-RAS and p53, which are known to be the most frequently occurring oncogenes and TSGs, respectively, are introduced. Among the accumulating amount of research on synthetic lethal interactions, the synthetic lethality between BRCA1/2 and PARP1 inhibition has been clinically proven. Thus, both preclinical and clinical data showing a preferential anti-tumor effect on BRCA1/2 deficient tumors by a PARP1 inhibitor are the best examples of the synthetic lethal approach of cancer therapeutics. Finally, methodological progress regarding synthetic lethal screening, including barcode shRNA screening and in vivo synthetic lethal screening, is described. Given the fact that an increasing number of synthetic lethal genes for major cancerous genes have been validated in preclinical studies, this intriguing approach awaits clinical verification of preferential benefits for cancer patients with specific genetic alterations as a clear predictive factor for tumor response.

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Introduction

Identification of therapeutic targets is one of the most imperative steps in cancer drug development. The emerging molecular cancer therapeutics, in addition to the classical cytotoxics, reiterate the importance of target identification approach as the primary upstream process of cancer drug development. Targets of molecular cancer therapeutics can be categorized into three classes. The first example of a cancer target class that has achieved successful outcomes is the one harboring genetic alterations, such as mutations or translocations. Clinical data have recently shown that epidermal growth factor receptor (EGFR) inhibitors provided benefits for non-small cell lung cancer (NSCLC) patients with an EGFR mutation, whereas a poor response was observed for patients with the wild-type EGFR gene (Maemondo et al. 2010; Mok et al. 2009). Despite the relatively recent clinical development of B-RAF inhibitors, targeting the mutated B-RAF gene by PLX-4032 resulted in a significant improvement in the overall response rate in melanoma patients (Smalley 2010). In in vitro enzyme kinase inhibition assays, PLX-4032 and its derivatives preferentially inhibited mutant B-RAF compared with the wild-type B-RAF, which could explain the promising results in the phase I trial of the B-RAF inhibitor (Tsai et al. 2008). One of the most successful

targets for molecular cancer therapeutics is imatinib for CML patients with a BCR-Abl translocation. After impressive data were reported that showed a drastically improved outcome in chronic myelogenous leukemia (CML) patients (Druker et al. 2001), imatinib was first approved for CML in 2001. This established a clinical proof of concept that cancer can be treated by targeting genetic defects that clearly differ from normal cells. Another target with a genetic alteration in clinical samples is the ALK inhibitor for NSLC with the EML4-ALK translocation. The EML4-ALK fusion oncogene has been reported to occur in about 4% of NSCLC (Janku et al. 2010). A phase II trial with an ALK inhibitor, PF-02341066, showed a remarkable response rate (OR 57%) in NSCLC patients harboring the EML4-ALK translocation (Bang et al. 2010). PF-02341066 is now being examined in phase III trials, which are comparing the inhibitor with a standard of care in second line treatment.

Although targeting the driver mutations in cancers is a straightforward approach, mutated genes are often not druggable; the gene products are not amenable to pharmacological intervention by small molecule compounds. Thus, a pathway focused approach is the second class of target identification that many researchers have pursued. K-RAS is the most frequently mutated oncogene known to date; however, it is relatively difficult to pharmacologically inhibit the activity of K-RAS compared with other classes of proteins, such as kinases, GPCRs or ion channels (Saxena et al. 2008). Examples include RAS farnesyl protein transferase inhibitors which showed little activity in tumors that frequently contain Ras mutation (Morgillo and Lee 2006). Therefore, MEK and B-RAF, which are downstream effectors of the RAS signaling pathway, have been targeted in a pathway focused approach, although the targets themselves were not mutated in the cell lines examined. The inhibitors showed a preferential anti-tumor effect on K-RAS pathway deregulated cells in preclinical research (Solit et al. 2006; Garon et al. 2010). mTOR is targeted to suppress the phosphoinositide 3-kinase (PI3K) pathway, and its allosteric inhibitors have been clinically approved (Shor et al. 2009). Although patient stratification was not conducted for the prescription of mTOR inhibitors, preclinical data indicated that tumors with an activated PI3K pathway show a higher response compared with inactivated ones (Neshat et al. 2001; Shi et al. 2002). In addition, PI3K pathways are known to be deregulated at high prevalence in the indications of drugs that include renal cell carcinoma (RCC) (Cho et al. 2007).

Recent years have witnessed a number of research studies focusing on synthetic lethal screening as the third class for cancer target identification (Mizuarai et al. 2008; Ferrari et al. 2010). With synthetic lethal interaction, either single mutation is compatible with viability, but the combinational disruption of these two non-essential genes leads to lethality. Targets with synthetic lethal interactions are expected to possess several advantages. In principle, targeting of synthetic lethal interactions would provide wider therapeutic windows. Since a cancer-related mutation of interest sensitizes tumor cells to the drug that inhibits its synthetic lethal partner, normal cells without the cancer-related mutation are not affected by the drug. In addition, this method can be applied to the mutation of tumor suppressor genes (TSGs) that cannot be addressed by other two approaches (Bommi-Reddy et al. 2008). Since mutations of TSGs account for more than 80% of cancer-related mutations, various synthetic lethal targets have been explored for TSGs.

Application of synthetic lethal genes has a large variety of possibilities, especially for the target identification of molecular therapeutics for cancers with non-druggable mutated genes, including p53, K-RAS and breast cancer gene 1/2 (BRCA1/2). In parallel with the recognition of synthetic lethal genes in the cancer field, great progress in methodology has been made for the establishment of high-throughput screening of synthetic lethal interactions.

Synthetic lethal genes with K-RAS

Several recent studies have independently identified synthetic lethal genes with K-RAS oncogenic mutations (Fig. 1). Barbie et al. (2009) have identified TANK-binding kinase 1 (TBK1) as a synthetic lethal gene with mutant K-RAS by RNA interference (RNAi) screening in a cell panel composed of wild-type and mutant K-RAS cells. The selective anti-cell growth effect was confirmed by another set of cell lines, demonstrating the generality of TBK1 as a synthetic lethal gene with mutant K-RAS. TBK1 is a non-canonical IkB that is known to regulate the NFkB pathway. To provide insight into the mechanism underlying the synthetic lethality of TBK1 and K-RAS, expression profiling by microarray analysis was performed for cancer cell lines and clinical samples. They found that both K-RAS and NFkB pathways were activated in mutant K-RAS cells when examined by pathway signatures, which consisted of two individual gene sets to measure the extent of K-RAS and NFkB deregulation, respectively.

Luo et al. (2009) performed a genome-wide RNAi assay to find genes whose inhibition selectively reduces cell viability in mutant K-RAS cells. The identified genes included cyclin A2 (CCNA2), KIF2C, PLK1, and anaphase-promoting complex/cyclosome (APC/C), suggesting that mutant K-RAS cells are vulnerable to mitotic perturbations. Microarray analysis of clinical lung adenocarcinoma showed an inverse correlation between the mitotic machinery genes with patient survival in a K-RAS status-dependent manner.

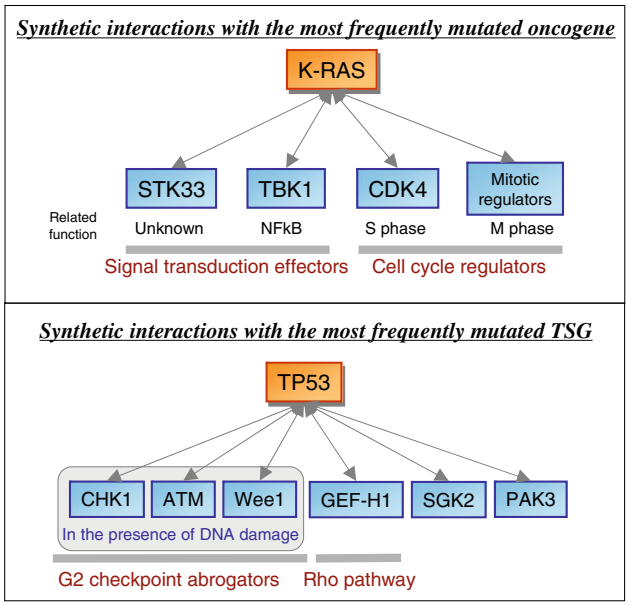

Fig. 1 Synthetic lethal interactions with the most frequently mutated oncogene or TSG. The *upper panel* shows the synthetic lethal interactions with the K-RAS oncogene. SKT33, TBK1, CDK4 and mitotic regulators, such as APC/C, were shown to be synthetically lethal with K-RAS in both in vitro and in vivo studies. STK33 and TBK1 are categorized into signal transduction notes, whereas CDK4 and mitotic regulators are cell cycle effectors. The *lower panel* shows the synthetic lethal interactions with p53-deficiency. CHK1, ATM and Wee1 were shown to be synthetically lethal with p53-deficiency in the presence of DNA damage, whereas GEF-H1, SGK2 and PAK3 showed the synthetic lethal interaction without DNA damage

Another research study, conducted by Scholl et al. (2009), also showed that mutant KRAS has a synthetic interaction with STK33 ablation in a large-scale RNAi screening containing kinase, phosphatase, and cancer-related genes. Interestingly, the selective anti-tumor effect of STK33-silencing on KRAS mutant cells was shown to be irrespective of tissue type through extensive studies with cell lines derived from AML, multiple myeloma, breast, colon pancreatic, and lung cancers. Although the synthetic lethal effect of STK33-silencing was mediated by modulating p70 ribosomal S6 kinase 1 dephosphorylation followed by BCL2-associated agonist of cell death (BAD) inactivation, it remains elusive why STK33 showed synthetic lethality with the K-RAS mutation. Therefore, understanding the molecular mechanism of the synthetic lethal interaction and elucidating the molecular function of STK33 are necessary.

Puyol et al. (2010) found an unexpected synthetic lethal interaction between mutant K-RAS and CDK4 ablation in a mouse NSCLC model. Among the three interphase cyclin-dependent kinases (CDKs: CDK2, CDK4, and CDK6) examined, only genetic disruption of CDK4 prevented the development of mutant K-RAS-induced NSCLC development in vivo. The approach was unique in that synthetic interaction was demonstrated with a transgenic mouse cancer model that recapitulates human NSCLC. This study suggested that CDK4 selective inhibitors should be examined in NSCLC patients with mutant K-RAS.

Since the discovery of an oncogenic mutation in the K-RAS gene in 1982 (Tabin et al. 1982), a large number of basic research articles that indicated the importance of K-RAS in tumorigenesis have been published. Until now, however, no anti-tumor drug targeted for K-RAS mutated cancers has been approved, although wild-type K-RAS is used as a predictor of non-response to EGFR targeted therapy (Van Cutsem et al. 2009). In order to develop novel therapeutics effective for K-RAS mutant cancer cells, it would be valuable to perform head-to-head comparisons of the selective anti-tumor effects on K-RAS mutated cancers among the candidate targets identified in the previous studies (Table 1) in the same experimental platform.

Synthetic lethal genes with p53

P53 is a tumor suppressor gene that is activated in response to various types of DNA damage, including chemotherapeutics, radiation, and oxidative stresses. The activation leads to the up-regulation of p53 regulatory genes, leading to arrest of the cell cycle at the G1 stage, and repair of the damaged DNA, which is known as G1 cell cycle checkpoint (Dasika et al. 1999). When the damage is detrimental, p53 transactivates additional genes involved in apoptosis to prevent the accumulation of the severe DNA damage. Among a variety of TSGs, p53 is the most frequently mutated one in multiple types of cancers. For instance, 33, 43, and 38% of mutations are found in pancreatic, colorectal and lung cancer, respectively. Therefore, the identification of cancer therapeutic targets with synthetic lethality with p53 deregulation attracts great attention since they are expected to exert selective lethal effects only in p53 mutant tumor cells, without causing side effects in normal wild-type somatic cells.

When DNA is damaged, both G1 and G2/M cell cycle checkpoints are activated to prevent the accumulation of DNA damage. Wee1 is a tyrosine kinase involved in the G2/M cell cycle checkpoint (Rowley et al. 1992). Wee1 kinase activated by DNA damage prevents cell cycle progression at the G2/M phase by inactivating phosphorylation of cyclin-dependent kinase1 (CDK1) (Lundgren et al. 1991). In p53 wild-type cells, DNA damage is repaired by both G1 and G2/M cell cycle checkpoints, whereas only the G2/M checkpoint mediated by Wee1 sustains cell cycle integrity in mutant p53 cells. In accordance with the pivotal role of Wee1 in the G2/M checkpoint, the inhibition of Wee1 was found to exert a preferential anti-tumor effect on p53-negative cells in the presence of DNA damage (Wang

Table 1 Summary of the identification and validation of K-RAS synthetic lethal genes

Validation method	TBK1	STK33	Mitotic regulators	CDK4
Isogenic cell lines	Immortalized lung epithelial cells: mutant K-RAS versus control	NA	DLD1: WT K-RAS versus mutant K-RAS	NA
Cancer cell panel	Primary screen: 19 cancer cell lines, secondary screen: 11 NSCLC cells	25 cancer cell lines and two untransformed cell type	14 NSCLC cell lines	10 human NSCLC cell lines
Confirmation in vivo	Xenograft tumors: two WT K-RAS versus two mutant K-RAS	Xenograft tumors: four WT K-RAS versus three mutant K-RAS	Xenograft tumors: DLD1 (WT) versus HCT116 (mutant)	A transgenic NSCLC mouse model: with and without inducible mutant K-RAS
Correlation in clinical samples	Positive correlation between RAS signature and NFkB signatures	NA	Expression of COPS3, CDC16 and EVI5 showed correlation with prognosis	NA
Tissue type	Lung	Irrespective of tissue origin	Lung adenocarcinoma, colorectal cancer	NSCLC

et al. 2004). The synthetic lethality with the p53 gene was also observed for the inhibition of other G2/M checkpoint-related genes, such as CHEK1 and ATM (Wang et al. 2004; Jiang et al. 2009), which leads to the establishment of a field of G2/M checkpoint abrogators as mutant p53-targeted therapies. In addition, MK2-silencing was shown to exhibit synthetic lethality with p53-deficiency in the presence of DNA damage (Reinhardt et al. 2007). MK2-depletion dramatically decreased the phosphorylation level of Cdc25A/B, providing initial evidence that MK2 plays an important role in cell cycle checkpoints. We also confirmed the synthetic lethal effect of Wee1 inhibition with mutant p53 by a selective small molecule inhibitor in the presence of DNA damaging agents (Hirai et al. 2009; Mizuarai et al. 2009). The context specificity of anti-tumor effects of Wee1 inhibition depending on p53 status was examined in detail with an isogenic pair of ovarian cancer cell lines (wild-type and mutant p53 TOV21G), followed by additional cells with either wild-type or mutant p53 genes. The effect was closely correlated with the indices of G2/M cell cycle abrogation that can be measured by phosphorylated-CDK1, phosphorylated-histone H3, and mRNA induction of Wee1 signature genes. With the accumulating evidence showing synthetic lethal interaction of p53 mutation with G2 checkpoint abrogators, development of Wee1 inhibitors holds promise for p53 mutant-targeted therapy in combination with DNA damaging agents.

Multiple studies have found that a missense mutation of the p53 gene provides gain-of-function for the mutated form of p53, in addition to well-recognized loss-of-function of p53 to transactivate mRNAs of genes related to apoptosis or cell cycle arrest (Frazier et al. 1998; Brosh and Rotter 2009). In p53 mutated cancers, numerous genes were reported to be up-regulated, promoting tumorigenesis. As one of the gain-of-function properties of p53 mutation, we and other groups have reported that the Rho pathway is more highly activated in mutant p53 cancer cells compared with wild-type p53 cells (Guo and Zheng 2004; Mizuarai et al. 2006). Expression of different types of mutant p53 was shown to induce mRNA expression of guanine exchange factor H1 (GEF-H1) for RhoA in cancer cells, implying that the Rho pathway that facilitates evasion or proliferation processes of tumor progression is relatively up-regulated by p53 mutation (Mizuarai et al. 2006). These data suggested that the gain-of-function of mutant p53 could be an Achilles' heel for cancer cells harboring its genetic alteration and thereby offers an alternative approach for p53 targeted therapy.

Additionally, an RNAi screen with a small set of 86 kinases also identified that SGK2 and PAK3 are selectively effective for the growth suppression of p53-deficient cells (Baldwin et al. 2010). The synthetic lethality was illustrated in a pair of human foreskin keratinocytes with or

without the HPV E6 protein that is known to inactivate p53. PAK3 interacts with the Rho family proteins, including CDC42 and RAC (Manser et al. 1995), and PAK3 is known to modulate actin filament that is also regulated in the downstream pathway of Rho family proteins (Kumar et al. 2006). Baldwin et al. (2010) also used the primary cells where p53 had been depleted by RNAi to confirm the synthetic lethal interaction between p53-deficiency and either SGK2 or PAK3. It would be interesting to further decipher the molecular mechanism of the synthetic lethal interaction with p53 deficiency.

Synthetic lethal genes with BRCA1/2

Poly (ADP-ribose) polymerase 1 (PARP-1) is a DNA repair enzyme that triggers the DNA damage signaling pathway by binding to DNA strand breaks. PARP1 cleaves NAD+ to produce ADP-ribose, which is followed by the addition of ADP polymers onto various proteins, including histones and other DNA repair enzymes (Peralta-Leal et al. 2008). The ADP ribosylated proteins at the site of DNA breaks enable the unwinding of the DNA helix and expose the matrix for the recruitment of proteins involved in DNA repair. PARP-1 inhibitor has gained attention, since it was the initial anti-tumor agent that was used to establish proof of concept of synthetic lethality in a clinical trial. Several years ago, two pioneering studies established the preclinical proof of concept of the selective anti-tumor effect of a PARP1 inhibitor on cancer cell lines devoid of BRCA1/2 (Bryant et al. 2005; Farmer et al. 2005). In xenograft tumor models, pharmacological intervention of PARP1 by AG14361 or KU0058684 resulted in profound tumor regression in BRCA1/2 deficient cell lines compared with wild-type or heterozygous cancer cells while sparing normal rodent cells. The preclinical studies also demonstrated the molecular mechanism of synthetic lethal interaction of PARP-1 inhibition with the BRCA1/2 mutation. Pharmacological intervention of PARP-1 inhibits the base-exchange-repair (BER) process, leading to the accumulation of single strand breaks (SSBs). This accumulation eventually results in the formation of detrimental double strand breaks (DSBs), which cannot be repaired in BRCA1/2 deficient cells. Given the prominent preclinical evidence of the efficacy of the PARP1 inhibitor for BRCA1/2 mutant cancer cells, clinical studies have been immediately adapted to target cancer patients who harbor these mutations. Preliminary results of a phase I clinical trial of a PARP1 inhibitor, olaparib, was reported with patients with a wide range of solid tumors, including BRCA1/2 deficient patients. Olaparib was well tolerated and showed single-agent anti-tumor efficacy only to BRCA1/2 mutation carriers (Fong et al. 2009). According to data obtained with the pharmacodynamic biomarker, which examined poly (ADP-ribose) (PAR) formation and γH2X in peripheral-blood mononuclear cells and plucked eyebrow-hair follicles, respectively, more than 90% of PARP inhibition was observed at tolerable doses. To further evaluate anti-tumor efficacy selective to subpopulations, phase II trials were conducted in patients of both chemotherapy-refractory breast and ovarian cancer patients harboring BRCA1/2 mutations. Olaparib showed striking clinical efficacy; the objective response rate (ORR) was 41 and 33%, respectively, as determined by RECIST criteria as an initial successful example of synthetic lethal interaction in clinical cancer research (Audeh et al. 2010; Tutt et al. 2010). Multiple ongoing phase I and II studies might further strengthen the clinical evidence of synthetic interactions between PARP1 suppression and BRCA1/2 deregulation. In addition, other defects in repair genes that exhibited synthetic lethal interaction with PARP inhibition in preclinical research could increase the number of responder patients (Löser et al. 2010; Weston et al. 2010).

Methodological advancement in synthetic lethal screening for cancer target identification

Cancer models used for synthetic lethal screens

In order to identify and validate synthetic lethal genes in the field of cancer research, cancer cell lines were used in three ways. First, isogenic cell lines have been most frequently used for synthetic lethal screening (Torrance et al. 2001; Bommi-Reddy et al. 2008). Since the paired cell lines differ only in the status of oncogene or tumor suppressor gene, it is quite useful to examine the effect of this genetic status on the knockdown of potential synthetic partners. Isogenic cell lines for research on most of the cancer-related genetic alterations are now available, including cell lines with alterations in the cell cycle, such as p53 (Bartz et al. 2006), RB1 (Eguchi et al. 2007) and CDKI (Geller et al. 2004) and ones with alterations in signaling transduction, such as PTEN (Lee et al. 2004), K-RAS (Barbie et al. 2009) and β-catenin (Kim et al. 2002). In an interesting study using isogenic cell lines, a pair of two isogenic cell lines with differing K-RAS statuses was marked by a distinct mutant of GFP that enables double-label reading (Torrance et al. 2001). The fluorescent labeling in each of the isogenic cell lines presented advantages: co-culturing of the two cell lines that creates an even growth environment and multiple time-point assessment in phenotypic assays. It is also worth noting that primary cells and their matched cells that were engineered to have a single oncogenic lesion have been successfully used to discover synthetic lethal

interactions (Baldwin et al. 2010). The second approach leverages a cell panel that harbors naturally occurring genetic alterations (Scholl et al. 2009). Since cell lines in the panel possess additional genetic alterations, as well as the mutation of interest, it would be important to use a larger number of cell lines to compare the approach with isogenic cell lines in order to exclude the possibility that observed differences in phenotype between the two groups are derived from genetic backgrounds other than genetic alterations under the examination of synthetic lethal interactions. In addition, the observation of synthetic lethal interactions in a cell panel assay generates more robust data compared with studies using only isogenic cell lines. Thus, candidates of synthetic lethal genes identified in large-scale RNAi screens with isogenic cell lines are often validated in cell panel assays. Conventionally, synthetic lethal genes are initially identified in in vitro studies with cultured cells and then examined in in vivo model animals, such as the rodent xenograft tumor model, for validation. However, some studies have attempted initially to identify the synthetic lethal interactions in vivo, which is the third method of synthetic lethal screening. Michiue et al. (2009) showed that simultaneous gene-silencing of both EGFR and AKT2 by siRNA synergized to induce apoptosis specifically in glioblastoma cells in vivo. In general, it is difficult to achieve sufficient gene-silencing by siRNA in vivo. However, efficient siRNA delivery for the gene-silencing of both genes was achieved by fusing the dsRNA binding domain with a TAT peptide transduction domain (PTD) delivery peptide. The fact that the PTD-mediated delivery into cells is utilized in multiple clinical trials with peptide and protein cargos also supports the idea that this technology could enhance the development of a research field of in vivo synthetic lethal assays by allowing simultaneous targeting of multiple cancer-related genes. Several other methods have also successfully delivered siRNA in vivo, which include lipid formation, polymer mediated delivery, and antibody fusion proteins. An alternative approach to examine in vivo synthetic lethality is to leverage genetically modified mouse models, as exemplified by mutant K-RAS and the CDK disruption mouse models (Puyol et al. 2010). Gene disruption of CDK4 prevented tumor progression in mice with oncogenic K-RAS. Various types of double knockout mice have been generated in other therapeutic fields that lead to the maturation of compound transgenic mice generation technology (Sainsbury 2002). Since synthetic lethal interaction screening examines growth inhibitory effects, conditional knockout or inducible gene expression systems that enable the site- and time-specific regulation of gene expression are essential for the application of double knockout mice technology.

Barcode shRNA pooled library for high-throughput synthetic lethal screens

Most of the synthetic lethal interactions in mammalian cells have been identified through large-scale screening with RNAi ranging from 500 to 20,000 covered genes (Berns et al. 2004; Siva et al. 2005; Schlabach et al. 2008). Whereas siRNA is used to perform high-throughput screening that enables knockdown for individual genes in single-well assays, shRNA screening allows researchers to investigate long-term knockdown effects on cancer-related phenotypes, such as viability, apoptosis, and cell cycle, in a pooled shRNA assay in which a single dish of cells is infected in culture with a large number of shRNA viral vectors. For example, in order to identify synthetic lethal targets, both isogenic cell lines are infected with the pooled shRNAs by the viral vectors. After cells were cultured in a selection medium, genomic DNA integrated with shRNA by retroviral or lentiviral infection is recovered and subjected to PCR amplification followed by DNA microarray analysis to determine the shRNA sequence. Comparing the relative abundance of individual shRNAs from the two isogenic cell lines using DNA microarray analysis enables the identification of novel synthetic lethal genes. In the process of PCR amplification of the integrated viral sequences in order to decode pooled shRNA, two methods have been employed to analyze the pool shRNA sequences. One employs a direct method by utilizing probe sequences complementary to shRNAs. However, since shRNAs are generally 21 nucleotides in length, self-annealing poses a problem during the process of recovery of shRNA from genomic DNA. To solve the annealing issue, the other method utilizes individual unique barcode sequences, which are an integral part of the shRNA cassette (Fig. 2). Since sequences of the 60 nucleotides barcode are different among shRNAs in a library, the barcode sequence enables the measurement of the abundance of each shRNA in a pool screen by subsequent shRNA microarray analysis (Silva et al. 2005). Alternatively, the current advancement of deep sequencing was also used for the analysis of quantification of pooled shRNA (Bassik et al. 2009). As compared with microarray analysis, this technique generates more accurate data for the analysis of barcode sequences.

Future direction

Since an increasing number of synthetic lethal genes have been proposed in basic research studies, it is hoped that synthetic lethal interactions will be demonstrated in clinical trials as well. For the clinical development of molecular cancer therapeutics, the identification of predictive

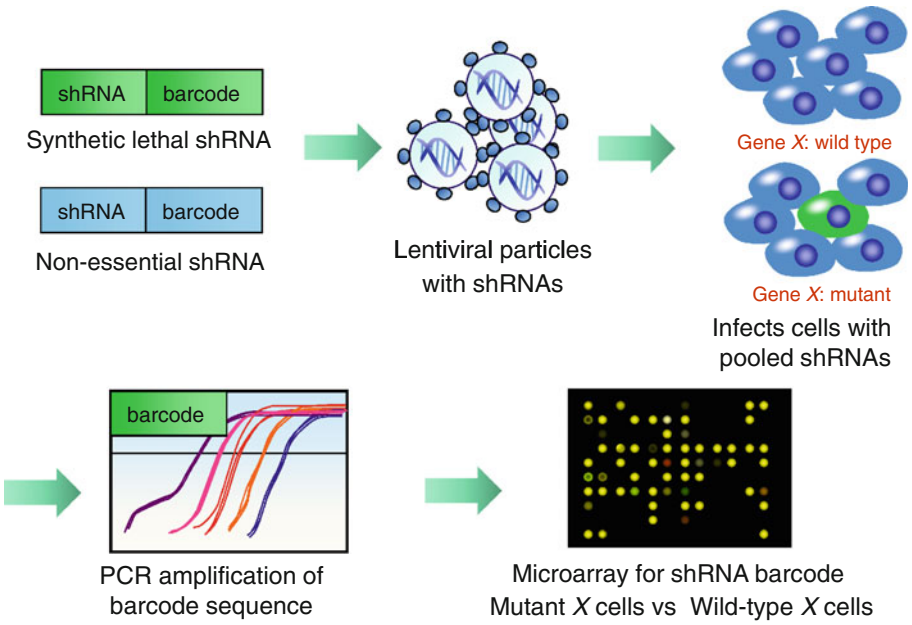

Fig. 2 Scheme of synthetic lethal screen with barcode shRNA. A number of shRNA expression vector constructs are available for lentiviral infection with high efficiency. Each shRNA vector contains a unique barcode sequence to identify the knockdown vector in a subsequent assay. Two types of cell lines with either a wild-type or mutant gene of interest as a synthetic lethal partner are used for synthetic lethal screening. The cells in a single dish for each type of cell line can be infected with a lentiviral shRNA library and incubated for phenotypic condition. After selection, barcode sequences are amplified by PCR. Change in shRNA abundance that corresponds to the abundance of barcode is measured by microarray analysis between selected and reference samples

biomarkers that can be used for the stratification of responder patients is of great significance. Imatinib and trastuzumab, which are recognized as milestones of molecular cancer therapeutics, have scored considerable success with the help of the predictive biomarkers of BCR-Abl translocation and Her2 over-expression, respectively. The promising candidate of predictive biomarkers for cancer therapeutic targets identified in synthetic lethal screens is the partner mutation associated with synthetic lethality. In general, the measurement of genetic alterations generates more accurate data compared with other molecular markers, such as protein over-expression and activation of proteins or pathways, offering an advantage for synthetic lethal targets. Furthermore, combination of two predictive biomarkers might be feasible for synthetic lethal genes, which predicts responder population more accurately compared with the cases with a single predictive biomarker. For example, a candidate predictive biomarker for CDK inhibitors could be a K-RAS mutation based on the aforementioned synthetic lethal study (Puyol et al. 2010). We and other groups also reported that deregulation of RB tumor suppressor pathway, which is composed of p18INK4C, RB1, and CDK4/6, affects responses to CDK inhibitors (Eguchi et al. 2009). The combination of K-RAS mutation and CDK pathway deregulation as predictive biomarkers could select hyper-responder populations or tumors. Since each of the two genes that exhibit synthetic lethality is frequently located in different cancer-related pathways, both dysfunction of the pathway in which the target is directly involved and the genetic mutation of the synthetic lethal partner could contribute to the improvement of the anti-cancer response rate for synthetic lethal target inhibitors. The unique characteristic of availability of the two predictive biomarkers holds great promise for the success of clinical evaluation of synthetic lethal genes.

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