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Mapping the protein–protein and genetic interactions of cancer to guide precision medicine

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Abstract

Massive efforts to sequence cancer genomes have compiled an impressive catalogue of cancer mutations, revealing the recurrent exploitation of a handful of 'hallmark cancer pathways'. However, unraveling how sets of mutated proteins in these and other pathways hijack pro-proliferative signaling networks and dictate therapeutic responsiveness remains challenging. Here, we show that cancer driver protein–protein interactions are enriched for additional cancer drivers, highlighting the power of physical interaction maps to explain known, as well as uncover new, disease-promoting pathway interrelationships. We hypothesize that by systematically mapping the protein–protein and genetic interactions in cancer—thereby creating Cancer Cell Maps—we will create resources against which to contextualize a patient's mutations into perturbed pathways/complexes and thereby specify a matching targeted therapeutic cocktail.

Protein–protein and genetic interactions fill the gap between genotype and phenotype

The advent of next-generation sequencing technology has fueled a massive accumulation of genomic sequences over the past decade. In fact, any individual can now sequence their entire genome for only $200, gaining unprecedented insight into their ancestry and predisposition for certain diseases, particularly those with mono-genetic or Mendelian drivers. Furthermore, genomic sequencing of cancerous tissues is practically commonplace in many clinics, from whole genome [1] to targeted arrays [2], bestowing oncologists large quantities of data from which to, possibly, guide treatment decisions [3]. However,

translating a list of patient-derived cancer mutations into patient-specific treatment decisions remains challenging, with only a few robust examples of success existing today (e.g. imatinib for the BCR–ABL fusion gene [4]).

Nevertheless, the analysis of genomic sequencing data has provided significant insight into our understanding of cancer biology. Numerous large-scale genomic studies, such as those from The Cancer Genome Atlas (TCGA) consortium, have demonstrated that cancer exploits a recurring set of 'hallmark cancer pathways'—pan-cancer analyses estimate 90% of tumors have a driver alteration in at least one of ten hallmark signaling pathways [5••]. However, complexity persists, as each patient's tumor possesses a heterogeneous mixture of mutations within these, as well as many other, pathways. Even two patients with the same tumor type (e.g. breast cancer) can possess strikingly different sets of mutations, with some patients possessing over 1000 mutations of unknown function, many of which may regulate these hallmark pathways in an unknown fashion. Consequently, and unsurprisingly, this mutational diversity between tumors drives differences in drug sensitivity, which are currently extremely difficult to reliably predict on a patient-by-patient basis.

The challenge of translating patient-derived mutational profiles into patient-tailored drug cocktails highlights a fundamental knowledge gap between genotype and phenotype in mammalian cell biology. In our view, navigating between genotype (e.g. mutations, copy number alterations, mRNA/protein expression) and phenotype (e.g. cell proliferation and drug sensitivity) requires maps depicting how each protein assembles into protein–protein interaction networks (by creating protein–protein interaction maps), and thus into particular pathways and protein complexes, and an understanding of how these interactions functionally compel cellular processes (by creating genetic interaction maps), such as cancer progression. In order to appropriately characterize disease-specific alterations, protein–protein and genetic interaction maps must be built by comparing 'healthy' (i.e. non-transformed) to diseased cells, contrasting mutant to wild-type networks, and evaluating differences across tissue types. The creation of 'differential' [6] cancer cell maps will provide a resource against which to contextualize new patient mutation data into known cancer pathways or propose synthetic lethal targeting strategies for pharmacological intervention (Figure 1).

Physical interactors of cancer drivers are often cancer drivers, too

Protein–protein interactions (PPIs) often implicate a functional relationship between proteins. For example, PPIs typically denote proteins that work together to accomplish a specific cellular task or co-drive disease [7]. Often times, interactions can mediate crosstalk between distinct cellular pathways. For example, the physical interaction between PIK3CA and KRAS, discovered over two decades ago [8], reveals a poignant mechanism of crosstalk between the ERK and PI3K signaling pathways. This finding highlights the capability of protein–protein interactions to reveal how the cancer signaling network is configured, with implications for cancer therapy [9]. Furthermore, the disruption or strengthening of PPIs by mutations, which are found to frequently occur at the interface between other proteins or ligands, highlights the wide role these interactions may play in regulating signaling activity

or downstream effectors [10]. In sum, proteins that physically interact with known disease genes are themselves, also, potential disease drivers.

Given this line of thinking, one might expect protein interactors of major cancer drivers to be enriched for additional cancer drivers. To probe this hypothesis, we ask: are physical interactors of major cancer drivers also frequently mutated in cancer? To answer this question, we assembled the union of several large protein–protein interaction networks, including BioPlex [11], IRefIndex [12], Mentha [13], Human Interactome [14], and HPRD [15], and extract physical interactors (first neighbors) for each of the fifteen most commonly mutated proteins (copy number variations excluded) in each of eleven distinct cancer types [16]. Interestingly, we found the average mutation frequency of these interactors to be significantly higher than size-matched random controls for each cancer type, with the exception of lung adenocarcinoma (Figure 2a). We observed the same trend when collapsing across all eleven cancer types—'Pan-Cancer' analysis (Figure 2b, top)—and also when assessing the interactomes of 12 canonical DNA damage proteins (Figure 2b, bottom). In sum, there appears to be a significant enrichment of frequently mutated cancer proteins that bind to other frequently mutated cancer proteins.

These results highlight an opportunity to uncover new biological processes and molecular mechanisms implicated in cancer by probing these physical interactions. In this article, we briefly review two such examples, which possess a dual physical/functional relationship, and showcase themes gaining momentum in the scientific literature. First, we explore the physical and functional interface between inflammatory proteins and oncogenes. Second, we highlight recent insights into the interrelationships between DNA damage proteins and upstream proliferation-driving oncogenes. Lastly, we review a few recent large-scale genetic interaction studies that demonstrate the overlap between genetic (i.e. functional) and protein–protein (i.e. physical) interactomes, illustrating the synergy between these mapping approaches.

Physical interactions between inflammatory proteins and oncogenes

The relationship between inflammation and cancer is not a recent discovery. In fact, the first known observation spans back to 1863, when Rudolf Virchow noticed cancer at sites of chronic inflammation [17]. Inflammation is well appreciated to play a role in several aspects of tumor development, from oncogenesis to metastasis, and patients with inflammatory diseases such as hepatitis, colitis, and pancreatitis display a higher incidence of cancer [18]. Conversely, treatment with non-steroidal anti-inflammatory drugs (NSAIDs) has displayed anti-cancer activity for several cancers [19–21], as have anti-inflammatory physiological processes, such as getting regular exercise [22,23]. Although many important observations over the years have linked cancer with inflammatory processes, the direct physical regulation of cancer driver proteins by inflammatory proteins is only recently being investigated [24]. Our cancer driver PPI analysis above identified several inflammatory proteins—including STAT3, SMAD3, NFKBIA, STAT5B, and LRP1—that bind to top cancer drivers. To what extent do physical interactions between inflammatory molecules and cancer drivers mediate inflammation-induced cancer pathology? This remains an open question in the field and highlights exciting avenues for future research.

As an example, our analysis highlights a relationship between EGFR and STAT3 signaling. Specifically, EGFR is known to interact with STAT3 as well as the protein tyrosine phosphatase PTPRD, a commonly mutated [25,26] known negative regulator of STAT3 [27]. EGFR is known to activate and maintain STAT3 signaling, driving transcriptome-rewiring events that promote cancer progression and drug resistance [28]. In addition, the EGFR-STAT3 system leads to the production of Il-6, an inflammatory cytokine, a feedback process which is thought to underlie tumor initiation [29] and metastasis [30]. Inhibition strategies that co-ablate phosphorylated EGFR and STAT3 may be a potent anti-cancer therapy in some contexts [31]. This is but one example of several representing a dual physical and functional relationship between an inflammatory protein and a cancer driver; though, many more are expected to emerge in the future.

Physical interactions between DNA damage response proteins and oncogenes that drive proliferation

Another signaling interface of interest is between DNA damage response (DDR) proteins and oncogenes that drive proliferation, an interrelationship increasingly reported in the scientific literature though not fully understood. A deeper understanding of this interface could help identify patient-specific therapies, such as new combinations of PARP and kinase inhibitors targeting angiogenesis [32], PI3K/AKT [33], RAS/MAPK [34•], WEE-1 [35] and ATR/CHEK1 [36] pathways in order to overcome PARP inhibitor resistance. In order to identify known physical interactions between DDR proteins and pro-proliferative oncogenes, we searched our PPI network for physical interactors of 12 canonical DDR proteins (CHEK1, CHEK2, RAD51, BRCA1, BRCA2, MLH1, MSH2, ATM, ATR, MDC1, PARP1, and FANCF). Interestingly, we find these DDR gene inter-actors to be more frequently altered than expected by chance, calling attention to the central role DDR proteins and their interactors play in cancer progression (Figure 2b, bottom).

Of particular interest, we observed several interactions between DDR proteins and both the Ras/MAPK and PI3K/AKT signaling pathways, interrelationships that are gaining increasing attention in the scientific literature. For instance, a recent study highlighted the interplay between PARP and MAPK signaling [34•], finding strong synergy between dual MEK and PARP inhibition for RAS mutant cancers *in vivo*. This interplay is corroborated by several known physical interactions between PARP1 and various MAPK pathway members, including MAPK1, MAPK3, and MAPK13. An additional example is the interplay between BRCA1 and AKT signaling; specifically BRCA1 deficiency was found to drive AKT activation [37], which was involved in mediating tumorigenesis in mice likely by promoting chromosome instability [38]. Activated AKT in BRCA-deficient cells was found to impair CHEK1 nuclear localization and CHEK1 interaction with RAD51, leading to defects in homologous recombination [39]. Furthermore, AKT inhibition displayed efficacy against BRCA1-mutated mammary tumors in mice [40]. Publicly available PPI data indicate a physical interaction between AKT1 and several DDR proteins, including BRCA1, CHEK1, and MSH2 as well as between PIK3CA and ATR. These examples illustrate how PPIs can help explain known relationships between distinct pathways, or even uncover new potential pathway relationships. Considering these insights, further defining how interactions

between DDR proteins and pro-proliferative oncogenes regulate cancer progression, growth suppression, and/or drug resistance is an exciting avenue for future research.

Genetic interaction mapping often reveals functional protein–protein interactions

Uncovering functional PPIs can be challenging, as many physical interactions, when perturbed, do not enact a phenotypic change. Genetic interaction (GI) mapping, pioneered in the early 2000s, is a powerful technique to systematically reveal functional relationships between genes, which often indicate the presence of a physical interaction. GI mapping involves the pairwise perturbation of genes (e.g. knockout, knockdown or overexpression) in order to elucidate how one gene modulates the phenotype of the other. Typically, cell viability is used as the phenotypic readout, where GIs that increase cellular fitness are said to be 'positive' and GIs that decrease cellular fitness are said to be 'negative'. GI mapping is often used to uncover new functions of genes [41,42], enabling a hierarchical organization of gene products into functional complexes and pathways, and to identify synthetic–lethal interactions with relevance to cancer combination therapy. As alluded to, the presence of a functional (i.e. genetic) interaction often indicates a physical interaction, and vice versa, highlighting the synergy between combined GI and PPI mapping initiatives.

The majority of GI studies were performed in the budding yeast *Saccharomyces cerevisiae* [43,44]. More recently, however, GI mapping has entered mammalian cell contexts. In human cells, GI screens have already tackled diverse topics, including chromatin regulation [45,46], ricin susceptibility [47], drug target interactions [48], and functioning of cancer oncogenes/tumor suppressors [49–51], among others. In one of the largest GI screens ever conducted in human cells, Horlbeck *et al.* assessed 222 784 gene pairs using a pooled dual-sgRNA CRISPR interference (dCas9-KRAB) loss-of-function lentiviral vector screen in K526 and Jurkat cells, two human immune cell lines [52••]. Interestingly, gene pairs with highly correlated GI fitness profiles were enriched for known physical interactions from the STRING physical interaction database. They further uncovered new mechanism, discovering TMEM261, a previously poorly characterized gene, as a critical regulator of oxidative phosphorylation similarly to core mitochondrial complex I proteins, to which TMEM261 is known to physically associate [53].

Computational analyses of GI datasets have also harnessed much insight, most of which further highlight the intersection between GIs and PPIs. Pan *et al.* [54] utilized data from Project Achilles [55], a compendium of lentivirus-based pooled shRNA and CRISPR/Cas9 genome-wide across cell lines, to cluster GI fitness profiles across cell lines ($N = 342$) [56•]. They found ~40% of complexes from the CORUM [57] protein complex data base to possess significantly correlated GI profiles. In addition, correlated GI profiles were also used to annotate previously unknown protein complexes. In another study, Rauscher *et al.* developed a computational framework ('MINGLE') to integrate 85 CRISPR/Cas9 screens with mutation, copy number, and mRNA expression data from 60 different cancer cell lines [58••]. This study used a random effects statistical model to identify significant relationships between normalized CRISPR scores and genetic alterations across cell lines (17 545

significant GIs). Again, genes with similar interaction profiles were enriched for co-complex membership. This study revealed PRKCSH, GANAB, and UGP2 as novel positive regulators of the Wnt/β-catenin pathway by searching for genes possessing a negative genetic interaction score with RNF43, a known negative Wnt pathway regulator. Interestingly, PRKCSH and GANAB also physically interact to form the glucosidase II complex.

These studies highlight the cohesion between GI and PPI mapping approaches—where the presence of one interaction type typically implicates the other—and depicts the synergy between the two approaches in systematically uncovering new biology with relevance to cancer and other diseases.

Building cancer cell maps for precision medicine

As mentioned above, genomic sequencing of cancerous tissues has led to an impressive catalogue of disease-driving mutations. Although many cancers harbor alterations in well-known cancer drivers, such as TP53 or EGFR, a tumor may additionally possess anywhere from 10 to over 1000 rare somatic genetic alterations that remain uncharacterized. We and others [59, 60, 61••] hypothesize that while these mutations may appear rare when viewed independently, they likely converge on a smaller number of protein complexes, signaling cascades, and transcriptional regulatory circuits. By recapitulating this at network-level, we anticipate significant pathway-based signals to emerge, signals likely imperceptible by considering individual mutations in isolation. Towards building a network-level view, we believe it critical to compare networks from 'healthy'/non-transformed cells to diseased cells, to study both mutant and wild-type protein interactomes, and to compare across distinct cancer types. The unbiased and systematic collection of network-level data in this way, utilizing both physical (e.g. large-scale AP-MS) and genetic (e.g. large-scale CRISPR screens) approaches, is needed to create Cancer Cell Maps that delineate the disease-specific molecular wiring of the cell and how it differs between cancer types. To probe these questions and hypotheses, we [61••] and others [62,63] have pioneered various cell mapping initiatives to delineate the molecular interactions that drive cancer. Once created and validated, we envision Cancer Cell Maps will be integrated into the oncologist's toolkit, against which a patient's specific mutations can be queried in order to identify the pathways and protein complexes that are perturbed, enabling the rational and precise selection of appropriate targeted therapies (Figure 1).

Acknowledgements

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•• of outstanding interest

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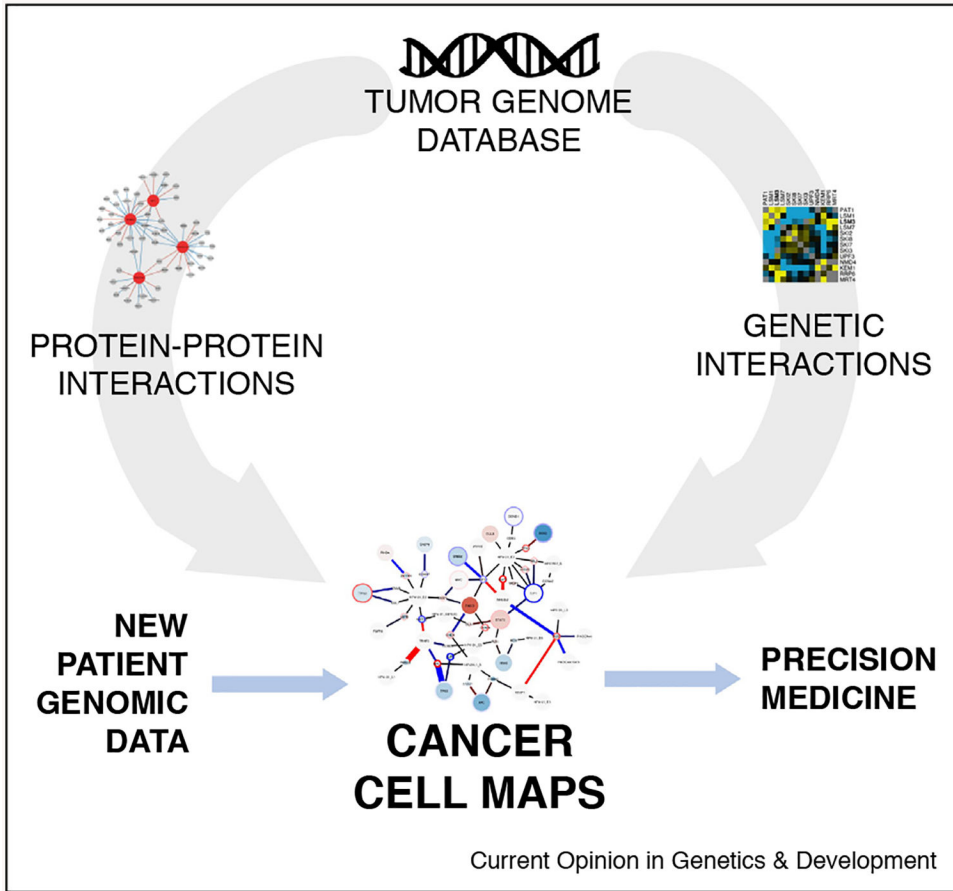
Current Opinion in Genetics & Development

Figure 1.
The cancer cell map for precision oncology. The analysis of large numbers of tumor genomes has revealed key mutations that drive cancer pathology. However, connecting the genotype of tumor mutational profiles to phenotypes of drug sensitivity is impeded by a fundamental lack of understanding into how protein–protein (physical) and genetic (functional) interactions are configured to drive cellular processes, such as proliferation and death. Seeded by this large-scale analysis of tumor genomes (top), what is needed is a systematic, context-diverse network mapping of the protein–protein interactions for mutated and wild-type oncogenes and tumor suppressors (left) as well as the identification of synthetic lethal genetic interactions (right). PPI and GI mapping, two highly complementary data sources, can then be assembled to form Cancer Cell Maps, providing a working scaffold of molecular interactions and the cell types/conditions under which they are active. New patient data can then be queried against this resource by mapping alterations to these hallmark cancer networks, which can inform precision medicine (blue arrows).
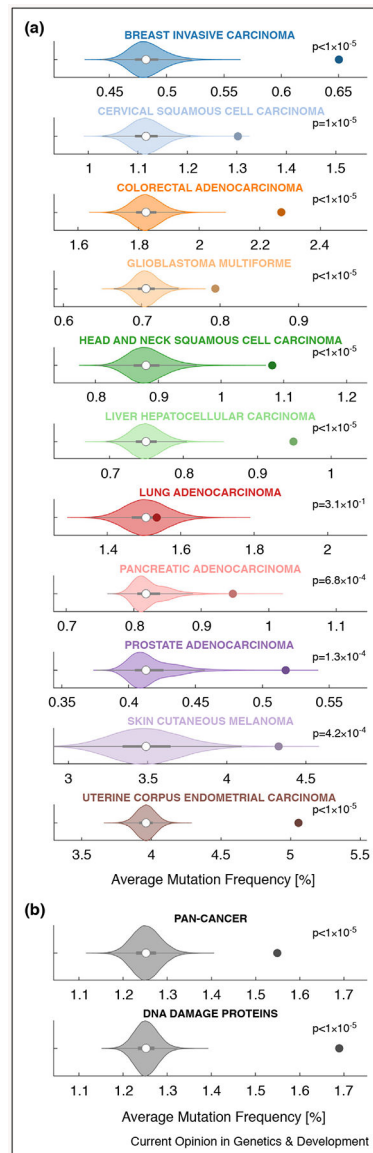
Figure 2.
Physical interactors of cancer drivers are often cancer drivers, too. (**a**) A human protein–protein interaction network was assembled from publicly available data (union of BioPlex, IRefIndex, Mentha, HumanInteractome, and HPRD networks, which amounted to 18 227 nodes and 333 490 edges) and probed for physical interactors (i.e. first neighbors) of the top 15 most frequently mutated proteins (copy number variations excluded) for each cancer type. This typically amounted to ~1500 interactors per cancer type. We then calculated the average mutation frequency across these cancer driver physical interactors (colored dots) for each cancer type (e.g. for breast invasive carcinoma, the average was ~0.65%) and compared them to the average mutation frequencies of 100 000 random size-matched permutations taken from the same dataset (violin histogram). We calculated an empirical p-value for each PPI set, defined as the fraction of random permutation sets of greater value. Interestingly, we find the average mutation frequency of the true cancer driver PPI sets for each cancer type,

with the exception of lung adenocarcinoma, to be mutated significantly more often than expected by chance. (**b**) A pan-cancer analysis, performed by calculating the mutation frequency for each gene across all 11 cancer types in (a), also showed the PPIs of the top 15 most frequently mutated proteins to be mutated significantly more often than expected by chance (top; 'Pan-Cancer'). In addition, we also assessed the average mutation frequency of known physical interactors of 12 canonical DNA damage proteins (CHEK1, CHEK2, RAD51, BRCA1, BRCA2, MLH1, MSH2, ATM, ATR, MDC1, PARP1, and FANCF), which we also found to be mutated significantly more often than expected by chance (mutation frequencies as calculated for 'Pan-Cancer' analysis). These results highlight the role of PPIs as potential drivers of cancer pathology and suggest an additional point of regulation for therapeutic intervention. Empty white dots denote the median of the randomly permuted sets, and the adjoining grey rectangles denote the interquartile range.