

Phenotypic screening using large-scale genomic libraries to identify drug targets for the treatment of cancer (Review)

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Abstract. During malignant progression to overt cancer cells, normal cells accumulate multiple genetic and non-genetic changes, which result in the acquisition of various oncogenic properties, such as uncontrolled proliferation, drug resistance, invasiveness, anoikis-resistance, the ability to bypass oncogene-induced senescence and cancer stemness. To identify potential novel drug targets contributing to these malignant phenotypes, researchers have performed large-scale genomic screening using various *in vitro* and *in vivo* screening models and identified numerous promising cancer drug target genes. However, there are issues with these identified genes, such as low reproducibility between different datasets. In the present study, the recent advances in the functional screening for identification of cancer drug target genes are summarized, and current issues and future perspectives are discussed.

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1. Introduction

Most human solid tumors develop through multi-step carcinogenesis (1,2). During this process, normal cells, presumed to be tissue-specific stem cells, accumulate multiple

molecular changes advantageous to step-wise growth, finally transforming into overt cancer cells (3). Previous advances in genome-wide profiling technologies have revealed a number of molecular changes in malignant cells at the genetic, epigenetic, transcriptional and translational levels (4-6). Genetic alterations in proto-oncogenes that significantly contribute to malignant phenotypes are called driver oncogenes, such as activated forms of epidermal growth factor receptor (*EGFR*), *HER2/Neu* and *BRAF* (7-10). There are multiple drugs that successfully target these driver oncogenes. For example, *EGFR*-targeted drugs, such as gefitinib and erlotinib, exhibit anti-cancer activity against lung cancer with activating mutations in *EGFR* (11,12).

However, it is difficult to pharmacologically inhibit oncogenic signaling of some driver oncogenes. For example, the development of mutant *KRAS*-targeted drugs has proven problematic over the previous three decades (13). Although recently, treatment with AMG510, a novel inhibitor against *KRAS G12C*, resulted in a promising response rate in patients with lung cancer harboring this specific type of mutation, development of drugs targeting other types of *KRAS* mutations have not yet been successful (14-16). In addition, mutations in driver oncogenes in a number of types of human cancer have not been identified (17). In such cases, cancer results from non-oncogenes conferring various malignant phenotypes, occasionally in a context-dependent manner (18) and these genes may serve as novel therapeutic targets. For example, a study demonstrated that cancer cells depend on non-oncogene Heat shock factor 1 (HSF1), which is the master regulator of the heat shock response in eukaryotes, for their proliferation and survival than their non-transformed counterparts (19). To identify drug target genes for cancer cells harboring oncogenes which are difficult to pharmacologically inhibit, or do not have known oncogenes, it is vital to perform an unbiased, large-scale functional screening (20). Two important gene modulating technologies, RNA interference (RNAi) and clustered regularly interspaced short palindromic repeats-associated protein 9 (CRISPR-Cas9) have emerged as powerful tools for evaluating gene function (21). In addition, technologies in next generation sequencing have improved. The combination of these advanced technologies has allowed investigation of gene function at genome-wide levels in a high-throughput manner.

Thus, functional screening based on cancer-specific characteristics has been extensively conducted. In the

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majority of cases, functional screening is a four-step process: i) Inducing loss-of-function via RNA interference (RNAi) or CRISPR-Cas9 in cells; ii) evaluating the effects of the loss of the selected gene on phenotypes critical to cancer cells; iii) quantifying short hairpin RNAs (shRNAs) or single-guide RNAs (sgRNAs) via next-generation sequencing or microarray hybridization; and iv) data analysis (Fig. 1). Malignant phenotypes used for functional screening include uncontrolled promoted proliferation, drug resistance, invasiveness and the ability to bypass oncogene-induced senescence (OIS). In the present study, the recent advances in functional screening to identify cancer drug target genes have been summarized, and current issues and future perspectives have been discussed.

2. Types of functional screening according to phenotypes used

Dropout viability screening. Using genome-wide methodologies to identify target genes that substantially contribute to the uncontrolled proliferation of cancer cells is a straightforward approach to discovering cancer drug target genes for new drug development. This type of assay is called dropout viability screening. Two pioneering studies have conducted genome-wide dropout shRNA screening in various human cancer cell lines and identified genes essential for cancer cells (22,23). The Project Achilles study (launched in 2011) systemically identified genes essential for proliferation and/or survival in particular cancer cell types (genetic vulnerabilities) by performing an integrative analysis involving two steps: i) Conducting a pooled shRNA screen that targeted 11,194 genes in 102 (updated to 216 in the latest study) human cancer cell lines, including ovarian, colon, pancreatic, esophageal and non-small cell lung cancers; and ii) combining these results with information on alterations of cancer genome through using publicly available databases (24,25). By analyzing such diverse types of cancer, the study identified a number of lineage-specific essential genes. Another similar study used a pooled shRNA library comprised of 72 breast, pancreatic and ovarian cancer cell lines (26). In addition, after a CRISPR-Cas9-mediated gene-knockout technology became available in the experimental cell biology field (27), two studies demonstrated the feasibility of using lentiviral CRISPR-Cas9 libraries for functional screening, with certain advantages over RNAi libraries in efficacy and reliability (28,29). Via negative screening with RNAi or CRISPR-Cas9, these studies identified genes essential for proliferation in cancer cells, of which certain genes were lineage-specific.

One critical issue resulting from the nature of dropout viability screening is that such identified essential genes for cancer cells may also be essential for normal cells; for example, housekeeping genes involved in the ribosomal, proteasomal and spliceosomal pathways (26). Nevertheless, such essential genes may serve as promising therapeutic targets, as cancer cells highly depend on them for proliferation and/or survival compared with normal cells. One way to identify general essential genes that are likely to serve as cancer drug targets is to integrate results of genomic library screening with gene expression data and copy number changes between cancer and normal cells (20). This helps identify the genes that are associated with proliferation and/or survival

in cancer cells (24). Using this approach, two housekeeping genes have been identified, proteasome 20S subunit alpha 6 (*PSMA6*; a proteasomal catalytic subunit) and eukaryotic translation initiation factor 2 subunit beta (*eIF2 β* ; a subunit of translation-initiation factor EIF2), as promising therapeutic targets for lung cancer (30,31).

Another way to identify essential genes that contribute to oncogenic phenotypes is to reveal the genes which cancer cells depend on in specific contexts; for example, with certain types of driver oncogenes (32). This situation is referred to as synthetic lethality and is described later. One study demonstrated that an essential gene *BUD31*, a component of the spliceosome is a potential therapeutic target specifically in MYC-driven cancers (33).

Synthetic lethality. A synthetic lethality refers to a phenomenon in which inhibition of one of two genes has no significant effects on cell viability but perturbation of both genes results in cell death (32). Synthetic lethality has attracted interest for the following reasons: i) If the synthetic lethality specifically occurs in cancer cells, treatments targeting genes involved in the synthetic lethality have a high therapeutic index; and ii) if the synthetic lethality involves driver oncogenes highly refractory to currently available treatment strategies, synthetic lethal genes may serve as good targets in types of cancer influenced by these oncogenes. A good example of such a gene is oncogenic *KRAS*, the most frequently mutated oncogene, although *KRAS*-targeted therapy is not used clinically (14). Using RNAi library screening, several studies have identified synthetic lethal genes in *KRAS*-mutated cancers, such as *STK33*, *TBK1*, *PLK1*, *SNAIL2*, *CDK1* and *GATA2* (34-39). However, these identified genes rarely overlapped between studies (40) and the identification of a synthetic lethal effect caused by *STK33* has not been reproduced (41,42). A recently conducted large-scale synthetic lethal RNAi screen, Project DRIVE, also failed to confirm significant synthetic interactions of mutant *KRAS* with these identified synthetic lethal genes (20). There are several possible reasons for such inconsistent results, including differences in methods of gene silencing (for example RNAi methodologies such as transient transfection of siRNAs or shRNA, and difference in types of library), and differences in types of cells used (for example variable dependencies on *KRAS* signaling). In particular, the latter seems to significantly influence screening results. Most studies of *KRAS* synthetic screens used cancer cell lines with or without mutant *KRAS* and/or isogenic cancer cell lines transfected with or without mutant *KRAS* (34-39). Cancer cell lines are highly variable in genetic changes (even those with the same driver oncogenes), which may result in inconsistent screening results (17,43).

Project DRIVE comprehensively assessed dependencies and synthetic lethal relationships using 398 cancer cell lines from different organs (20). To minimize false-positive rates, an average of 20 shRNAs per gene were used and, although synthetic lethal genes could not be confirmed for mutant *KRAS*, a number of novel findings regarding synthetic lethality which are translatable to developing novel therapeutics were identified. For example, reduced expression levels of an anti-apoptotic protein *BCL2L1*, and increased expression levels of pro-apoptotic protein *BIM*, were the

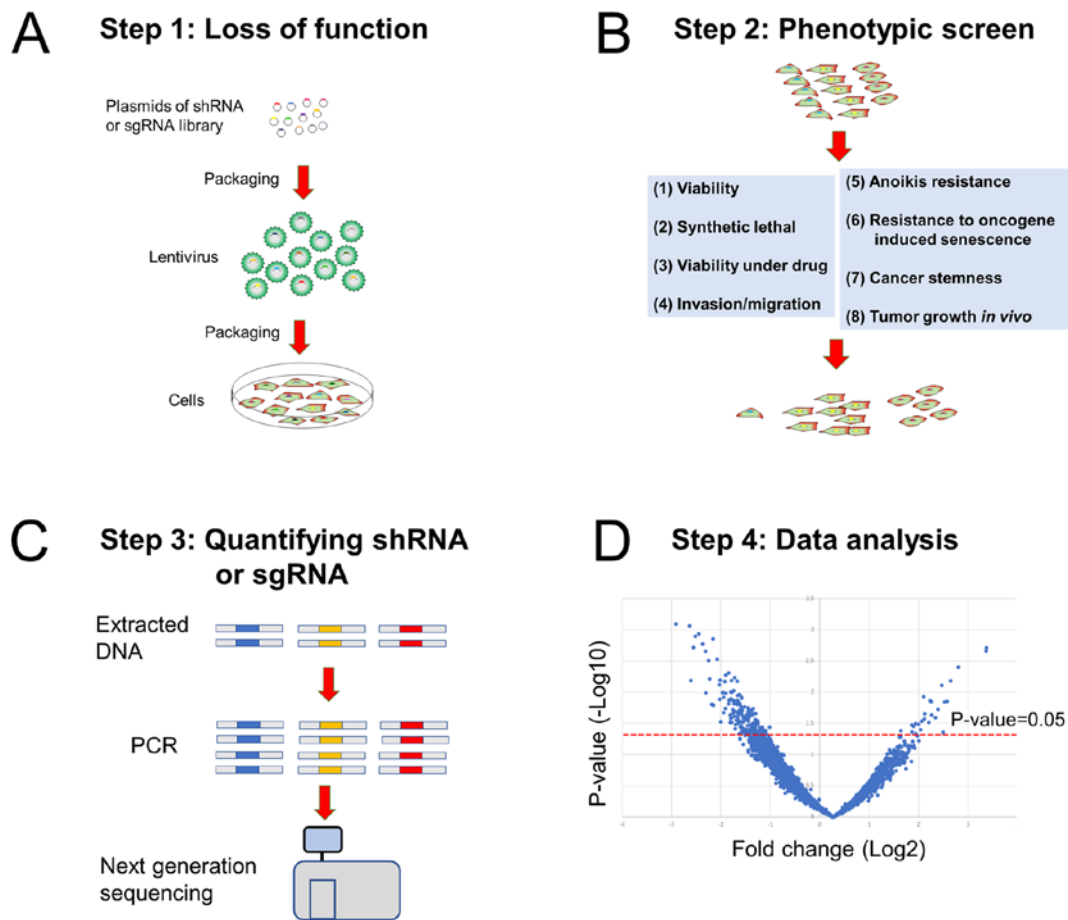


Figure 1. Flow diagram of the steps of phenotypic library screening with a genomic library for identifying cancer drug target genes. (A) Step 1: Loss of function, which is obtained by RNAi-mediated gene knockdown or Cas9-mediated gene knockout in cells. (B) Step 2: Phenotypic screen. Cells are subjected to various assays with different selection pressures including: 1, viability; 2, synthetic lethal; 3, viability under drug; 4, invasion/migration; 5, anoikis-resistance; 6, resistance to oncogene-induced senescence; 7, cancer stemness; and 8, tumor growth *in vivo*. (C) Step 3: Quantifying shRNA or sgRNA. DNA is extracted from harvested cells. Abundance of each shRNA or sgRNA is quantified using next-generation sequencing. (D) Step 4: Data analysis. Data are analyzed to generate ranked lists of promising cancer drug target genes. shRNA, short hairpin RNA; sgRNA, single-guide RNA.

strongest predictors of the growth-inhibiting effects following knockdown of anti-apoptotic protein myeloid cell leukemia sequence 1 (*MCL1*).

Recently, via genome-wide CRISPR-Cas9 screening, two independent groups identified WRN helicase as a synthetic lethal target in microsatellite unstable cancer types (44,45). Moreover, a small molecule inhibitor of WRN helicase (NSC617145) has been revealed to exhibit cytotoxic effects in cells derived from patients with Fanconi anemia, in a synthetic lethal manner (46).

Dropout viability screening under drug treatment. Drug resistance is a critical problem in chemotherapy (47). Cancer can be resistant to a number of types of drugs, such as cytotoxic, molecular-targeted drugs and immune checkpoint inhibitors (47-49). Therefore, researchers focus extensively on finding therapeutic approaches to overcoming the development of drug resistance.

Dropout viability screening in the presence of anti-cancer drugs is a powerful approach to identifying genes responsible for drug resistance and several potentially chemo-sensitizing targets have been reported (Table I). Using a genome-wide an arrayed RNAi library, Whitehurst *et al* (50) identified several

genes influencing resistance to paclitaxel in a lung cancer cell line. Lin *et al* (51) identified *MCL1* as a potential drug target gene that sensitizes a small cell lung cancer cell line to ABT-737, an inhibitor of the antiapoptotic molecules Bcl-2, Bcl-X(L) and Bcl-w. After the development of pooled RNAi library technology, numerous investigators began using such libraries. For example, Prahallad *et al* (52) revealed genes responsible for resistance to a *BRAF* inhibitor PLX4032 (vemurafenib) in types of cancer harboring *BRAF* V600E mutations. It was revealed that *EGFR* activation, which is rapidly induced by vemurafenib treatment, induces resistance to vemurafenib treatment, suggesting that combination therapy of vemurafenib and an *EGFR* inhibitor may be beneficial. Previously, studies using CRISPR-Cas9 libraries were published. Most of these studies used the same type of genome-wide library, GeCKO CRISPR Library version 1 or 2, comprising of >120,000 sgRNAs targeting nearly the entire genome (53-56). For example, Sustic *et al* identified the endoplasmic reticulum to nucleus signaling 1 (*ERN1*)-*JNK*-*JUN* pathway as a potential target for improving the anti-cancer effects of MET inhibitors in *KRAS*-mutated colon cancer (56). *KRAS*-targeted therapy has not been successfully developed previously and, therefore, these findings are promising.

Table I. Studies identifying target genes for the treatment of cancer-associated drug resistance using large scale libraries.

Authors, year	Type of library	Size of library	Cancer type	Drug(s)	Identified genes or the pathways	(Refs.)
Bartz <i>et al.</i> , 2006	Pooled shRNA	20,000 genes	Non-small cell lung, cervical and ovarian	Cisplatin	<i>BRCA1</i> , <i>BRCA2</i> , <i>TP53</i>	(97)
Whitehurst <i>et al.</i> , 2007	Arrayed RNA oligos	21,127 genes	Non-small cell lung	Paclitaxel	<i>ACRBP</i> , <i>ATP6V0D2</i> , <i>FGD4</i> , <i>HS6ST2</i> , <i>PSMA6</i> , <i>TUBGGCP2</i>	(50)
Lin <i>et al.</i> , 2007	Arrayed RNA oligos	4,000 druggable genes	Small cell lung	ABT-737	<i>MCL1</i>	(51)
Lam <i>et al.</i> , 2008	pooled shRNA	500 kinase genes	Diffuse large B-cell lymphoma	IKK β inhibitors	<i>CARD11</i>	(98)
Xu <i>et al.</i> , 2011	Arrayed RNA oligos	22,000 genes	Cervical	Cytotoxic nucleoside analog 2', 2'-difluorodeoxycytidine u	<i>SRSF3</i> <i>SFPQ</i>	(99)
Guerreiro <i>et al.</i> , 2011	Arrayed RNA oligos	719 kinase genes	Medulloblastoma	Cisplatin	<i>ATR</i> , <i>LYK5</i> , <i>MPP2</i> <i>PIK3CG</i> , <i>PIK4CA</i> , <i>WNK4</i>	(100)
Liu-Sullivan <i>et al.</i> , 2011	Arrayed RNA oligos	1,657 genes	Non-small cell lung	GSK461364A (PLK1 inhibitor)	97 genes	(101)
Prahallad <i>et al.</i> , 2012	Pooled shRNA	518 kinase and 17 kinase-related genes	Colorectal, prostate and thyroid	Vemurafenib	<i>EGFR</i>	(52)
Fredebohm <i>et al.</i> , 2013	Pooled shRNA	1,000 genes	Pancreatic	Gemcitabine	<i>RAD17</i>	(102)
Milosevic <i>et al.</i> , 2013	Pooled shRNA	779 kinase genes	Pancreatic	Erlotinib	<i>RPS6KA2</i>	(103)
Wetterskog <i>et al.</i> , 2014	Arrayed RNAi	369 genes	ERBB2-amplified breast	Lapatinib	<i>RAB34</i> , <i>TP53INP1</i> , <i>RAC1</i> , <i>ATP6CIV1</i> , <i>C11ORF73</i> , <i>MLLT6</i> , <i>NIBP</i> (<i>TRAPPC9</i>), <i>NUFIP</i> , <i>PROCA1</i> , <i>RAB7L1</i> , <i>RAD21</i> , <i>SCRN2</i> and <i>SPOP</i>	(104)
MacKay <i>et al.</i> , 2014	Arrayed RNA oligos	1,067 genes	Osteosarcoma	Cisplatin	<i>HOIP</i>	(105)
Maryyama <i>et al.</i> , 2014	Pooled shRNA	10,000 shRNAs	Prostate	Bicalutamide	<i>RPL31</i>	(106)
Sudo <i>et al.</i> , 2015	Pooled shRNA	16,000 genes	Non-small cell lung	gefitinib	<i>PRKCSH</i> and genes involved in the <i>CD27</i> signaling cascade	(107)
Prahallad <i>et al.</i> , 2015	Pooled shRNA	298 phosphatases or phosphatase-related genes	Colorectal	Vemurafenib	<i>PTPN11</i>	(108)
Kobayashi <i>et al.</i> , 2015	Pooled shRNA	~15,000 genes	Cervical, colorectal and non-small cell lung	2-deoxyglucose (2DG) (glycolytic inhibitor)	<i>COPB1</i> , <i>ARCN</i>	(109)
Yamaguchi <i>et al.</i> , 2016	Pooled shRNA	2,924 genes	Head and neck squamous cell carcinomas	Rapamycin	Genes involved in the <i>ERK</i> pathway	(110)

Table I. Continued.

Authors, year	Type of library	Size of library	Cancer type	Drug(s)	Identified genes or the pathways	(Refs.)
Yamanoi <i>et al.</i> , 2016	Pooled shRNA	~15,000 genes	Ovarian	Cisplatin	<i>ABHD2</i>	(111)
Kurata <i>et al.</i> , 2016	Pooled CRISPR	19,050 genes	Acute myeloid leukemia	Ara-C	<i>DCK, SLC29A</i>	(53)
Hou <i>et al.</i> , 2017	Pooled CRISPR	18,080 genes	Acute myeloid leukemia	FLT3 inhibitor AC220	<i>SPRY3, GSK3</i>	(54)
Sun <i>et al.</i> , 2018	Pooled CRISPR	19,050 genes	hepatocellular carcinoma	Sorafenib	<i>SGOLI</i>	(55)
Sustic <i>et al.</i> , 2018	Pooled CRISPR	65,383 sgRNAs	<i>KRAS</i> -mutant colon	MEK inhibitors	The <i>ERN1-JNK-JUN</i> pathway	(56)
Combes <i>et al.</i> , 2019	Pooled CRISPR	518 kinase genes	Colorectal	Oxaliplatin	<i>ATR</i>	(112)

RNAi, RNA interference; CRISPR, clustered regularly interspaced short palindromic repeats; shRNA, short hairpin RNA; sgRNA, single-guide RNA.

Immune therapy using immune checkpoint inhibitors provides significant clinical benefit to patients with various types of cancer, including melanoma, lymphoma, and lung cancer (57). However, intrinsic or acquired resistance inevitably occurs, limiting the clinical benefits (49). Using genome-wide CRISPR-Cas9 or siRNA libraries, two studies identified *APLNR* (encoding the apelin receptor) and C-C motif chemokine receptor 9 as genes that may cause resistance to immune checkpoint inhibitors (58,59).

Invasion and migration. Metastasis is significantly associated with a poor patient prognosis, and patients with metastatic cancer exhibit poor survival outcomes (60). Metastasis comprises several sequential steps: i) Migration from a primary site; ii) intravasation; iii) passage by blood flow; iv) extravasation; v) and final settlement at distant sites. To complete this process, cancer cells must acquire the ability to invade and migrate and cancer cells exhibit these oncogenic properties. Previous studies demonstrated that epithelial-mesenchymal transition (EMT) significantly contributes to metastasis in cancer cells (61,62). EMT, and its reverse phenomenon MET, were initially identified during embryonic development, in which embryonic cells transform into terminally differentiated, specialized cells via several cycles of EMT and MET (61). A number of studies suggest a central role of EMT in metastasis (63-65). Previous studies have identified target genes for inhibiting migration and/or invasion ability of cancer cells through library screening. Pavan *et al* (66) developed a system combining RNAi library screening with a microscopy-based high-throughput quantitative analysis to identify a signaling pathway contributing to EMT in breast cancer. The group identified 59 genes whose inhibition suppressed transforming growth factor β -induced EMT in immortalized epithelial normal murine mammary gland cells. In addition, Pavan *et al* (66) focused on *MEK5* and *ERK5* belonging to the same signaling pathway and demonstrated the potential of targeting *MEK5* and *ERK5* as an anti-metastatic mechanism. Another study used migration ability as a phenotype for functional screening, identifying genes contributing to migration in glioblastoma, a highly invasive cancer (67). The authors performed a genome-wide RNAi screening in glioblastoma cells with a functional selection of cells able to migrate through Matrigel, identifying two genes [KH-type splicing regulatory protein (*KHSRP*) and host cell factor C1 (*HCF1*)] as targets of invasion-suppressing therapeutics for glioblastoma.

Resistance to anoikis: Anchorage-independent growth. Upon detachment from the extracellular matrix or neighboring cells, normal epithelial cells undergo a type of apoptosis called anoikis (68). Anoikis prevents normal epithelial cells from colonizing at different organ sites, thereby maintaining the integrity of the body (68). Most cancer cells acquire resistance to anoikis, which is called anchorage-independent growth (AIG). The ability of AIG allows cancer cells to metastasize to different organs and is considered a hallmark of cancer cells (64). Several different molecular mechanisms underlying AIG have been identified, including the induction of intrinsic and extrinsic anti-apoptotic signaling, often triggered by changes in the expression patterns of integrin family members (68,69). In addition, previous studies have

demonstrated the role of EMT in AIG (68,70); however, the underlying molecular mechanisms of AIG are yet to be elucidated.

Eskiocak *et al* (71) used the immortalized untransformed colon epithelial cell line HCEC as a model system to evaluate the effects of shRNA-mediated knockdown of selected genes on AIG. The effects of the knockdown of 151 candidate cancer genes (*CAN*-genes), which have been identified as genes most likely to be drivers in breast and colorectal cancers, via a comprehensive statistical and bioinformatic analysis (72), were evaluated and revealed that *CAN*-genes are enriched in AIG suppressors. In addition, Simpson *et al* conducted a genome-wide shRNA screening to identify anoikis-resistant genes by culturing immortalized prostate and nasopharyngeal untransformed cell lines in a suspension condition, which identified α/β hydrolase domain containing 4 (*ABHD4*) as a promising target for inducing anoikis (73).

Resistance to oncogene-induced senescence (OIS). Activation of certain types of oncogenes, such as mutant *KRAS* and *BRAF*, causes normal cells to undergo senescence (74,75). This type of senescence is stress-induced and is termed OIS (74,75). OIS functions as a barrier to carcinogenesis initiated by normal cells, whereas senescence-associated secretory phenotype (*SASP*) is a carcinogenesis-promoting aspect of OIS (76). OIS was discovered by Serrano *et al* (77), who demonstrated that oncogenic *ras* induces premature senescence in experimental cell cultures. Subsequently, the occurrence of OIS in human disease was demonstrated in a developmental process of melanoma. Two studies revealed that OIS prevents benign melanocytic nevi, presumed to be the origin of melanoma, from transforming to overt melanoma (78,79). These studies demonstrated that proliferation of nevi cells is suppressed at very low levels despite harboring the highly oncogenic mutation *BRAFV600*. Studies have also revealed that *BRAFV600*-induced OIS is associated with p16INK4A upregulation (78,79); however, other unidentified changes may also be involved because of the complex mechanisms governing senescence (80). Therefore, several studies have attempted to identify genes that may facilitate cells to bypass senescence induced by oncogenic *RAS* or *BRAF*. Vicent *et al* (81) performed RNAi screening to identify genes that facilitate bypassing *ras*-induced OIS in mouse models, reporting that Wt1 transcription factor (*Wt1*) is an OIS-bypassing gene using both *in vitro* and *in vivo* models. Vicent *et al* (81) also demonstrated *Wt1* to be an independent prognostic factor in patients with *KRAS*-mutated lung cancer. Another study performed a near-genome-wide screening (~15,000 genes) to identify regulators of *BRAFV600*-induced senescence and identified *RASSF* as an OIS suppressor (82). Some screenings searching for OIS-bypassing genes used primary normal culture, and immortalized untransformed normal cell lines as model systems (82,83) because these cells are more prone to exhibit OIS, primarily due to their intact senescent machinery. However, the ability of cancer cells to undergo OIS may be impaired because of alterations in genes involved in senescence (74). Therefore, the applicability of the identified OIS-bypassing genes needs to be validated in multiple human cancer cell lines before exploring their usefulness as drug targets.

Cancer stemness. The cancer stem cell (CSC) theory hypothesizes that CSCs have the ability to self-renew and to differentiate into phenotypically diverse cancer cells (84). Although the CSC concept has not been demonstrated, accumulating evidence suggests that a number of types of cancer harbor CSCs (84,85). Notably, CSCs are hypothesized to be resistant to chemotherapy and irradiation (84). Therefore, the development of CSC-targeted therapeutics is attracting attention because of its potential to eradicate cancer cells. A functional library screening based on the sphere-forming ability of breast cancer cell lines identified *ATG4* as a promoter of the breast CSC-like phenotype (86). However, the usefulness of a sphere-formation assay for evaluating the self-renewal capacity is based on the assumption that the assay developed for normal neural stem cells can be accurately used for CSCs. Therefore, validation of genes identified as cancer stemness genes by other assays, such as a transplantation assays and lineage-tracing approaches, are required.

Genomic Instability. A phenotype of genomic instability facilitates diverse oncogenic properties because it causes numerous mutations resulting from the activation of oncogenic genes or inactivation of tumor suppressive genes (87). A previous study performed a genome-wide RNAi screen to identify the pathways and specific genes mediating genomic stability (88). A screen using elevation of γ H2A.X variant histone (H2AX; a marker of double strand DNA damage) as an indicator for detecting DNA damage was conducted in HeLa cancer cells, identifying genes involved in DNA replication, checkpoint activation and DNA repair. The identified genes included *TIMELESS* and *TIPIN* encoding proteins that form a complex, leading to activation of the replication checkpoint. The identified genes may serve as promising drug targets to restore genomic stability in cancer cells (88).

Tumor growth in vivo. Tumor growth *in vivo* represents a more accurate screening method because it accounts for several aspects of real tumor growth, including 3-D condition, requirements for angiogenesis and the microenvironment (89).

One critical issue of *in vivo* pooled library screening is the difficulty in ensuring appropriate representation of the entire library in the initial population inoculated into model animals (89). There is a limitation to the maximum number of cells that can be inoculated, which limits the size and complexity of the library. Notably, the minimum cell numbers required for each shRNA differ significantly, depending on whether tumor suppressor genes or oncogenic genes are targeted for screening (89). In the case of oncogenic gene-targeted screening, low library representation tends to result in false-positive results, so instead of using genome-wide libraries, researchers used libraries focused on specific types of genes in order to identify oncogenic genes. For example, Singh *et al* (90) used an shRNA library of 150 genes associated with brain metastasis to conduct a library screening consisting of both *in vivo* (intracranial injection) and *in vitro* (tumor sphere-forming assay) assays to identify metastasis-promoting genes. The group successfully identified SPARC (osteonectin), *cwcv* and *kazal* like domains proteoglycan 1 (*SPOCK1*) and twist family bHLH transcription factor 2 (*TWIST2*) as

regulators of brain metastasis-initiating cells. In addition, most studies using *in vivo* genome-wide or near-genome-wide RNAi models discovered tumor-suppressive genes (91,92).

3. Conclusions and future perspectives

Shortly after RNAi technology for gene knockdown was developed in the laboratory, attempts to conduct large-scale functional screenings with RNAi were initiated (93). In addition, a gene knockout technique, CRISPR-Cas9 was also introduced for laboratory use (94). For >10 years, researchers have extensively conducted functional genomic screening to identify better targets and to develop new therapeutics for cancer. The present paper reviewed and summarized knowledge obtained by these studies, which has the potential to be used for drug development. Nevertheless, breakthroughs that can be immediately translated into clinical use are yet to be made. In particular, despite many reported studies, *KRAS* synthetic genes that have been reproducibly confirmed have not been successfully identified; therefore, development of *KRAS*-synthetic lethal drugs has not been successful. Project DRIVE suggested that no single synthetic lethal genes for *KRAS* exist. However, there may be certain strategies potentially enabling the identification of true *KRAS* synthetic genes; for example, one approach may be using more realistic modeling systems to evaluate malignant phenotypes. Such models may include a 3-D culture of cell lines and patient-derived xenografts (95,96), although such models are usually difficult to manage for large-scale screening. Due to the large heterogeneity in coexisting genomic alterations among *KRAS*-mutated tumors, studies using cancer cells may suffer from the presence of high background of noise during screening. Therefore, focus is needed on cancer cells which have higher similarities in harbored genetic alterations in addition to mutant *KRAS*.

An improvement in consistency of identified genes from a genome-wide screen has been revealed in CRISPR-Cas9 knockout compared with shRNA techniques (28). However, pharmacological inhibition of gene function with compounds is usually incomplete; thus, target genes identified through partial knockdown with RNAi represent improved targets. Therefore, results from CRISPR-Cas9 and RNAi screens need to be regarded as complementary.

In conclusion, advances in the technology of gene silencing and next generation sequencing have enabled researchers to conduct large-scale high-throughput phenotypic screenings, resulting in the identification of numerous potential novel drug targets for cancer. However, there are several issues, such as low reproducibility in the identified genes (40). Thus, substantial effort is required to adequately address these problems in order to identify novel cancer drug target genes.

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Author's contributions

MS designed the review, researched the literature and wrote the manuscript.

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Not applicable.

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Competing interests

The author declares that he has no competing interests.

References

1. Armitage P and Doll R: The age distribution of cancer and a multi-stage theory of carcinogenesis. *Br J Cancer* 8: 1-12, 1954.
2. Vogelstein B and Kinzler KW: The multistep nature of cancer. *Trends Genet* 9: 138-141, 1993.
3. Chaffer CL and Weinberg RA: How does multistep tumorigenesis really proceed? *Cancer Discov* 5: 22-24, 2015.
4. Barretina J, Caponigro G, Stransky N, Venkatesan K, Margolin AA, Kim S, Wilson CJ, Lehár J, Kryukov GV, Sonkin D, *et al*: The cancer cell line encyclopedia enables predictive modelling of anticancer drug sensitivity. *Nature* 483: 603-607, 2012.
5. Bailey MH, Tokheim C, Porta-Pardo E, Sengupta S, Bertrand D, Weerasinghe A, Colaprico A, Wendl MC, Kim J, Reardon B, *et al*: Comprehensive characterization of cancer driver genes and mutations. *Cell* 173: 371-385 e18, 2018.
6. Garraway LA and Lander ES: Lessons from the cancer genome. *Cell* 153: 17-37, 2013.
7. Seshadri R, Matthews C, Dobrovic A and Horsfall DJ: The significance of oncogene amplification in primary breast cancer. *Int J Cancer* 43: 270-272, 1989.
8. Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, Teague J, Woffendin H, Garnett MJ, Bottomley W, *et al*: Mutations of the BRAF gene in human cancer. *Nature* 417: 949-954, 2002.
9. Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW, Harris PL, Haserlat SM, Supko JG, Haluska FG, *et al*: Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N Engl J Med* 350: 2129-2139, 2004.
10. Paez JG, Jänne PA, Lee JC, Tracy S, Greulich H, Gabriel S, Herman P, Kaye FJ, Lindeman N, Boggon TJ, *et al*: EGFR mutations in lung cancer: Correlation with clinical response to gefitinib therapy. *Science* 304: 1497-1500, 2004.
11. Hirsch FR, Scagliotti GV, Mulshine JL, Kwon R, Curran WJ Jr, Wu YL and Paz-Ares L: Lung cancer: Current therapies and new targeted treatments. *Lancet* 389: 299-311, 2017.
12. Sato M, Shames DS, Gazdar AF and Minna JD: A translational view of the molecular pathogenesis of lung cancer. *J Thorac Oncol* 2: 327-343, 2007.
13. Murugan AK, Grieco M and Tsuchida N: RAS mutations in human cancers: Roles in precision medicine. *Semin Cancer Biol* 59: 23-35, 2019.
14. Ryan MB and Corcoran RB: Therapeutic strategies to target RAS-mutant cancers. *Nat Rev Clin Oncol* 15: 709-720, 2018.

15. Govindan R, Fakih M, Price T, Falchook G, Desai J, Kuo J, Strickler J, Krauss J, Li B, Denlinger C, *et al*: OA02.02 Phase I study of safety, tolerability, PK and efficacy of AMG 510, a novel KRASG12C inhibitor, evaluated in NSCLC. *J Thorac Oncol* 14 (Suppl): S208, 2019.
16. Canon J, Rex K, Saiki AY, Mohr C, Cooke K, Bagal D, Gaida K, Holt T, Knutson CG, Koppada N, *et al*: The clinical KRAS(G12C) inhibitor AMG 510 drives anti-tumour immunity. *Nature* 575: 217-223, 2019.
17. Vogelstein B, Papadopoulos N, Velculescu VE, Zhou S, Diaz LA Jr and Kinzler KW: Cancer genome landscapes. *Science* 339: 1546-1558, 2013.
18. Nagel R, Semenova EA and Berns A: Drugging the addict: Non-oncogene addiction as a target for cancer therapy. *EMBO Rep* 17: 1516-1531, 2016.
19. Dai C, Whitesell L, Rogers AB and Lindquist S: Heat shock factor 1 is a powerful multifaceted modifier of carcinogenesis. *Cell* 130: 1005-1018, 2007.
20. McDonald ER III, de Weck A, Schlabach MR, Billy E, Mavrakis KJ, Hoffman GR, Belur D, Castelletti D, Frias E, Gampa K, *et al*: Project DRIVE: A compendium of cancer dependencies and synthetic lethal relationships uncovered by large-scale, deep RNAi screening. *Cell* 170: 577-592 e10, 2017.
21. Schuster A, Erasmus H, Fritah S, Nazarov PV, van Dyck E, Niclou SP and Golebiewska A: RNAi/CRISPR Screens: From a pool to a valid hit. *Trends Biotechnol* 37: 38-55, 2019.
22. Schlabach MR, Luo J, Solimini NL, Hu G, Xu Q, Li MZ, Zhao Z, Smogorzewska A, Sowa ME, Ang XL, *et al*: Cancer proliferation gene discovery through functional genomics. *Science* 319: 620-624, 2008.
23. Silva JM, Marran K, Parker JS, Silva J, Golding M, Schlabach MR, Elledge SJ, Hannon GJ and Chang K: Profiling essential genes in human mammary cells by multiplex RNAi screening. *Science* 319: 617-620, 2008.
24. Cheung HW, Cowley GS, Weir BA, Boehm JS, Rusin S, Scott JA, East A, Ali LD, Lizotte PH, Wong TC, *et al*: Systematic investigation of genetic vulnerabilities across cancer cell lines reveals lineage-specific dependencies in ovarian cancer. *Proc Natl Acad Sci USA* 108: 12372-12377, 2011.
25. Cowley GS, Weir BA, Vazquez F, Tamayo P, Scott JA, Rusin S, East-Seletsky A, Ali LD, Gerath WF, Pantel SE, *et al*: Parallel genome-scale loss of function screens in 216 cancer cell lines for the identification of context-specific genetic dependencies. *Sci Data* 1: 140035, 2014.
26. Marcotte R, Brown KR, Suarez F, Sayad A, Karamboulas K, Krzyzanowski PM, Sircoulomb F, Medrano M, Fedyshyn Y, Koh JLY, *et al*: Essential gene profiles in breast, pancreatic, and ovarian cancer cells. *Cancer Discov* 2: 172-189, 2012.
27. Ran FA, Hsu PD, Wright J, Agarwala V, Scott DA and Zhang F: Genome engineering using the CRISPR-Cas9 system. *Nat Protoc* 8: 2281-2308, 2013.
28. Shalem O, Sanjana NE, Hartenian E, Shi X, Scott DA, Mikkelsen T, Heckl D, Ebert BL, Root DE, Doench JG and Zhang F: Genome-scale CRISPR-Cas9 knockout screening in human cells. *Science* 343: 84-87, 2014.
29. Wang T, Wei JJ, Sabatini DM and Lander ES: Genetic screens in human cells using the CRISPR-Cas9 system. *Science* 343: 80-84, 2014.
30. Kakumu T, Sato M, Goto D, Kato T, Yogo N, Hase T, Morise M, Fukui T, Yokoi K, Sekido Y, *et al*: Identification of proteasomal catalytic subunit PSMA6 as a therapeutic target for lung cancer. *Cancer Sci* 108: 732-743, 2017.
31. Tanaka I, Sato M, Kato T, Goto D, Kakumu T, Miyazawa A, Yogo N, Hase T, Morise M, Sekido Y, *et al*: eIF2 β , a subunit of translation-initiation factor EIF2, is a potential therapeutic target for non-small cell lung cancer. *Cancer Sci* 109: 1843-1852, 2018.
32. O'Neil NJ, Bailey ML and Hieter P: Synthetic lethality and cancer. *Nat Rev Genet* 18: 613-623, 2017.
33. Hsu TY, Simon LM, Neill NJ, Marcotte R, Sayad A, Bland CS, Echeverria GV, Sun T, Kurley SJ, Tyagi S, *et al*: The spliceosome is a therapeutic vulnerability in MYC-driven cancer. *Nature* 525: 384-388, 2015.
34. Kumar MS, Hancock DC, Molina-Arcas M, Steckel M, East P, Diefenbacher M, Armenteros-Monterroso E, Lassailly F, Matthews N, Nye E, *et al*: The GATA2 transcriptional network is requisite for RAS oncogene-driven non-small cell lung cancer. *Cell* 149: 642-655, 2012.
35. Luo J, Emanuele MJ, Li D, Creighton CJ, Schlabach MR, Westbrook TF, Wong KK and Elledge SJ: A genome-wide RNAi screen identifies multiple synthetic lethal interactions with the Ras oncogene. *Cell* 137: 835-848, 2009.
36. Scholl C, Fröhling S, Dunn IF, Schinzel AC, Barbie DA, Kim SY, Silver SJ, Tamayo P, Wadlow RC, Ramaswamy S, *et al*: Synthetic lethal interaction between oncogenic KRAS dependency and STK33 suppression in human cancer cells. *Cell* 137: 821-834, 2009.
37. Barbie DA, Tamayo P, Boehm JS, Kim SY, Moody SE, Dunn IF, Schinzel AC, Sandy P, Meylan E, Scholl C, *et al*: Systematic RNA interference reveals that oncogenic KRAS-driven cancers require TBK1. *Nature* 462: 108-112, 2009.
38. Wang Y, Ngo VN, Marani M, Yang Y, Wright G, Staudt LM and Downward J: Critical role for transcriptional repressor Snail2 in transformation by oncogenic RAS in colorectal carcinoma cells. *Oncogene* 29: 4658-4670, 2010.
39. Costa-Cabral S, Brough R, Konde A, Aarts M, Campbell J, Marinari E, Riffell J, Bardelli A, Torrance C, Lord CJ and Ashworth A: CDK1 is a synthetic lethal target for KRAS mutant tumours. *PLoS One* 11: e0149099, 2016.
40. Downward J: RAS synthetic lethal screens revisited: Still seeking the elusive prize? *Clin Cancer Res* 21: 1802-1809, 2015.
41. Babij C, Zhang Y, Kurzeja RJ, Munzli A, Shehabeldin A, Fernando M, Quon K, Kassner PD, Ruefli-Brasse AA, Watson VJ, *et al*: STK33 kinase activity is nonessential in KRAS-dependent cancer cells. *Cancer Res* 71: 5818-5826, 2011.
42. Fröhling S and Scholl C: STK33 kinase is not essential in KRAS-dependent cells-letter. *Cancer Res* 71: 7716; author reply 7717, 2011.
43. Forbes SA, Beare D, Boutselakis H, Bamford S, Bindal N, Tate J, Cole CG, Ward S, Dawson E, Ponting L, *et al*: COSMIC: Somatic cancer genetics at high-resolution. *Nucleic Acids Res* 45D: D777-D783, 2017.
44. Behan FM, Iorio F, Picco G, Gonçalves E, Beaver CM, Migliardi G, Santos R, Rao Y, Sassi F, Pinnelli M, *et al*: Prioritization of cancer therapeutic targets using CRISPR-Cas9 screens. *Nature* 568: 511-516, 2019.
45. Chan EM, Shibue T, McFarland JM, Gaeta B, Ghandi M, Dumont N, Gonzalez A, McPartlan JS, Li T, Zhang Y, *et al*: WRN helicase is a synthetic lethal target in microsatellite unstable cancers. *Nature* 568: 551-556, 2019.
46. Aggarwal M, Banerjee T, Sommers JA, Iannascioli C, Pichierri P, Shoemaker RH and Brosh RM Jr: Werner syndrome helicase has a critical role in DNA damage responses in the absence of a functional fanconi anemia pathway. *Cancer Res* 73: 5497-5507, 2013.
47. Holohan C, Van Schaeybroeck S, Longley DB and Johnston PG: Cancer drug resistance: An evolving paradigm. *Nat Rev Cancer* 13: 714-726, 2013.
48. Gottesman MM, Lavi O, Hall MD and Gillet JP: Toward a better understanding of the complexity of cancer drug resistance. *Annu Rev Pharmacol Toxicol* 56: 85-102, 2016.
49. Jackson CM, Choi J and Lim M: Mechanisms of immunotherapy resistance: Lessons from glioblastoma. *Nat Immunol* 20: 1100-1109, 2019.
50. Whitehurst AW, Bodemann BO, Cardenas J, Ferguson D, Girard L, Peyton M, Minna JD, Michnoff C, Hao W, Roth MG, *et al*: Synthetic lethal screen identification of chemosensitizer loci in cancer cells. *Nature* 446: 815-819, 2007.
51. Lin X, Morgan-Lappe S, Huang X, Li L, Zakula DM, Vernetti LA, Fesik SW and Shen Y: 'Seed' analysis of off-target siRNAs reveals an essential role of Mcl-1 in resistance to the small-molecule Bcl-2/Bcl-XL inhibitor ABT-737. *Oncogene* 26: 3972-3979, 2007.
52. Prahallad A, Sun C, Huang S, Di Nicolantonio F, Salazar R, Zecchin D, Beijersbergen RL, Bardelli A and Bernards R: Unresponsiveness of colon cancer to BRAF(V600E) inhibition through feedback activation of EGFR. *Nature* 483: 100-103, 2012.
53. Kurata M, Rathe SK, Bailey NJ, Aumann NK, Jones JM, Veldhuijzen GW, Moriarity BS and Largaespada DA: Using genome-wide CRISPR library screening with library resistant DCK to find new sources of Ara-C drug resistance in AML. *Sci Rep* 6: 36199, 2016.
54. Hou P, Wu C, Wang Y, Qi R, Bhavanasi D, Zuo Z, Dos Santos C, Chen S, Chen Y, Zheng H, *et al*: A Genome-wide CRISPR screen identifies genes critical for resistance to FLT3 inhibitor AC220. *Cancer Res* 77: 4402-4413, 2017.
55. Sun W, He B, Yang B, Hu W, Cheng S, Xiao H, Yang Z, Wen X, Zhou L, Xie H, *et al*: Genome-wide CRISPR screen reveals SGOL1 as a druggable target of sorafenib-treated hepatocellular carcinoma. *Lab Invest* 98: 734-744, 2018.
56. Sustic T, van Wageningen S, Bosdriesz E, Reid RJD, Dittmar J, Liefink C, Beijersbergen RL, Wessels LFA, Rothstein R and Bernards R: A role for the unfolded protein response stress sensor ERN1 in regulating the response to MEK inhibitors in KRAS mutant colon cancers. *Genome Med* 10: 90, 2018.

57. Sharma P and Allison JP: The future of immune checkpoint therapy. *Science* 348: 56-61, 2015.
58. Khandelwal N, Breinig M, Speck T, Michels T, Kreutzer C, Sorrentino A, Sharma AK, Umansky L, Conrad H, Poschke I, *et al*: A high-throughput RNAi screen for detection of immune-checkpoint molecules that mediate tumor resistance to cytotoxic T lymphocytes. *EMBO Mol Med* 7: 450-463, 2015.
59. Patel SJ, Sanjana NE, Kishton RJ, Eidizadeh A, Vodnala SK, Cam M, Gartner JJ, Jia L, Steinberg SM, Yamamoto TN, *et al*: Identification of essential genes for cancer immunotherapy. *Nature* 548: 537-542, 2017.
60. Steeg PS: Targeting metastasis. *Nat Rev Cancer* 16: 201-218, 2016.
61. Thiery JP, Acloque H, Huang RY and Nieto MA: Epithelial-mesenchymal transitions in development and disease. *Cell* 139: 871-890, 2009.
62. Sato M, Shames DS and Hasegawa Y: Emerging evidence of epithelial-to-mesenchymal transition in lung carcinogenesis. *Respirology* 17: 1048-1059, 2012.
63. Chaffer CL, San Juan BP, Lim E and Weinberg RA: EMT, cell plasticity and metastasis. *Cancer Metastasis Rev* 35: 645-654, 2016.
64. Yilmaz M and Christofori G: EMT, the cytoskeleton, and cancer cell invasion. *Cancer Metastasis Rev* 28: 15-33, 2009.
65. Heerboth S, Housman G, Leary M, Longacre M, Byler S, Lapinska K, Willbanks A and Sarkar S: EMT and tumor metastasis. *Clin Transl Med* 4: 6, 2015.
66. Pavan S, Meyer-Schaller N, Diepenbruck M, Kalathur RKR, Saxena M and Christofori G: A kinome-wide high-content siRNA screen identifies MEK5-ERK5 signaling as critical for breast cancer cell EMT and metastasis. *Oncogene* 37: 4197-4213, 2018.
67. Yang J, Fan J, Li Y, Li F, Chen P, Fan Y, Xia X and Wong ST: Genome-wide RNAi screening identifies genes inhibiting the migration of glioblastoma cells. *PLoS One* 8: e61915, 2013.
68. Paoli P, Giannoni E and Chiarugi P: Anoikis molecular pathways and its role in cancer progression. *Biochim Biophys Acta* 1833: 3481-3498, 2013.
69. Taddei ML, Giannoni E, Fiaschi T and Chiarugi P: Anoikis: An emerging hallmark in health and diseases. *J Pathol* 226: 380-393, 2012.
70. Takeyama Y, Sato M, Horio M, Hase T, Yoshida K, Yokoyama T, Nakashima H, Hashimoto N, Sekido Y, Gazdar AF, *et al*: Knockdown of ZEB1, a master epithelial-to-mesenchymal transition (EMT) gene, suppresses anchorage-independent cell growth of lung cancer cells. *Cancer Lett* 296: 216-224, 2010.
71. Eskiocak U, Kim SB, Ly P, Roig AI, Biglione S, Komurov K, Cornelius C, Wright WE, White MA and Shay JW: Functional parsing of driver mutations in the colorectal cancer genome reveals numerous suppressors of anchorage-independent growth. *Cancer Res* 71: 4359-4365, 2011.
72. Wood LD, Parsons DW, Jones S, Lin J, Sjöblom T, Leary RJ, Shen D, Boca SM, Barber T, Ptak J, *et al*: The genomic landscapes of human breast and colorectal cancers. *Science* 318: 1108-1113, 2007.
73. Simpson CD, Hurren R, Kasimer D, MacLean N, Eberhard Y, Ketela T, Moffat J and Schimmer AD: A genome wide shRNA screen identifies α/β hydrolase domain containing 4 (ABHD4) as a novel regulator of anoikis resistance. *Apoptosis* 17: 666-678, 2012.
74. Larsson LG: Oncogene- and tumor suppressor gene-mediated suppression of cellular senescence. *Semin Cancer Biol* 21: 367-376, 2011.
75. Gorgoulis VG and Halazonetis TD: Oncogene-induced senescence: The bright and dark side of the response. *Curr Opin Cell Biol* 22: 816-827, 2010.
76. Faget DV, Ren Q and Stewart SA: Unmasking senescence: Context-dependent effects of SASP in cancer. *Nat Rev Cancer* 19: 439-453, 2019.
77. Serrano M, Lin AW, McCurrach ME, Beach D and Lowe SW: Oncogenic ras provokes premature cell senescence associated with accumulation of p53 and p16INK4a. *Cell* 88: 593-602, 1997.
78. Michaloglou C, Vredeveld LC, Soengas MS, Denoyelle C, Kuilman T, van der Horst CM, Majoor DM, Shay JW, Mooi WJ and Peeper DS: BRAF^{V600E}-associated senescence-like cell cycle arrest of human naevi. *Nature* 436: 720-724, 2005.
79. Gray-Schopfer VC, Cheong SC, Chong H, Chow J, Moss T, Abdel-Malek ZA, Marais R, Wynford-Thomas D and Bennett DC: Cellular senescence in naevi and immortalisation in melanoma: A role for p16? *Br J Cancer* 95: 496-505, 2006.
80. He S and Sharpless NE: Senescence in health and disease. *Cell* 169: 1000-1011, 2017.
81. Vicent S, Chen R, Sayles LC, Lin C, Walker RG, Gillespie AK, Subramanian A, Hinkle G, Yang X, Saif S, *et al*: Wilms tumor 1 (WT1) regulates KRAS-driven oncogenesis and senescence in mouse and human models. *J Clin Invest* 120: 3940-3952, 2010.
82. Kaplon J, Hömig-Hölzel C, Gao L, Meissl K, Verdegaal EM, van der Burg SH, van Doorn R and Peeper DS: Near-genomewide RNAi screening for regulators of BRAF(V600E)-induced senescence identifies RASEF, a gene epigenetically silenced in melanoma. *Pigment Cell Melanoma Res* 27: 640-652, 2014.
83. Tordella L, Khan S, Hohmeyer A, Banito A, Klotz S, Raguz S, Martin N, Dhamarlingam G, Carroll T, González Meljem JM, *et al*: SWI/SNF regulates a transcriptional program that induces senescence to prevent liver cancer. *Genes Dev* 30: 2187-2198, 2016.
84. Battle E and Clevers H: Cancer stem cells revisited. *Nat Med* 23: 1124-1134, 2017.
85. Nassar D and Blanpain C: Cancer stem cells: Basic concepts and therapeutic implications. *Annu Rev Pathol* 11: 47-76, 2016.
86. Wolf J, Dewi DL, Fredebohm J, Müller-Decker K, Flechtenmacher C, Hoheisel JD and Boettcher M: A mammosphere formation RNAi screen reveals that ATG4A promotes a breast cancer stem-like phenotype. *Breast Cancer Res* 15: R109, 2013.
87. Hanahan D and Weinberg RA: Hallmarks of cancer: The next generation. *Cell* 144: 646-674, 2011.
88. Paulsen RD, Soni DV, Wollman R, Hahn AT, Yee MC, Guan A, Hesley JA, Miller SC, Cromwell EF, Solow-Cordero DE, *et al*: A genome-wide siRNA screen reveals diverse cellular processes and pathways that mediate genome stability. *Mol Cell* 35: 228-239, 2009.
89. Gargiulo G, Serresi M, Cesaroni M, Hulsman D and van Lohuizen M: In vivo shRNA screens in solid tumors. *Nat Protoc* 9: 2880-2902, 2014.
90. Singh M, Venugopal C, Tokar T, Brown KR, McFarlane N, Bakhshinyan D, Vijayakumar T, Manoranjan B, Mahendram S, Vora P, *et al*: RNAi screen identifies essential regulators of human brain metastasis-initiating cells. *Acta Neuropathol* 134: 923-940, 2017.
91. Lin L, Chamberlain L, Pak ML, Nagarajan A, Gupta R, Zhu LJ, Wright CM, Fong KM, Wajapeyee N, Green MR: A large-scale RNAi-based mouse tumorigenesis screen identifies new lung cancer tumor suppressors that repress FGFR signaling. *Cancer Discov* 4: 1168-1181, 2014.
92. Iorns E, Ward TM, Dean S, Jegg A, Thomas D, Murugaesu N, Sims D, Mitsopoulos C, Fenwick K, Kozarewa I, *et al*: Whole genome in vivo RNAi screening identifies the leukemia inhibitory factor receptor as a novel breast tumor suppressor. *Breast Cancer Res Treat* 135: 79-91, 2012.
93. Elbashir SM, Harborth J, Lendeckel W, Yalcin A, Weber K and Tuschl T: Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. *Nature* 411: 494-498, 2001.
94. Wang H, La Russa M and Qi LS: CRISPR/Cas9 in genome editing and beyond. *Annu Rev Biochem* 85: 227-264, 2016.
95. Nyga A, Cheema U and Loizidou M: 3D tumour models: Novel in vitro approaches to cancer studies. *J Cell Commun Signal* 5: 239-248, 2011.
96. Hidalgo M, Amant F, Biankin AV, Budinská E, Byrne AT, Caldas C, Clarke RB, de Jong S, Jonkers J, Mælandsmo GM, *et al*: Patient-derived xenograft models: An emerging platform for translational cancer research. *Cancer Discov* 4: 998-1013, 2014.
97. Bartz SR, Zhang Z, Burchard J, Imakura M, Martin M, Palmieri A, Needham R, Guo J, Gordon M, Chung N, *et al*: Small interfering RNA screens reveal enhanced cisplatin cytotoxicity in tumor cells having both BRCA network and TP53 disruptions. *Mol Cell Biol* 26: 9377-9386, 2006.
98. Lam LT, Davis RE, Ngo VN, Lenz G, Wright G, Xu W, Zhao H, Yu X, Dang L and Staudt LM: Compensatory IKK α activation of classical NF- κ B signaling during IKK β inhibition identified by an RNA interference sensitization screen. *Proc Natl Acad Sci USA* 105: 20798-20803, 2008.
99. Xu Y, Karlsson A and Johansson M: Identification of genes associated to 2',2'-difluorodeoxycytidine resistance in HeLa cells with a lentiviral short-hairpin RNA library. *Biochem Pharmacol* 82: 210-215, 2011.
100. Guerreiro AS, Fattet S, Kulesza DW, Atamer A, Elsing AN, Shalaby T, Jackson SP, Schoenwaelder SM, Grotzer MA, Delattre O and Arcaro A: A sensitized RNA interference screen identifies a novel role for the PI3K p110 γ isoform in medulloblastoma cell proliferation and chemoresistance. *Mol Cancer Res* 9: 925-935, 2011.

101. Liu-Sullivan N, Zhang J, Bakleh A, Marchica J, Li J, Siolas D, Laquerre S, Degenhardt YY, Wooster R, Chang K, *et al*: Pooled shRNA screen for sensitizers to inhibition of the mitotic regulator polo-like kinase (PLK1). *Oncotarget* 2: 1254-1264, 2011.
102. Fredebohm J, Wolf J, Hoheisel JD and Boettcher M: Depletion of RAD17 sensitizes pancreatic cancer cells to gemcitabine. *J Cell Sci* 126: 3380-3389, 2013.
103. Milosevic N, Kühnemuth B, Mühlberg L, Ripka S, Griesmann H, Lölkes C, Buchholz M, Aust D, Pilarsky C, Krug S, *et al*: Synthetic lethality screen identifies RPS6KA2 as modifier of epidermal growth factor receptor activity in pancreatic cancer. *Neoplasia* 15: 1354-1362, 2013.
104. Wetterskog D, Shiu KK, Chong I, Meijer T, Mackay A, Lambros M, Cunningham D, Reis-Filho JS, Lord CJ and Ashworth A: Identification of novel determinants of resistance to lapatinib in ERBB2-amplified cancers. *Oncogene* 33: 966-976, 2014.
105. MacKay C, Carroll E, Ibrahim AFM, Garg A, Inman GJ, Hay RT and Alpi AF: E3 ubiquitin ligase HOIP attenuates apoptotic cell death induced by cisplatin. *Cancer Res* 74: 2246-2257, 2014.
106. Maruyama Y, Miyazaki T, Ikeda K, Okumura T, Sato W, Horie-Inoue K, Okamoto K, Takeda S and Inoue S: Short hairpin RNA library-based functional screening identified ribosomal protein L31 that modulates prostate cancer cell growth via p53 pathway. *PLoS One* 9: e108743, 2014.
107. Sudo M, Mori S, Madan V, Yang H, Leong G and Koeffler HP: Short-hairpin RNA library: Identification of therapeutic partners for gefitinib-resistant non-small cell lung cancer. *Oncotarget* 6: 814-824, 2015.
108. Prahallad A, Heynen GJ, Germano G, Willems SM, Evers B, Vecchione L, Gambino V, Lieftink C, Beijersbergen RL, Di Nicolantonio F, *et al*: PTPN11 is a central node in intrinsic and acquired resistance to targeted cancer drugs. *Cell Rep* 12: 1978-1985, 2015.
109. Kobayashi H, Nishimura H, Matsumoto K and Yoshida M: Identification of the determinants of 2-deoxyglucose sensitivity in cancer cells by shRNA library screening. *Biochem Biophys Res Commun* 467: 121-127, 2015.
110. Yamaguchi K, Iglesias-Bartolomé R, Wang Z, Callejas-Valera JL, Amornphimoltham P, Molinolo AA, Cohen EE, Califano JA, Lippman SM, Luo J and Gutkind JS: A synthetic-lethality RNAi screen reveals an ERK-mTOR co-targeting pro-apoptotic switch in PIK3CA+ oral cancers. *Oncotarget* 7: 10696-10709, 2016.
111. Yamanoi K, Matsumura N, Murphy SK, Baba T, Abiko K, Hamanishi J, Yamaguchi K, Koshiyama M, Konishi I and Mandai M: Suppression of ABHD2, identified through a functional genomics screen, causes anoikis resistance, chemoresistance and poor prognosis in ovarian cancer. *Oncotarget* 7: 47620-47636, 2016.
112. Combes E, Andrade AF, Tosi D, Michaud HA, Coquel F, Garambois V, Desigaud D, Jarlier M, Coquelle A, Pasero P, *et al*: Inhibition of ataxia-telangiectasia mutated and RAD3-related (ATR) overcomes oxaliplatin resistance and promotes antitumor immunity in colorectal cancer. *Cancer Res* 79: 2933-2946, 2019.



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