



Towards unraveling cancer's Achilles heel: a massive RNAi screen exposes tumor cell weaknesses

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Cancer arises as a result of a multi-step evolutionary process that is manifested on a genetically and epigenetically heterogeneous population of cells and currently is one of the leading causes of morbidity and mortality worldwide (1,2). In a Darwinian sense, “survival of the fittest” correlates with the selection of the most proliferative, invasive and tumorigenic cell type (3). Despite recent advances in diagnosis as well as development of targeted therapies and therapeutic interventions, there is a projection for a 50% increase in new cancer cases and a 60% increase in cancer associated deaths in the following two decades according to the World Health Organization (WHO). As the majority of cancer related deaths are due to the development of metastatic disease, this greatly reflects our limited understanding of the key processes that drive human cancer and lead to disease progression. Moreover, it highlights the ineffectiveness of current therapies that are most commonly followed by the development of resistance, as well as the inability to reinstate tumor suppressor gene function.

From a therapeutic standpoint, the ultimate goal is to develop therapies that target cancer-relevant genetic alterations and benefit cancer patients by prolonging their life expectancy. Collaborative efforts such as The Cancer Genome Atlas (TCGA) and the International Cancer Genome Consortium (ICGC) initiative, employ next generation sequencing to catalogue the genetic as well as the molecular and epigenetic heterogeneity in different cancers and metastatic sites in a large number of patients (4,5). The emerging data can be better exploited by understanding how genetic driver events rewire the tumor cells' transcriptional network to impact survival and phenotypic fitness of different cancer types

in accordance to their unique tumor microenvironment. Interestingly, successful therapeutic approaches suggest that the genetic drivers that sustain tumor malignancy are actually quite few, with most cancers being dependent on one or few oncogenes or oncogenic pathways. For example, tumors can be commonly classified by a single genetic driver event such as estrogen receptor (ER) or epidermal growth factor receptor 2 (HER2) expressing breast cancer, BRAF expressing melanoma and so forth. This phenomenon has been termed as “oncogene addiction” and it highlights the central role of oncogenes in rewiring transcriptional networks in dependent cancer cells (6,7). Therefore, unraveling the new network nodes and interactions in cancer cells and understanding the interplay with the tumor microenvironment as well as the effect of current treatments in reshaping these networks is fundamental for the development of more effective targeted and combination therapies (6). With a prerequisite for a well-characterized genetic background, functional genomic screens can help unravel these novel network relationships and growth vulnerabilities in cancer cells.

To this end, in the recent publication of McDonald *et al.*, a large RNAi screen was performed in order to uncover cancer dependencies and to define protein interactions and networks that are pivotal for cancer survival. This project is termed DRIVE (deep RNAi interrogation of viability effects in cancer) and in detail, the authors targeted 7,837 genes with an average of 20 shRNAs per gene in 398 genetically sequenced and well-characterized cancer cell lines as part of the Cancer Cell Line Encyclopedia (CCLE) joint research program (8). With this approach, a global genetic interaction map has been recreated

where individual genes or sets of genes can be queried for their local network neighborhood and correlations can be identified in different cancers. Within this global network, essential genes denote a robust neighborhood. A great example of the power of this approach is the reconstruction of p53 network. Known positive (MDM2, MDM4) and negative (TP53BP1, USP28 and CDKN1A) regulators are allocated in the close neighborhood of 2–4 interactors, however new dependencies can also be identified—and explored therapeutically—when expanding the interactor neighborhood to include up to 10 genes. As proof of principle in the network reconstruction, genes with functional similarities form tight interactions therefore, node proximity between certain regulators, such as those identified therein between DNA methylation and histone acetylation protein mediators could be indicative of interplay and similar dependencies in cancer cells.

As mentioned above, the CCLE cell lines are well characterized genetically and this allows for dependence correlations of genetic and expression data to be drawn. Not surprisingly among the most robust dependencies observed with the DRIVE project are those related to the shRNA gene target itself. These include mutated oncogenes such as *BRAF* and *KRAS*, amplifications of either mutated oncogenes or copy number alterations (CNAs) of wild-type genes such as *ERBB2* that act as oncogenes as well as highly expressed gene targets. Among the latter are known genetic drivers and cell cycle regulators such as D-type cyclins that interestingly, in order to mediate G1/S cell cycle transition, display a therapeutically exploitable lineage specific dependency with either CDK6 or CDK4 in hematopoietic malignancies and solid tumors respectively. Novel dependencies, such as the dependence on *RRAS2* in breast and ovarian cancer as well as the identification of oncogenic gene modifiers, that is genes that can modify oncogenic dependence, can also be uncovered. For example, in lung cancer and other solid tumors, dependence on *EGFR* relies on concomitant upregulation of the growth factor amphiregulin (*AREG*). Therefore, *AREG* expression should be taken into account when stratifying patients to achieve better response with anti-*EGFR* treatment regimens but also to identify those patients who are more likely to develop resistance.

A major hurdle with targeted therapies is that in most cases, tumors are expected to develop resistance. To some extent, genetic determinants that lead to acquired resistance preexist in the treated tumors and as such, they should be anticipated and accounted for in the initial therapeutic design. A promising approach to counteract this is the development of combination therapies. The rationale for the effectiveness

of this approach is that targeting the same oncogene or pathway using multiple drugs is expected to minimize the incidence of resistance, as the probability of multiple resistant phenotypes co-existing in a given tumor is very small. To this end, the DRIVE project has been invaluable for identifying synthetic lethal (SL) relationships, where disruption of a gene is only lethal when combined with the disruption of a pathway interacting partner, a paralog gene or a result of collateral incidence (9). Examples include the pairs of *APC/CTNNB1* and *CDKN2A/CDK2* for pathway, *ARID1A/B* for paralog and *CDKN2A/PRMT5* for collateral SL. Moreover, of great importance is the identification of those SL dependencies that involve tumor suppressor genes. These genes have not been exploited therapeutically since it is lack of their expression that correlates with the malignant phenotype. With DRIVE, the authors could link mostly the homozygous deletion of tumor suppressor genes *p53*, *CDKN2A* and *SMAD4* with collateral SL relationships involving a number of genes such as *UBC*, *POLR2A*, *AURKB* and *PRMT5*. As these dependencies create opportunities for the development of novel therapeutic efforts, they also create the basis for better understanding their network biology. To this direction, the network analysis reported in DRIVE also uncovered two metabolic neighborhoods, one for lipid biosynthesis (*SCAP/SCD/SREBF1*) and one for amino acid homeostasis (*ASNS/ATF4/EIF2AK4*). Both networks include several SL interconnections that can help define the metabolic pathways in cancer, as currently there is no molecular evidence to corroborate them.

The DRIVE project and similar functional genetic screens are invaluable for validating (or not) cancer mutational profiles and in parallel provide the possibility for new therapeutic targets to be exploited. However, as much as these functional datasets are attractive and create a great resource for the development of novel therapeutic approaches, they still require a validation step before clinical applications can be considered. This has proven to be a bottleneck as the only clinically approved and therefore successfully exploited SL interaction to date is that of *BRCA1/2* and *PARP* in cancer patients (10). In part this is due to the *in vitro* monolayer models that are currently employed for genetic screens and functional validation and their inherent inability to accurately interrogate the survival phenotype since they do not allow for the interplay with the tumor microenvironment to be evaluated. In addition, a number of cell lines that are routinely used for *in vitro* validation are poorly characterized. This is also evidenced by the DRIVE project for a number of *KRAS* mutant cell lines where unexpectedly no dependency

for KRAS itself was identified.

Moving forward towards the development of successful therapies that truly benefit cancer patients, we will also need to reevaluate previous data generated on well-established cell lines using more elaborate *in vitro* models. To this direction, 3-dimensional (3D) co-culture tumor spheroid assays and patient derived organoids that interrogate the tumor 3D architecture and interaction with the tumor stroma could be invaluable for identifying bona fide cancer dependencies and SL interactions before more extensive functional validation is performed in patient derived mouse xenograft models. In addition, we also need to employ data from various genetic functional screens to identify robust genetic targets. For example, with CRISPR screens we can assess the complete knockout phenotype, as opposed to shRNA gene expression knockdown, and therefore this might allow us to uncover different phenotypes. In parallel, we need to continue to characterize tumor heterogeneity, identify low frequency mutations and discover more targets for therapeutic applications. The interrogation at single cell resolution of both genomic and transcriptomic profiles will greatly advance our understanding of how cancer evolves. Moreover, “liquid biopsy” approaches, such as the ability to non-invasively, serially isolate and functionally characterize circulating tumor cells and cell free DNA from a large number of patients with progressive metastatic disease will also help us to narrow down the relevant genotypes for metastasis initiation. Synergistically, these approaches should identify and exploit true cancer vulnerabilities and help us fight this noxious disease.

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aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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