Methods 131 (2017) 66–73

Contents lists available at ScienceDirect

Methods

journal homepage: www.elsevier.com/locate/ymeth

Identifying synthetic lethal targets using CRISPR/Cas9 system

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ARTICLE INFO

Article history:
Received 29 April 2017
Received in revised form 6 July 2017
Accepted 8 July 2017
Available online 12 July 2017

Keywords:
Synthetic lethality
CRISPR/Cas9
Knockout library
Arrayed screen
Pooled screen

ABSTRACT

Synthetic lethality occurs when co-occurrence of two genetic events is unfavorable for the survival of the cell or organism. The conventional approach of high throughput screening of synthetic lethal targets using chemical compounds has been replaced by RNAi technology. CRISPR/Cas9, an RNA guided endonuclease system is the most recent technology for this work. Here, we have discussed the major considerations involved in designing a CRISPR/Cas9 based screening experiment for identification of synthetic lethal targets. It mainly includes CRISPR library to be used, cell types for conducting the experiment, the most appropriate screening strategy and ways of selecting the desired phenotypes from the complete cell population. The complete knockdown of genes can be achieved using CRISPR/Cas9 knockout libraries. For higher quality loss-of-function screens, haploid cells with defective homology-directed DNA repair mechanism could be used. Two widely used screening formats include arrayed and pooled screens followed by negative or positive selection of the cells with desired phenotype. However, pooled screening format with negative selection of cells serves the best. The advantages of using CRISPR/Cas9 system over the other RNAi approaches have also been discussed. Finally, some studies using CRISPR/Cas9 for genome-wide knockout screening in human cells and computational approaches for identification of synthetic lethal interactions have been discussed.

1. Introduction

The term ‘synthetic lethality’ is used to define genetic interactions in which co-existence of two genetic events leads to the death of the cell or the organism. This concept was first described by an American geneticist Calvin Bridges [1]. He observed that certain combinations of non-allelic genes in *Drosophila melanogaster* were lethal for the fruit flies, though individual occurrence had no impact on their survival. The term ‘synthetic lethality’ was however coined much later by Theodore Dobzhansky [2]. Synthetic sickness, another phenomenon where such genetic combinations do not kill the cells but impair the growth, is also sometimes grouped together with synthetic lethality [3].

The best studied examples of combinations of genetic perturbations leading to synthetic lethality arise due to loss-of-function mutants. To maintain genetic robustness, cells generally have redundant or back up pathways. So the impaired function of one of the genes can be compensated by the product of another gene with similar function. But simultaneous mutations in these co-essential genes will result in lethality [4]. The loss of function of the gene can be achieved by either chemical or genetic means (Fig. 1). Genetic means generally include functional interference using RNAi, mutation or deletion in the DNA sequence, perturbations of upstream regulators, or changes induced by environmental factors. The function of the gene may also be affected using chemical compounds at DNA or protein level. The mode of inhibition for both the genes forming the synthetic lethal pair may be different or the same. For example, one gene can be inactive due to deletion in the coding region while the inhibition of other can be due to the action of a chemical compound [4]. Mutations in genes leading to gain of function has also been attributed to the occurrence of synthetic lethal interactions [5,6].

One of the momentous clinic implications in synthetic lethal targeting is the use of Poly (ADP-ribose) polymerase (*PARP*) inhibitors for the treatment of breast and ovarian cancers with characteristic mutations in *BRCA*1 and *BRCA2* genes. *BRCA*1 and *BRCA*2 are involved in repair of DNA double strand break by the mechanism of homologous recombination. In two independent studies, this information was combined with the fact that loss-of-function of *PARP* leads to introduction of single strand breaks at replication fork [7,8]. Thus, it was found that tumors lacking functional *BRCA* genes were unable to repair breaks in DNA strand induced extrinsically using chemical or genetic means of inhibition of *PARP*. This

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http://dx.doi.org/10.1016/j.ymeth.2017.07.007
1046-2023/

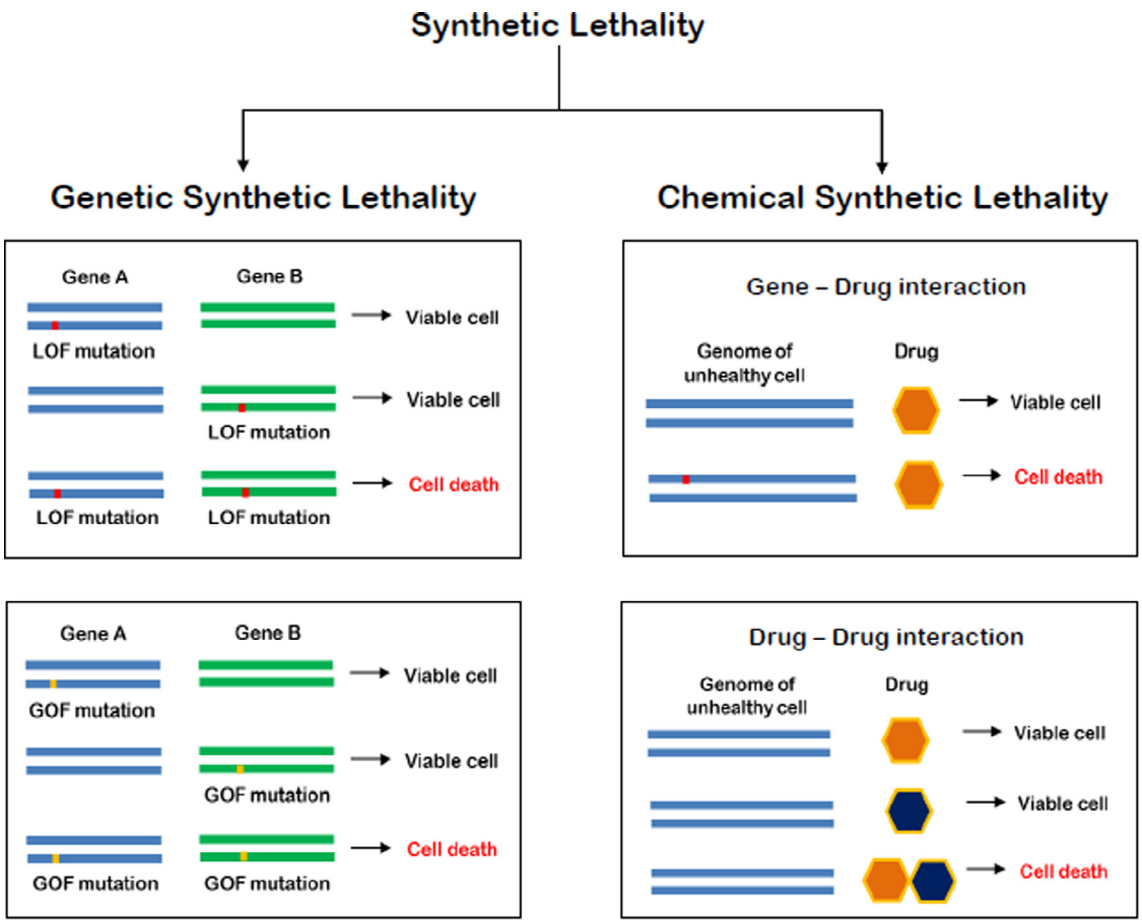

Fig. 1. Genetic and chemical means of synthetic lethality. In genetic synthetic lethality, simultaneous mutational events in two different genes resulting in either gain of function or loss of function prove out to be lethal for the cell. Chemical synthetic lethality involves gene-drug or drug-drug interaction. In some cases, single nucleotide change in the DNA sequence can make cells susceptible to the cytotoxic action of a drug. Also a combination of drugs can be synthetic lethal for the cells.

synthetic lethal pair was tested *in vivo*, which further got extended to clinical trials [9–11].

Drugs also display synthetic lethal interactions with genes. Therefore, another approach is to combine the effect of chemical drugs and dysfunctional gene to selectively kill a group of cells. For instance, cisplatin is still one of the most widely accepted chemotherapeutic agents despite having side effects. It belongs to the family of platinum-based anti-neoplastic drugs. Chemotherapy with such a family of drugs has been commonly associated with neurological complications. Furthermore, owing to the resistance of cells to such platinum-based chemical compounds, attempts are being made to uncover more combinatorial-targeted therapies. It has been found that cells with mutated *BRCA* genes are hypersensitive to cisplatin [12]. *AMBRA* and *PRKAB*1 are among the other potential target genes that can make cancer cells susceptible to cisplatin treatment [13,14].

Attempts are being made to explore synthetic lethal interactions to get insights into functional relationships between genes. These interactions can further be used for studying various cellular processes. Synthetic lethal targeting has gained the attention of cancer biologists since it has the potential to open new ways for designing cancer therapy and to explain the selective effect of drugs on certain types of cancer.

2. Strategies for the identification of synthetic lethal targets

Until recently, the high throughput method for screening synthetic lethal targets involved the use of a library of chemical compounds to treat a cell line comprising of genetic alteration under study. However, with the advancement in RNA interference technology, various screening approaches have been developed to understand gene-gene synthetic lethal interactions at human genome level. All these strategies are discussed below:

2.1. Chemical library screening

Chemical libraries are classified into two categories as (i) non-annotated and (ii) annotated chemical libraries. In non-annotated libraries, the molecular targets of the compounds are already known. Cells with known genetic perturbation are cultured in multi-well plate. Each compound from the library is used to treat the cells lining a different well. Cell viability assay is carried out to obtain the potential 'hit'. Thus this 'hit' is a compound that shows synthetic lethal interaction with the genetic mutation harbored by the cell line used and thus leads to the death of the cells. The disadvantage of using non-annotated chemical screens is the difficulty in obtaining the information about the molecular target of the hit. In case of annotated chemical library, identification of hits requires an additional screening process. If the identified hit has been previously explored as potential drug target, further RNAi is carried out to confirm whether the hit is acting 'on target'. Despite being more informative, the annotated chemical library is comparatively smaller in size, which is often considered as disadvantageous over non-annotated chemical library. For instance, to inhibit the fanconi anemia pathway, a chemical library consisting of 16,000 compounds was screened for re-sensitizing cancer cells to DNA damaging agents like cisplatin [15].

2.2. siRNA based screening

Small interfering RNAs enable genome-wide investigation of specific mutations. siRNAs have remarkable ability to silence specific genes. This can be used in harnessing drugs that interfere with disease-causing or disease-promoting genes. Synthetic lethal target screening using siRNAs is also based on plate format where different siRNAs are transfected in separate wells. Cell Titer-Glo luminescent cell viability assay from Promega is commonly used to evaluate the cell viability and identify hits [4]. In a study, siRNA library targeting 5000 human genes was used to explore genetic mutations in glioblastoma cells susceptible to tumor necrosis factor-related apoptosis-inducing ligand (TRAIL). TRAIL is an active factor that participates in the death receptor-mediated apoptosis pathway in normal cells but is found to be inactivated in many types of cancerous cells. The cells in which siRNAs silenced the expression of *FAT*1 gene showed decreased survival in response to TRAIL [16]. Thus *FAT*1 was found to be synthetic lethal with TRAIL treatment.

2.3. shRNA based screening

Short hairpin RNAs (shRNAs) are synthesized within the cell by DNA vector-mediated production and can be introduced into cells via plasmid transfection or viral transduction. shRNA vector libraries are used to identify genes associated with similar phenotype, to identify genes required in a variety of processes such as cancer cell survival and proliferation, tumor suppressor pathway components. There are several strategies for shRNA library screening. Single cell-based arrays have certain limitations and are used to identify shRNA vectors which induce phenotypes that can be measured in well-based or individual cell-based readouts [17]. In polyclonal screens, entire library of shRNA vectors is infected in cells in a pooled format. The cells are then subjected to conditions such as exposure to cytostatic drug, induced cell growth arrest or apoptosis. This method has proven effective in the isolation of several genes that encode proteins in cancer-relevant pathways such as the *p*53 pathway and RAS-dependent transformation [18,19]. Another reliable method is bar-code screening. In this method, selective enrichment of certain shRNAs is performed due to which there is random distribution of shRNA vectors in the population. The active shRNA vectors are enriched to a much higher degree because of their specific effect on cellular phenotype under the conditions of the screen. This results in increase of selective shRNA vectors in the population. Microarray hybridization is used to measure the abundance. For instance, this approach was used to perform shRNA library screening on cancer cell lines such as pancreas, ovarian, colon and others to investigate synthetic lethality with *CCNE*1. The data was retrieved from a microarray shRNA experiment. The shRNA library consisted of a pool of 54,020 shRNAs targeting 11,194 genes. The screen identified 835 genes and 25 confidential hits. This result was further validated by siRNA screen. *BRCA*1 and *CDK*2 were among the best hits including genes involved in DNA damage repair and homologous recombination. Furthermore chemical library screening was also included and Bortezomib was found to be synthetically lethal to *CCNE*1 amplification in multiple ovarian cell line. Hence, it was selected to target fanconi anemia pathway and interrupt homologous recombination [20].

2.4. CRISPR/Cas9 based screening

CRISPR/Cas9 system refers to a target specific single-guide RNA (sgRNA) that is used in conjugation with an RNA-guided endonuclease like Cas9 [21,22]. CRISPR stands for clustered regularly interspaced short palindromic repeats. The sgRNA sequence guides the non-specific cleavage domain to a specific location within the genome, where the endonuclease introduces a double-strand break in the DNA. The targeted location is generally a stretch of 17–20 nucleotides complementary to the sequence of sgRNA. Presence of protospacer adjacent motif (PAM) just adjacent to the target site is a prerequisite for the cleavage activity of Cas9 endonuclease. The double strand break introduced by Cas9 gets repaired endogenously through either non-homologous end joining (NHEJ) or homology-directed repair (HDR). NHEJ is more active than HDR and hence observed more often resulting in frame shift indels that disrupt the function of the gene [23]. Similar to the other RNA interference approaches, CRISPR/Cas9 system can also be applied for the identification of the synthetic lethal targets.

CRISPR/cas9 system offers certain advantages over the other RNAi methods of identification of synthetic lethal targets [24]:

i. Endogenous pathway of RNAi can target only mRNA molecules for silencing based on the sequence complementarity. However, CRISPR/Cas9 system can theoretically be used for targeting any specific location within the complete genome of any organism.
ii. The success of screens using RNAi often gets limited due to incomplete knockdown of the target gene. On the other hand, CRISPR with the help of Cas9 permanently disrupts the function of the gene by introducing frame shift mutations in the coding sequence.
iii. Extensive off-target activity due to RNAi molecules in comparison to CRISPR/Cas9 system also makes it difficult to study the phenotypic changes to be observed as the result of the screen.

A brief overview of various strategies that are available for identification of synthetic lethal targets, along with their advantages and limitations is given in Table 1.

3. CRISPR/Cas9 based screening strategies

Some of the major considerations while designing the CRISPR/Cas9 screening for identification of synthetic lethal targets include

Table 1
Overview of the strategies being used for the identification of synthetic lethal targets.

Features	RNAi	CRISPR/Cas9
Mechanism of action	• Gene silencing occurs at the level of mRNA or non-coding RNA • Utilizes endogenous mammalian miRNA machinery for gene silencing	• Modification occurs at DNA level • Uses Cas9 endonuclease for gene disruption • CRISPR can be fused to other functional domains for other regulatory functions
Site of action	Cytoplasm	Nucleus
Knock-in/knock-out	Knock-out	Knock-in and knock-out
Duration of effect	Short term effect (siRNA) to long term effect (shRNA), with incomplete knockdown	Permanent modification due to change in DNA; also allows heritable changes
Possible target sites	Can be used only to target the transcriptome	Any site adjacent to PAM region can be targeted in the genome

the choice of i) type of CRISPR library to use, ii) cell type for performing the experiment, iii) the most appropriate screening strategy, and iv) the selection of hits from the screen.

3.1. CRISPR/Cas9 libraries for synthetic lethality screening

For the screening of synthetic lethality, one requires the complete knockdown of the expression of the gene. CRISPR/Cas9 knockout libraries are thus the best option for carrying out such loss-of-function screens. The sgRNAs are designed to target in general the early constitutively expressed exons or regions coding for functional protein domains for all known genes for the organism under study [25]. The Cas9 nuclease from *Streptococcus pyogenes* is further used to introduce a double strand break within the targeted site. The result of this molecular manipulation is either a non-functional or dominant negative gene product. The most updated versions of commercially available sgRNA knockout libraries for humans have been reported to target over 18,000 genes in the whole genome [25]. The most widely used knockout libraries are the ones generated by Zhang lab and Sabatini/Lander lab at MIT [26–28]. The design of sgRNA for targeting the genes is the most crucial step. Though designed for a unique site, CRISPR has the ability to interact with other locations differing from the intended site by a few bases. Many studies have been carried out to investigate the parameters that govern this degenerate binding of CRISPRs [29–32]. To further help the experimentalists to choose the target sites, many computational tools are available [33–36]. These tools take into consideration the features like number of mismatches and buldges tolerated by CRISPR, positional preference of the bases, acceptable mutations in PAM site, number of possible off-targets, and suggest the most optimal target sites within each gene. So, second generation of knockout libraries has also been developed that utilize the sgRNAs that have utmost one off-target. They make use of design rules to get maximum cleavage efficiency with minimal effect at unintended locations. There are more focused libraries like the one that can specifically target genes with related functions, like kinases or cell cycle proteins. These kinds of libraries prove beneficial when exploring a narrow hypothesis [25].

The other kind of library, not so common, for this work includes repression or CRISPRi library. The sgRNAs used in these libraries either contain an inactivate Cas9 (dCas9) to block the RNA polymerase binding site or dCas9 conjugated to a repressive effector domain (like *KRAB*) to repress the transcription of the targeted gene. One such CRISPRi library, developed by Weissman lab, consists of sgRNAs targeting around 16,000 human genes [37]. The targeted gene is transcriptionally silenced using a chimeric protein dCas9-*KRAB*. However, the effect of CRISPRi is not a permanent change in the DNA sequence. As the level of transcriptional repression can vary depending upon the experimental conditions, CRISPRi may not be as efficient as gene knockout approach.

3.2. The choice of cell type

For studying synthetic lethality, the cell line used will already be lacking a functional gene. All possible gene knockout cells are made to check for the combination that can lead to cell death.

The efficiency of genetic manipulation carried out using the desired CRISPR library, to a large extent, depends upon the number of target genetic locations to be altered. Most of the human cells are diploid in nature, carrying two copies of a gene. Also cancer cell lines may have aberrant number of chromosomes or multiple copies of the same gene due to genetic instability. Thus, it is difficult to ascertain if all the loci gets disrupted by the complementary sgRNAs. Therefore, higher quality loss-of-function screens can better be carried out in haploid cell lines [25].

Another point of consideration is DNA repair mechanism used by a specific cell line. As Cas9 used in knockout libraries leads to a double strand break at the site targeted by sgRNA, the natural mechanism of cell calls out for the DNA repair mechanism. There exist two pathways for this damage repair-Homology-directed repair (HDR) and Non-homologous end pair joining (NHEJ). HDR using the sister chromatid can precisely repair the fault thereby reversing the knockdown effect of sgRNA. NHEJ tries to re-ligate the broken ends, but leads to insertion or deletion of small DNA fragments during the process. These incorporated indels cause shift in the reading frames or lead to premature codons, resulting in a non-functional gene product. Gene knockout thus depends on the NHEJ process. So while using the cell types, which harbor both HDR and NHEJ process of DNA repair, the probability of complete knockout at every locus is much less. Therefore, transducing cells lines with defective HDR mechanism can yield better hits during a loss-of-function screen using CRISPR knockout libraries [25].

Finally, the efficiency of transduction of a cell line is also an important factor. The cell line should be susceptible to one or multiple lentiviruses, which are generally used for transferring sgRNA into the pool of cells. Multiplicity of infection (MOI) is a parameter that helps in estimating the number of virion particles that will be added per cell during the infection. Alternatively, MOI can be defined as the ratio of number of virus particles to the number of target cells in a defined space. In general, MOI ranging from 0.4 to 0.6 transduction units/cell is used for lentiviral transduction of sgRNA libraries to ensure that every cell contains a single sgRNA [26,27].

3.3. Screening strategy

Two different screening formats are widely used, namely arrayed screen and pooled screen (Fig. 2). In arrayed screen, a different sgRNA with the required pool of reagents is added to each well of the multi-well plate. As the target for the sgRNA in each well is already known, it can easily be correlated to the observed cellular phenotype [23]. On the contrary, in pooled screening format, the complete sgRNA library is synthesized, cloned and transfected altogether into a pool of cells. Various genetic perturbations get introduced into the cells. The cells are then separated based on the observed phenotype; the genetic change is investigated and linked to the change in the cell morphology, chemistry or cell viability [23]. So, there are two ways in which cells can be separated after the screening: positive selection and negative selection. Positive selection works when genetic manipulation is in the favor of the cell and allows it to survive under the created selective pressure. In the negative selection, focus is on the cells that deplete from the pool of cells over the treatment period. This can be attributed to the genetic perturbations affecting the working of the genes essential for cell survival [23].

For study of synthetic lethal targets, pooled screening format with negative selection of cells seems to be the most suited strategy. Typically, cell pellets are collected from the reaction mixture at different points throughout the course of screen. The number of cells should be sufficient enough to represent the diverse cell population. It should also be able to take care of the under-represented sgRNAs, responsible for the cell depletion. DNA is extracted using the standard protocol. The sgRNAs present in the DNA sample are amplified using PCR primers designed specifically for the portion of lentiviral backbone containing sgRNA. Next generation sequencing is then used to sequence the sgRNA available in the pool. Subsequently, the sequences obtained are mapped to the

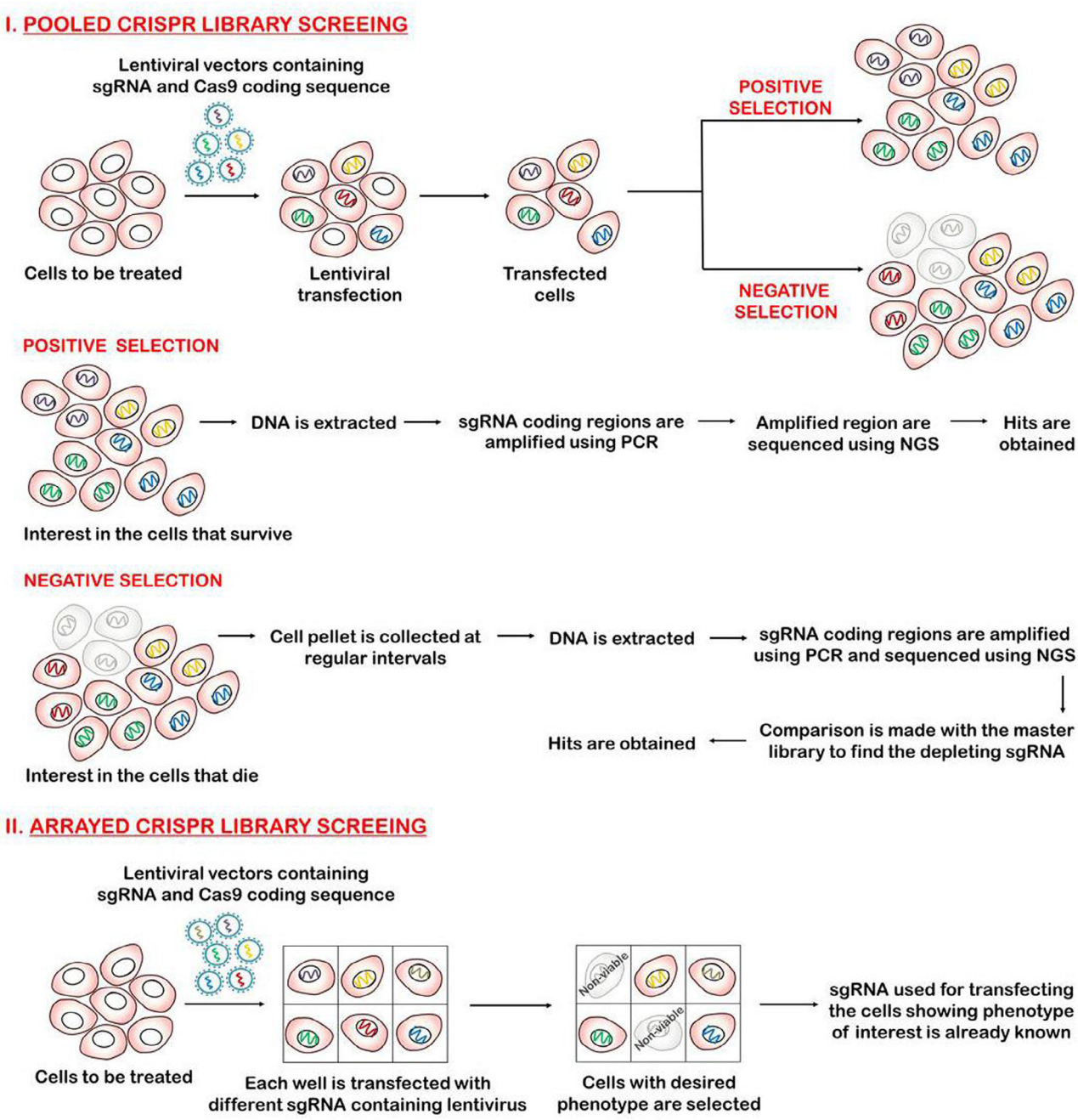

Fig. 2. Schematic representation of strategies used for CRISPR/Cas based genomic screening. There are two main screening formats: I. Pooled library screening- All the sgRNAs comprising a library are synthesized, cloned and transfected to get a pooled population of cells. The transformed cells with desired phenotypic change can be separated from the entire pooled population using either positive or negative selection (based on the objective of the experiment). The genetic cause underlying the phenotypic alteration is then investigated and linked to the observed outcome. II. Arrayed library screen-Multiple reactions for transformation of cells using different sgRNAs are carried out simultaneously, but each in a separate well of multi-well plate. As the target sgRNA for each well is already known, the observed phenotype can easily be associated with responsible genetic perturbation.

initial sgRNA library to read out sgRNA representation in the selected cell subpopulation. The under-represented sgRNAs thus are the synthetic lethal target candidates [25–27].

4. Applications of CRISPR-Cas9 based screening of synthetic lethal targets

CRISPR/Cas has become the system of interest in genome engineering after the successful genome editing using this system was reported in human cell cultures [38–40]. The potential of sgRNA-based genome screening technology was explored in a genome-wide knockout screening in human cells [41]. Genome-wide recessive genetic screening with a lentiviral CRISPR-gRNA library in human cells has also been carried out [42]. Attempts are being made to improve the packing capacity of lentiviral vectors and construct more specific and genome-wide sgRNA libraries for CRISPR/Cas based screening [43]. Another experiment employing the potential of CRISPR/Cas9 for genomic studies was to understand the differential genetic vulnerabilities in cancer cells [44]. All such

similar studies have paved the way for using CRISPR/Cas system for genome-wide exploration of synthetic lethal targets in human cancer cells.

A recent study investigating synthetic lethal interactions in cancer cells using genome wide CRISPR/Cas9 knockout screens has revealed potential targets for Glioblastoma (GBM) [45]. Scientists have screened a genome wide CRISPR/Cas9 library against patient-derived Glioblastoma stem-like cells (GSCs) and healthy neural stem/progenitors cells (NSCs) to identify therapeutic targets for GBM [45]. 18,080 genes have been targeted in two adult GSC isolates and two control NSC lines using a CRISPR/Cas9 library comprising 64,751 sgRNAs by lentiviral transduction. It was found that *PKMYT*1 and *WEE*1 are synthetic lethal in NSCs but not GSCs. Cells were transduced using a "shot gun" approach. sgRNA read counts were obtained by deep sequencing. A Bioconductor software package, called edgeR that analyses differential expression of replicated count data, was used to identify significantly scoring sgRNAs. Bayesian classifier was employed to determine functional genetic screening quality. It was found that out of 946 GSC specific hits, only 10 overlapped core pathways altered in Glioblastoma. These results were compared with previously performed shRNA screens in GSCs [46] and there was an agreement between GSC sensitive hits from both methods. Further sgRNA hits were targeted in ten different GSC isolates. The results showed that eight of these isolates required *PKMYT*1 for their viability. Hence this gene was selected as a candidate therapeutic target for further validation. *PKMYT*1 codes for a dual specificity protein kinase homologous to *WEE*1. On examination of the effects of *PKMYT*1 and *WEE1* inhibition, it was found that these proteins act redundantly to phosphorylate *CDK*1-Y15 in NSCs. It was found that in NSCs, knockout of either *PKMYT*1 or *WEE*1 led to modest increases in mitotic transit time (MTT), whereas knockout of both led to drastic increase in MTT which led to cell death. MTT here was the estimate of time for completion of one mitotic cycle, starting from the breakdown of nuclear envelope to the end of cytokinesis. But in GSCs, *PKMYT*1 knockout or *WEE*1 knockout alone led to cell death. This shows that *PKMYT*1 and *WEE*1 act redundantly in NSCs whereas this redundancy in absent in GSCs. Hence, this study suggested *PKMYT*1 and *WEE*1 as potential therapeutic targets for Glioblastoma.

Another study used siRNA based screening in conjunction with CRISPR-based knockdown experiment to identify atypical cadherin *FAT*1 for synthetic lethal interactions with death receptor-mediated apoptosis. A genome wide pooled siRNA library was used in human glioblastoma cell line U251MG to search for proteins that can sensitize the cells for death receptor-induced apoptosis. The aim was to find the siRNAs that were synthetic lethal with TRAIL treatment but had no effect on viability in absence of this extrinsic factor. It was observed that silencing of *FAT*1 was resulting in increased apoptosis in the presence of TRAIL. It was further investigated that *FAT*1 knockdown and TRAIL stimulation together upheld the recruitment of procaspase-8 to the death-inducing signaling complex leading to the formation of molecular complexes containing caspase-8. This helped in uncovering the mechanism controlling caspase activation and thus death receptor-mediated apoptosis under physiological conditions. The results were further validated using *FAT1* mutant cell lines generated by employing CRISPR/Cas9-mediated genome engineering. Here, CRISPR/Cas9 system was not used to identify the synthetic lethal targets, but to validate the results obtained using RNAi screening. However, it is believed that the approach of CRISPR-mediated mutagenesis could alone have been sufficient to study and confirm knockdown phenotypes without using the RNAi screen for selection of synthetic lethal target candidates [16].

Being a new player in the field, there are not many reports to discuss here; however, the potential of this revolutionary genome editing tool for synthetic lethal target identification can be well appreciated with the above examples.

5. Computational approaches for prediction of synthetic lethal partners

With the advancement in RNAi screening technology, a plethora of literature is available on synthetic lethal partners. The collection and organization of this data for clinical application thus became a major challenge. The first attempt towards integrating this data was made with the development of GenomeRNAi database. It was first created in 2007 by manually curating the RNAi screening data from literature [47,48]. The updated version of GenomeRNAi comprises of 127 and 170 RNAi screens performed in humans and *Drosophila* respectively. This database also provides the information related to the reagents used for carrying out the screening experiments, which includes details of sequence, primer and RNAi reagent library. Further, it was also a challenge to follow a standard format for representation of data. Using the guidelines laid out by 'Minimum Information About an RNAi Experiment' effort, the developers of the tool have now come up with a common template to be used for the submission of new data to be incorporated into the database [49].

With further increase in the synthetic lethality related data, a more comprehensive dataset, called SynLethDB was proposed [50]. A wide range of resources has been used to integrate data into this database. The first source included the research papers focusing on identification of synthetic lethal pairs of genes using biochemical experiments. This section also included the synthetic lethal gene pairs identified using various high-throughput RNAi screening experiments or their combination with drug screening. The second source involved the assimilation of genetic interactions already annotated as synthetic lethal in BioGRID [51] and GenomeRNAi database [49]. The third source was the computational predictions, mainly from DAISY [52]. To further ensure that all synthetic interactions scattered in literature have been covered, text-based mining tools like MedlineRanker [53] and PESCADOR [54] were employed. Finally, the publications in PubMed with 'synthetic lethality' or 'synthetic lethal' in title were manually curated for finding the gene pairs still not available in the database. In the current version of SynLethDB, there are 19,952 synthetic lethal interactions of humans, 366 of mouse, 423 of *Drosophila*, 107 of *C. elegans* and 13,421 of *S. cerevisiae*. For all the collected synthetic lethal pairs, supporting evidence and a confidence score depicting the reliability based on the source of evidence has also been provided. This database also gives an estimation of druggability of the reported gene pairs as potential drug targets. This reflects the efficiency with which cancer cell viability can be affected by targeting these genes with specific drugs. The prediction in this context is made using statistical analysis based on genomic data pertaining to a) copy number alterations, gene expression profiles and mutations retrieved from Catalogue of Somatic Mutations in Cancer database; b) drug-protein interactions collected from DrugBank [55], STITCH [56] and drug-kinase binding affinity profiles [57]; and c) drug sensitivity profiles of different cancer cell lines from CCLE [58], GDSC [59] and NCI-60 [60]. Using this data, the authors compiled the IC_{50} of 19,017 unique drugs in more than 1000 cancer cell lines. Furthermore, this database includes six functional modules, namely query, filtering, ranking, statistical analysis of drug-sensitivity, drug-SL partner interaction query and gene set enrichment analysis to help the users get a better insight into the enormous data [50].

Although these screens for identification of synthetic lethal partners are quite useful, they can mainly be employed for identification of synthetic lethal partners for one gene or one drug at a

time. Therefore, the next challenge was to design a more systematic approach for identification of synthetic lethal interactions in humans on a large scale. Computational prediction came up as a good alternative to complement the existing elaborate screening technology. The initial attempts were based on extrapolating the synthetic lethal partners found in yeast to their human orthologs [61]. An *in silico* tool called DAISY, Data mining synthetic lethality identification pipeline, was developed for exploring synthetic lethal interactions in humans, specifically for cancer [52]. DAISY works on three main principles- i) a pair of genes whose co-activation has not been observed in tumors or cancers can be assumed to be synthetically lethal, as their co-expression will be a survival disadvantage for the cells, ii) it incorporated the data mined from genome-wide shRNA screens carried out in various human tumor cell lines with known genome and transcriptome profiles, and iii) it exploits the observation that synthetic lethal genes are often functionally related. All the three aspects are firstly used separately to score the interaction of gene pairs using the knowledge gained from expression profiles, copy number data, and shRNA screening results [52,62]. The generated scores are then integrated to predict the potential synthetic lethal pair of genes. The tool also envisages the synthetic dosage lethal pairs, where hyper activity of one gene partner makes the other gene essential for the cell. These genetic interactions can prove beneficial in cases where over-expressed oncogenes like *KRAS* are difficult to be targeted directly and the other gene from the pair can be targeted for selectively killing the cancer cells. Probing these interactions is also based on the same principles as discussed earlier. These discovered synthetic dosage lethal interactions were further used to predict the response of different cancer cell types to anticancer drugs. The hypothesis underlying this prediction was that if a drug target has more number of overactive synthetic dosage lethal partners in a cancer cell line, the cancer type will be more responsive to that particular drug. To comment on the reliability of the tool, it was first made to predict some of the experimentally verified synthetic lethal interactions. Then the predictions made by DAISY for *VHL*, a tumor suppressor were tested *in vitro*. DAISY was further used to construct genome-wide synthetic lethal and synthetic dosage lethal interaction networks in cancer, predict gene essentiality in cancer cell lines and verify that counderexpression of synthetic lethal pairs can be used for better prognosis in breast cancer [52]. As this tool relies on sequencing and gene expression data, it has a wide scope of improving the accuracy and network coverage of its predictions with increase in patient and cell line data.

Soon another computational tool to predict synthetic lethal interactions in cancer was reported [63]. The rationale behind the working was that the genes that are altered in mutually exclusive fashion in cancer cells are likely to fall in the category of synthetic lethal partners. The authors have used the gene expression profile of 3980 patient samples (retrieved from The Cancer Genome Atlas) forming a pool of four types of cancers, namely breast, prostate, ovarian and uterine cancer. This data was combined with information on genomic copy number to identify 718 genes that can be probable synthetic lethal candidates for six important DNA-damage response (*DDR*) genes in these cancer types. To further validate the significance of these genes, the results obtained were compared against the data available on essential genes from ten *DDR*-deficient cancer cell lines. The identified genes were found enriched among the most essential genes in these cell lines suggesting that their knockout can result in cell death. Tousled-like kinase 2 (*TLK2*) and Ubiquitin-specific-processing protease 7 (*USP7*) are examples of such genes whose overexpression in DDR^{-} cell lines correlates with poor survival of cancer patients and hence can be targeted for cancer intervention in such cases.

These examples thus illustrate the support of computational databases and tools in organizing the enormous amount of existing data in the form that can be used by researchers for clinical applications and further predicting the genetic interactions; these are otherwise difficult to be explored using elaborate, expensive and time consuming experimental screenings.

6. Conclusion

CRISPR/Cas9 based high throughput screening of synthetic lethal targets is gaining the attention of scientist community for the ease and simplified use it offers. This system allows complete knockdown of the genes being targeted, a major limitation associated with other RNAi approaches. Highly efficient genome wide sgRNA knockout libraries are commercially available for human cells. Haploid cells carrying only a single copy of each gene are best suited for screening to ensure complete disruption of targeted locus. Cell lines with defect in HDR mechanism of DNA damage repair further enhance the quality of screening results. It has also been observed that pooled screening with negative selection of cells works best for studying synthetic lethal targets. CRISPR/Cas9 system thus offers an immense potential for exploring various synthetic lethal combinations for the designing of new interventions for combating cancer.

Acknowledgements

JKD acknowledges the award of Senior Research Fellowship (SRF) from Council of Scientific and Industrial Research (CSIR), Govt. of India.

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