Methods xxx (2017) xxx–xxx

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 xxxx

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 The conventional approach of high throughput screening of synthetic lethal targets using chemical compounds has been replaced by RNAi technology.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/

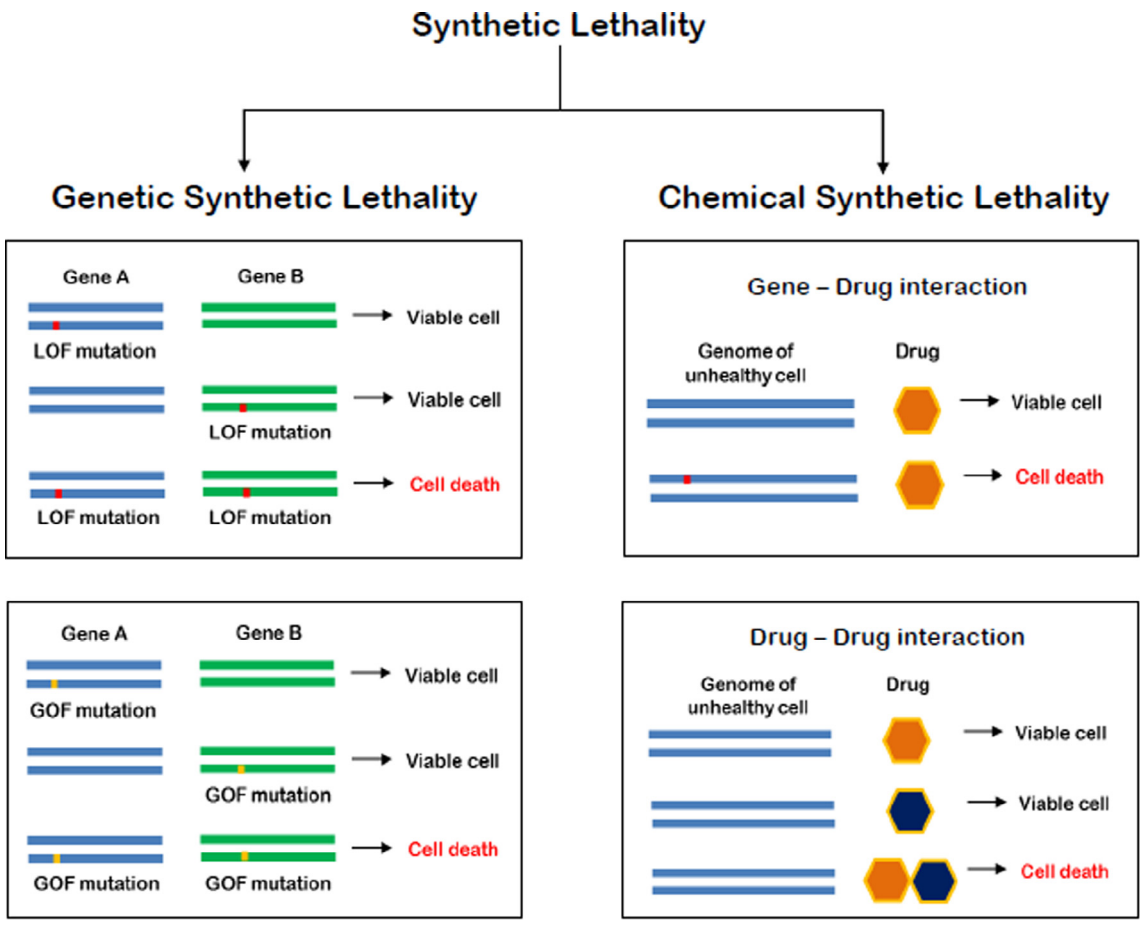

Fig. 1. Genetic and chemical means of synthetic lethality. In genetic synthetic lethality, simultaneous mutational events in two different genes resulting in either gain of function or loss of function prove out to be lethal for the cell. Chemical synthetic lethality involves gene-drug or drug-drug interaction. 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].