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Mini-review

Application of the concept synthetic lethality toward anticancer therapy: A promise fulfilled?

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ABSTRACT

Back in 1997, a suggestion to apply the concept of synthetic lethality towards the development of selective, less toxic, cancer drugs and anticancer targets, was brought forward. The availability of large scale synthetic, low-molecular-weight chemical libraries seemed to lend itself to the concept. Human/mouse genome-wide siRNAs and shRNA-expressing libraries allowing high throughput screening for target genes synergistic lethal with defined human cancer aberrations, also raised high hopes of a soon to be established selective therapy. Sixteen years later, the major experimental aspects relating to the implementation of this more focused approach to cancer drug discovery, is briefly and critically reviewed.

1. Introduction

1.1. The basics of synthetic lethality

Synthetic lethality, initially reported in bacteria [1] and yeast [2], describes a cellular condition in which two (or more) non-allelic and non-essential mutations, which are not lethal on their own, become deadly, when present simultaneously within the same cell. The synthetic lethal mutations may constitute partial mutations present together in a single linear essential pathway (Fig. 1a), reside in parallel pathways leading to the synthesis of a common essential gene product (Fig. 1b), or constitute independent parallel survival pathways, each serving as salvage pathway in the absence of the other (Fig. 1c). Other intermediate and quite common situations, in which two mutant genes may generate a "synthetic sickness" condition, is also known to exist [3]. The occurrences of "synthetic sickness" can be exacerbated, when combined with one or more additional non-essential mutation/s.

Although tentatively, the two mutant synthetic lethal genes are presumed to represent loss-of-function mutations, a condition where synthetic lethality between an over expressed 'gene of interest' and a mutant null gene, should also be taken into account. This scenario has been initially described in yeast and termed "synthetic dosage lethality" phenotype [4].

The Hartwell and Friend groups were first to suggest the usage of chemical and genetic synthetic lethality screening, for the development of cancer therapy [5]. However, realizing that the state of genetic manipulations in mammalian and human-cell systems, was unripe for genome-wide genetic synthetic lethality screening (a situation dramatically changed by the introduction of synthetic siRNAs in 2001), they suggested to perform such screenings in model genetic systems, such as *Saccharomyces cerevisiae* [2], the nematode *Caenorhabditis elegans* [6] and the *Drosophila melanogaster* fruit fly [7]. Extrapolation to human cancers was to follow.

On the face of it, implementation of the concept of synthetic lethality should lead to the development of selective less toxic cancer drugs and anticancer targets.

The purpose of this mini-review is to describe the experimental approaches which examine synthetic lethality relationships (chemical and genetic) in mammalian cells. Agents used in the study of these synthetic lethality relationships are then critically evaluated, in an attempt to boost the pace of future targeted therapy.

2. Experimental approaches for identification of synthetic lethality relationships: synthetic lethality screening in yeast, as a model system

In their search for cancer specific genetic changes which could form potential selective therapeutic targets, Hartwell and Friend relied on the fact that one of the hallmarks of cancer is genetic instability. This instability is primarily contributed by defects in DNA repair pathways, in cell cycle checkpoints and in other cell cycle controls. Because these cellular functions have been well conserved between yeast and humans, thus for the chemical screening, these investigators have used a panel of tens of isogenic yeast strains, each defective in either a DNA repair or cell cycle

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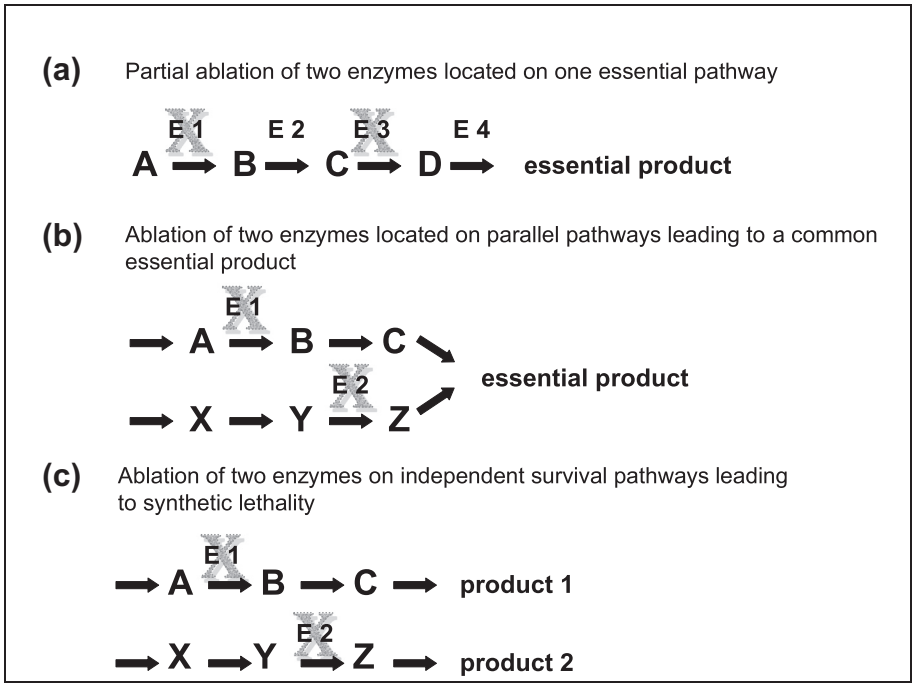

Fig. 1. Three modes of cell survival pathways amenable for analysis by the synthetic lethality screening approach. (a) Partial ablation of two enzymes located on one essential pathway. (b) Ablation of two enzymes located on parallel pathways leading to a common essential product. (c) Ablating two enzymes on independent survival pathways leading to synthetic lethality.

control gene/genes. The results of their systematic testing of the 33 most common FDA-approved anticancer cytotoxic drugs have been since reported [5,8], as well as a HTS of more than 85,000 compounds from the collection of the Developmental Therapeutics Program (DTP) branch of NCI [9]. The results of this large scale chemical screen (which was part of the Seattle project, based at the Fred Hutchinson Cancer Research Center) is available at the DTP website http://www.dtp.nci.nih.yacds/index.html under "NCI Yeast Anticancer Drug Screen".

Finalist drugs from the DTP collection screen, which resulted in yeast synthetic lethality phenotypes, were further tested on mammalian cells. Out of eight compounds analyzed, seven were toxic to mammalian cells; five were classified as topoisomerase I poisons and two as topoisomerase II inhibitors. This feasibility/proof of concept study demonstrated, in part, the pros and cons of using a model organism for identification of anticancer drugs, in humans.

A boost to the usage of synthetic lethality screens in yeast, has been achieved by integrating drug-sensitivity profiles, with large scale genetic interaction data, obtained through genome-wide genetic synthetic lethality screens performed by either SGA [10] or dSLAM [11,12]. The consolidated clustering of the two profiles has linked compounds to their protein targets and/or target pathways [13,14]. Extrapolation of gene product-interactions across species has been made easier by the establishment of BioGRID: Biological General Repository for Interaction Datasets [15,16], http://thebiogrid.org). This repository covers 45 organisms, currently encompassing close to 475,000 non-redundant physical and genetic interactions (out of which 131,624 represent interactions in Homo Sapiens).

Aided in part, by the already existing yeast interaction dataset, McManus et al. [17] showed, that in colorectal cancer cells, RAD54B is synthetic lethal with the Flap Endonuclease 1 (FEN1). Both genes are involved in homologous recombination repair. This observation was recently further extended to synthetic lethality between the human FEN1 and the genes RFN20, MRE11A, CDC4, 5MC1A and 5MC3, which frequently occur to be mutated in human cancers, marking FEN1 as potential target for cancer therapy [18].

3. Experimental approaches for identification of synthetic lethality relationships directly in mammalian cells: general outline

Over the past two decades, several general methods of mammalian cell-based high throughput screening (HTS) for synthetic lethal compounds or genes, have been reported. The first is the classical screening for genotype-specific chemical/genetic inhibitors that are differentially toxic to mutant vs. wild type cell lines, grown in parallel multi well plates. Toxicity was measured by anyone of a number of viability assays, such as respiration monitoring via colorimetric reduction of Resazurin, (Alamar blue), or measurement of replication via BrdU incorporation by cytoblotting [19].

To circumvent the problem of a potential uneven growth condition between the tested cell line and its control, Kinzler and colleagues co-cultured these two isogenic human cancer cell lines, each tagged by a distinct GFP mutant, allowing for multiple time point, double label assessments, of the relative cell viability through GFP fluorescence monitoring [20].

The second method was specifically developed to address high throughput chemical and genetic synthetic lethality screenings, in human cells [21] and mouse embryo fibroblasts [22]. In this approach, our group has implemented the concept underlying the original yeast methodology, onto human/MEF cells; i.e., use of a tagged, low copy number episomal replicon, carrying an expression cassette of a complementing gene of interest. The episomal replicon, being naturally unstable, is selected for its retention, under a drug-induced insult, or due to suppression of a gene synthetic lethal with the gene of interest. Under synthetic lethal conditions, retention of the episomal plasmid, expressing the wild type gene of interest, becomes indispensable for cell viability. Permanent tagging of the chromosomal host DNA, alongside tagging of the Epstein-Barr Virus

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