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Synthetic lethal approaches for assessing combinatorial efficacy of chemotherapeutic drugs

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ABSTRACT

The recent advances in pharmacogenomics have made personalized medicine no longer a pipedream but a precise and powerful way to tailor individualized cancer treatment strategies. Cancer is a devastating disease, and contemporary chemotherapeutic strategies now integrate several agents in the treatment of some types of cancer, with the intent to block more than one target simultaneously. This constitutes the premise of synthetic lethality, an attractive therapeutic strategy already demonstrating clinical success in patients with breast and ovarian cancers. Synthetic lethal combinations offer the potential to also target the hitherto "undruggable" mutations that have challenged the cancer field for decades. However, synthetic lethality in clinical cancer therapy is very much still in its infancy, and selecting the most appropriate combinations—or synthetic lethal pairs—is not always an intuitive process. Here, we review some of the recent progress in identifying synthetic lethal combinations and their potential for therapy and highlight some of the tools through which synthetic lethal pairs are identified.

Contents

Abbreviations: ATM, ataxia telangiectasia, mutated; ATR, ATM and Rad3-related; ATRIP, ATR-interacting protein; BAF, Brg1-associated factor; BER, base excision repair; BRCA1, breast cancer 1, early onset; BRCA2, breast cancer 2, early onset; CDK1, cyclin-dependent kinase 1; CRISPR, clustered regularly interspaced short palindromic repeats; DDR, DNA damage response; DNA-PK, DNA-dependent protein kinase; DSBs, double strand breaks; EGFR, epidermal growth factor receptor; EML4-ALK, echinoderm microtubule associated protein like 4–anaplastic lymphoma kinase; ER, estrogen receptor; ERBB2, receptor tyrosine-protein kinase erbB-2; ERK, extracellular signal-regulated kinase; EZH2, enhancer of zeste homology 2; FA, Fanconi anemia; FGFR, fibroblast growth factor receptor; HDAC, histone deacetylases; HDACi, histone deacetylase inhibitor; HR, homologous recombination; ICL, interstrand crosslinks; LTM, logical transformation of model; MAPK, mitogen-activated protein kinase; Mdm2, murine double minute 2; MMEJ, microhomology-mediated end joining; miR, micro RNA; MRE11, Meiotic recombination 11 homolog 1; MRN, MRE11, RAD50 and NBS1 complex; NBS1, Nijmegen breakage syndrome protein 1; RAD50, RAD50 homology (*S. cerevisiae*); NHEJ, non-homologous end joining; NER, nucleotide excision repair; NFκB, Nuclear factor kB; PALB2, partner and localizer of BRCA2; PAR, poly(ADP-ribose); PARP, poly(ADP-ribose) polymerase; PARPi, poly(ADP-ribose) polymerase inhibitor; PI5P4Ks, Type 2 phosphatidylinositol-5-phosphate 4-kinases alpha and/or beta; PLK1, polo-like kinase 1; PR, progesterone receptor; RNAi, RNA interference; ROS, reactive oxygen species; RPA, replication protein A; SAHA, suberoylandilide hydroxamic acid; shRNA, short hairpin RNA; siRNA, small interfering RNA; SNPs, single nucleotide polymorphisms; SOD1, superoxide dismutase 1; SOS, son of sevenless; SWI/SNF, SWItch/Sucrose Non-Fermentable; TNBC, triple-negative breast cancers; Top, topoisomerase; VHL, Von Hippel–Lindau.

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1. Introduction

In the United States, cancer is the second leading cause of all death, with lung cancer the most common cause in both men (28%) and women (26%). Current projected estimates expect that over 1.6 million new cases of cancer will have been diagnosed by the end of 2015, of which prostate (26%) and breast (29%) cancers will represent the most common new cases for men and women, respectively (American Cancer Society, 2015). There is no known cure for most cancers; albeit, a number of early-stage cancers can be cured by surgery and, according to risk factors for relapse, hormone therapy and chemotherapy, or radiotherapy alone or in combination with chemotherapy can be applied to successfully treat patients with early-stage disease. More recently, breakthroughs in the use of immunotherapies have given hope to terminally ill patients, with treatment combinations that harness the activity of the immune system shown to work against a wide array of cancers. However, as is commonly noted, these drugs do not work for everyone, with some patients failing to respond, particularly those with lung cancer. Others, who may initially show good response, almost invariably relapse and develop resistance (Thomas & Giaccone, 2015).

Cancer and its resurgence arise as a result of various genetic and epigenetic changes that lead to selective cellular advantages, such as unrestricted proliferation and migration, immune avoidance, angiogenesis, tissue invasion, and metabolic changes, some of the so-called "hallmarks of cancer" (Hanahan & Weinberg, 2011). Numerous somatic mutations have been identified through large-scale genomics sequencing: "passenger" mutations, which are innocuous and unlikely to play a role in cancer, versus "driver" mutations, which are fewer in number but linked to the cause or the progression of the disease (Hanahan & Weinberg, 2011). These driver mutations tend to occur in high frequency and are found in a range of tumors, making up approximately 20% to 30% of known mutations (Hanahan & Weinberg, 2000). Most tumors tend to have just two to eight mutations, suggesting that the number of driver mutations required for tumor transformation is small (Kandoth et al., 2013; Vogelstein et al., 2013). Identifying such mutations is key to the development of broad-acting anti-cancer therapeutics.

Missense mutations or chromosomal translocations result in the production of activated, inhibited, or even chimeric proteins that alter normal cellular signaling pathways and often result in the activation of alternative or compensatory pathways, such as stress response or metabolic flux pathways; these alternate pathways support the survival and unchecked growth, respectively, of the cells (Pawson & Warner, 2007; Wood et al., 2007). These changes can cause the cells to become reliant on the mutations and the resultant pathway rewiring, a phenomenon referred to as "oncogenic addiction". For example, numerous KRAS-mutant cell lines demonstrate addiction to the KRAS oncogene (Cox et al., 2014) and tumors with BCR-ABL chimeras or epidermal growth factor receptor (EGFR) overexpression also show oncogenic addiction (Sharma et al., 2006). This addiction helps the cells maintain their highly proliferative phenotype, which is not observed in normal cells (Mair et al., 2014).

EGFR overexpression and other types of activating mutations provide clear targets for drug design. However, cancers also arise as a result of loss-of-function or loss-of-expression mutations, and it is these mutations that tend to be more difficult to treat, as they lack targetable sites. Indeed, several of the classical loss-of-function driver mutations are regarded as "undruggable" for this reason, such as the RAS family members, which are found in approximately 20% of all malignancies (Cox et al., 2014; Downward, 2015). The bulk of treatment strategies against these so-called undruggable targets tend to fall short of clinical success.

A revolutionary paper by Hartwell, Friend and colleagues in 1997 (Hartwell et al., 1997) first proposed the concept of synthetic lethality as a way to reform cancer therapeutic strategies. Synthetic lethality describes the combination of two genetics events that leads to a lethal phenotype when neither event alone has a significant effect on the cell. For example, the delivery of a drug to a cell harboring a certain mutation leads to a loss of viability or cell death but the drug or mutation alone are compatible with viability (Fig. 1). Similarly, the deletion of two genes or the delivery of two drugs can be lethal to the cells but only in combination.

Almost all cancer cells comprise a "safety net" of overlapping genes that endow them with the ability to tolerate mutations and the activation of alternative or additional pathways. This overlap provides the cells with a certain degree of robustness that prevents mutations from causing large changes to vital cellular processes (Nijman, 2011). It is this robustness that is hijacked in the synthetic lethal concept, such that ablation of the expression or function of a certain gene pair will cause cell death. Consequently, research is now geared up toward identifying synthetic lethal pairs that could be exploited for the design of targeted therapies. The exploration of synthetic lethal combinations also offers the opportunity to understand the essentiality of specific gene combinations in cells, particularly *induced essentiality*, a concept coined by Ashworth and colleagues that describes the process by which certain genes or pathways become essential to tumor cells following a drug treatment or gene change (Ashworth et al., 2011). However, as we will demonstrate, the identification of these synthetic lethal pairs is not a clear-cut task. In the next sections, we shall discuss some of the successes that have been attained by taking advantage of this concept.

2. DNA damage repair proteins — common targets for synthetic lethality

Genomic integrity is essential for eukaryotic cell viability, and is ensured by various cell cycle checkpoint proteins and a set of carefully orchestrated DNA repair mechanisms, collectively referred to as the DNA damage response (DDR). The DDR is extremely complex, and increasing evidence points to extensive cross-talk and feedback among the factors that promote apoptosis, growth, and cell fate (Lee et al., 2012).

Myriad extracellular and intracellular signals result in the activation of DDR pathways (Fig. 2), with double-strand breaks (DSBs) regarded as the most severe form of DNA damage. Under normal conditions, DSBs are repaired via one of two mechanisms: (1) homology-directed repair, most commonly, homologous recombination (HR), an error-free repair strategy that employs a homologous template for accurate DNA repair; or, (2) non-homologous end joining (NHEJ), which involves the direct ligation of the disjointed DNA ends but risks the possibility of sequence loss or the introduction of others errors [for specific details, see review by (Ciccia & Elledge, 2010)]. HR and NHEJ are held in delicate balance, and how the cell decides which pathway to use is still debated. However, it is known that the cell cycle dictates this choice, with evidence to suggest that the repair pathway chosen is dependent on the timing of a break (i.e., S-phase versus other phases of the cell cycle) as well as the proximity of homologous templates for repair during the S-G2 phase (Lieber, 2010; Ceccaldi et al., 2016). Nevertheless, proteins from both pathways, as well as other important components of DNA repair, have been highlighted as being synthetically lethal to cells in certain combinations. Table 1 lists just some of the synthetic lethal pairs that have been identified from DDR pathways, with several of these interactions discussed in more detail below.

DNA damage is sensed by one of several sensing complexes (MRN, ATRIP, RPA) within the cell. These, in turn, initiate the activation of checkpoint kinases, ataxia telangiectasia-mutated (ATM) and ATM and Rad3-related (ATR), which then activate one of several pathways to induce cell cycle arrest, DNA repair or apoptosis, depending on the type and degree of repair required for cells at different stages of the cell