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A Bounty of New Challenging Targets in Oncology for Chemical Discovery

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Since the connection between oncogenic molecular features and specific genetic vulnerabilities was initially made between the Philadelphia chromosome and the fusion oncoprotein BCR-ABL, inhibitors directed against these selective dependencies have been successfully applied in multiple therapeutic development efforts for the treatment of cancer. These inhibitors are both effective and relatively well tolerated by patients because cancer cells require the function of these driver oncogenes to a greater degree than normal tissue, creating a therapeutic window. This targeted therapeutic approach has largely relied upon the initial recognition of cancer-associated genetic perturbations followed by an investigation of the functions of these proteins and consequences of their inhibition. Functional genomics inverts this framework by first systematically assessing the functional relevance of perturbing (e.g., using CRISPR-Cas9) any gene in the genome of a particular cancer cell.[1] Phenotypes determined from multiple large-scale screens can then be associated with specific oncogenic molecular features to identify potentially tractable targets.

Behan et al. tackled the challenge of target prioritization by performing genome-scale CRISPR knockout screens in 324 human cancer cell lines representing 30 different cancer types.[2] The authors developed a computational framework to integrate their functional genomics data with genetic biomarker information to identify both broadly required ("pan-cancer") and highly specific ("cancer-type-specific) genetic dependencies. The authors then assigned each of 628 priority targets to one of three tractability groups (Figure 1). Tractability group 1 (40 genes) contained genes that had already been targeted by clinically approved or advanced clinical/preclinical compounds. Group 2 (277 genes) encompassed genes with some features that supported their tractability despite not having compounds in clinical development. Genes without strong evidence of tractability were placed in group 3 (311 genes). These efforts nominated a large number of potentially interesting targets, and the authors further investigated a selected example to validate their approach and highlight a novel cancer vulnerability uncovered by their functional genomics data.

The RecQ helicase family member WRN, a tractability group 2 gene, was shown to be selectively essential in the context of microsatellite instability (MSI) in multiple cancer types. MSI results from a deficiency in DNA mismatch repair, which can promote the accumulation of pro-oncogenic mutations that support tumor growth and survival. Functional genomics screens are well equipped to determine synthetic lethal (SL) interactions, such as those between WRN and MSI, which formally occur when perturbation of either of two genes alone remains viable, but perturbation of both results in a loss of viability. In this case, MSI is not itself a specific gene but a molecular marker reflective of a particular cancer cell state. Genetic knockout (protein removal), however, is fundamentally distinct from pharmacological inhibition (typically occupancy of an active site), and the authors provided further evidence to bolster WRN's attractiveness as a drug target in MSI cancers. They demonstrated that expression of point mutant variants of WRN that were helicase deficient was insufficient to rescue knockout of WRN in MSI cells, offering a rationale for the development of chemical inhibitors against the specific functional domain of relevance (Figure 2). The authors concluded by validating the essentiality of WRN in an *in vivo* xenograft model. Notably, a related manuscript from Chan et al. also nominated WRN as a SL vulnerability in MSI cancers through a large-scale functional genomics approach, and

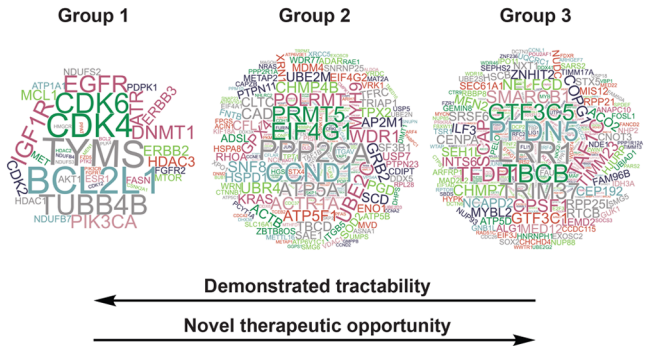

Figure 1. Priority cancer targets identified through large-scale functional genomics screens grouped according to tractability for chemical inhibition.

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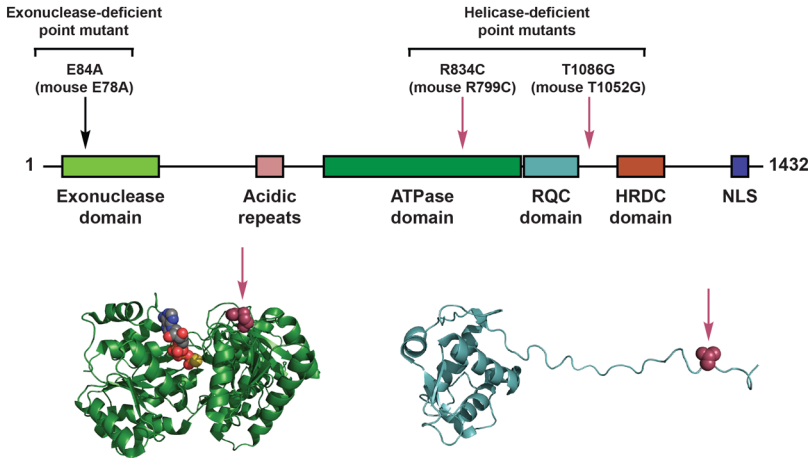

Figure 2. Domain architecture of WRN and three different point mutants used to determine the function of WRN relevant to its SL interaction with MSI. A black arrow indicates that the point mutant could rescue the SL phenotype, while a red arrow indicates that the point mutant could not rescue the SL phenotype. Homologous R306 of *Escherichia coli* RecQ helicase bound to ATP-γ-S (Protein Data Bank entry 1OYY) and T1086 of human WRN partial RQC domain (Protein Data Bank entry 2AXL) are colored red.

several others have arrived at similar conclusions through independent means.[3]

Upon broader examination of the three tractability groups, group 1 concurs with decades of prior efforts (e.g., increased PIK3CB dependency in PTEN mutant breast cancer) while groups 2 and 3 provide a wealth of opportunities for new therapeutic development (e.g., increased SEC61A1 dependency in ASH1L mutant cancer). These results motivate further biochemical and cellular characterization of group 2 and 3 targets, particularly WRN for which a lack of knowledge about the precise shape of the helicase domain precludes structure-guided drug discovery efforts. There is reason to believe, however, that application of existing pharmacology against group 2 (e.g., ARF1, EIF4A1, KRAS, and SHP2/PTPN11) and even group 3 (e.g., MYCN and SEC61A1) targets can be informed by these data to accelerate the development of molecules toward their appropriate clinical scenarios (genotype/tissue of origin).

The vast majority of group 2 and 3 targets, however, will likely require novel pharmacological approaches to even consider initiating drug discovery efforts. Such targets offer a challenge to emerging therapeutic discovery modalities: proteolysis-targeting chimeras (PROTACs), cysteine tethering, DNA-encoded chemical libraries, mRNA display of natural and unnatural peptides, and others. Even the least tractable group 3 targets are not entirely impervious, as strategies that utilize allostery or natural products have been applied to successfully target MYCN and SEC61A1. For example, the small molecule CD532 binds to Aurora kinase A, inducing an allosteric change to disrupt a protein interface that stabilizes MYCN, resulting in its proteolytic degradation.[4] Additionally, several natural products and their derivatives such as HUN-7293 and cotransin target the Sec61 translocon complex by trapping nascent transmembrane domains prior to endoplasmic reticulum membrane integration.[5] These data offer one of the most comprehensive hit lists for single-target drug discovery and will serve to inspire the further creative development of chemical matter for the treatment of cancer.

Despite the value of single-target inhibition in cancer, however, targeted therapies face major clinical challenges related to mechanisms of resistance due to the selection of resistant subpopulations and even nonmutational bypass mechanisms. Further functional genomics efforts will need to address the complexity of multilayered functional perturbations in the form of genetic interaction maps and chemical–genetic screens. Combining broad-coverage chemical inhibitors with functional genomics could be particularly useful in revealing the biology of functionally redundant paralogs, whose important functions could be masked by compensation of other closely related genes. Nevertheless, the work of Behan et al. and Chan et al. marks a cornerstone in the application of functional genomics toward target identification in cancer. The monumental functional genomics data made publicly available by the Wellcome Sanger Institute (https://score.depmap.sanger.ac.uk/) and the Broad Institute (https://depmap.org/portal/) empower any scientist or clinician to initiate genomically informed campaigns to identify and validate new targets of potential therapeutic value. As the discovery of new oncogenic drivers has slowed, functional genomics will play an important role in the identification of actionable targets beyond the traditional oncogene addiction paradigm (e.g., SL interactions and combination therapies). We anticipate that this work will serve as a basis for future biochemical, cellular, and clinical studies of genetic dependencies in cancer and accelerate the development of targeted therapeutics for the advancement of patient care.

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