Expanding the computational toolbox for interrogating cancer kinomes

“Kinase addiction ranker is a novel computational algorithm that integrates high-throughput drug-screening data, comprehensive quantitative drug–kinase binding data and transcriptomics data to predict kinase dependencies in cancer cells.”

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Kinase dependency in cancer

Recent large-scale cancer genome projects such as The Cancer Genome Atlas and the International Cancer Genome Consortium have systematically characterized genes and pathways that are implicated in tumor initiation and progression across various cancers [1]. Numerous studies have demonstrated that some cancers are dependent on these oncogene-driven signals for survival and maintenance. Many of these oncogenes are protein kinases, which are frequently mutated or amplified in cancer and acquire oncogenic properties to drive tumorigenesis. These cancer cells are often ‘addicted’ to the mutated or amplified oncogenes (e.g., kinases). Targeted cancer therapies have exploited this ‘oncogene addiction,’ and small molecules that can inhibit these oncogenic kinases have been deployed to kill cancer cells – a concept known as ‘kinase dependency’. For example, chronic myeloid leukemia is dependent on the constitutive activation of the *ABL* kinase as a consequence of fusion to the *BCR* gene. Imatinib, the first kinase inhibitor that targets the *BCR-ABL* gene in chronic myeloid leukemia, shows dramatic efficacy in the treatment of this disease and represents the poster child of targeted cancer therapy [2]. To date, the US FDA has approved 30 small molecular inhibitors and seven therapeutic antibodies targeting kinases as cancer treatments, and there are multiple clinical trials currently undergoing early evaluation of

novel small-molecule inhibitors for an array of kinases implicated in cancer and other diseases.

A considerable body of recent evidence demonstrates that kinase dependency could occur in the absence of a kinase mutation. For example, *FGFR1* mRNA expression predicts FGFR tyrosine kinase inhibitor sensitivity in lung, head and neck cancers [3,4]. Furthermore, the signaling pathways driven by these oncogenic kinases rarely act in isolation but rather cooperate as networks that undergo extensive crosstalk and feedback. It is therefore no longer sufficient to examine kinases as single entities; instead they should be investigated as complex networks working in a concerted fashion [5]. More importantly, one of the common resistance mechanisms in response to kinase inhibitors is the reprogramming of kinase dependency via crosstalk and activation of bypass signaling pathways. Therefore, deploying kinase inhibitor combinations that target different nodes in cancer signaling networks as a means of eliminating oncogenic signaling in cancer cells represents an exciting research trend in personalized medicine in oncology [6,7].

Computational tools to dissect kinase dependency in cancer

Next-generation sequencing is the state-of-the-art technology to identify candidate kinase dependencies in cancer that arise as a result of kinase mutations (i.e., point

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mutation, insertion/deletion and fusions). Novel technologies beyond sequencing are needed to unravel kinase dependencies in cancers that may be independent of kinase mutations. Large-scale functional genomics using RNA interference (RNAi) screens provide a systematic approach to uncover kinase dependency and new therapeutic targets in cancer cells [8–10]. Large-scale pharmacological screening using selective kinase inhibitors also provide an alternative chemical biology approach to interrogate kinase dependencies in cancer cells [11,12]. However, due to unexpected drug–kinase interactions (polypharmacology) and off-target effects, target deconvolution for large-scale pharmacological screening data remains a significant challenge in chemical systems biology. New technology in medicinal chemistry such as the comprehensive high-throughput kinase profiling provides a parallel approach to interrogate compounds and drugs against hundreds of targets (kinases) in a single screen. Both potency and selectivity of a compound can be determined simultaneously by such screens, providing a tool to survey the complex drug–kinase interactions in an unbiased manner [13]. Deconvoluting candidate drug targets as well as determining the functional relevance of kinase dependencies from diverse screening datasets represents a prerequisite for performing precision oncology. Therefore, the challenge for cancer genomics has shifted the bottleneck from data generation to data analysis and interpretation.

“Kinase inhibitor connectivity map is a novel and user-friendly web-based program that systematically connects a set of query kinases to kinase inhibitors based on quantitative profiles of the kinase inhibitor activities.”

Recently, several computational tools have been developed to overcome this challenge [14,15] including the Kinase Addiction Ranker (KAR) [16]. KAR is a novel computational algorithm that integrates high-throughput drug-screening data, comprehensive quantitative drug–kinase binding data and transcriptomics data to predict kinase dependencies in cancer cells. By integrating the comprehensive kinase profiling data with the pharmacological screening data, KAR computes a score for each kinase within a cancer cell. For each cancer cell, KAR first assigns compounds in the high-throughput pharmacological screening data to one of five bins based on drug sensitivity. The bin number determines how many points each kinase target of the drug receives by the scoring algorithm. For example, targets of compounds in Bin 1 (most sensitive) receive 20 points and Bin 5 (most resistant) targets receive -10 points. Next,

quantitative kinase-binding data are dichotomized as inhibited or not inhibited for each compound based on user-defined threshold. Transcriptomics data are then used to filter out low expressing kinases in the specific cell line of interest. Finally, p-values are computed by using Chi-square and Fisher's exact tests to determine if there is a significant association between kinase inhibition and drug sensitivity in the cell line. As an output, KAR generates a 'kinase dependency' list, which is a ranked list of kinases with high correlation to phenotypic outputs such as cell proliferation or survival based on the pharmacological screening data. As a proof of concept, KAR was applied to rank kinase dependencies in 21 lung cancer cell lines using publicly available pharmacological screening data from the Genomics of Drug Sensitivity in Cancer. KAR predictions of *FGFR1* and *MTOR* dependencies in a lung large cell carcinoma line H1581 were experimentally validated by genetic (knock-down) and chemical (drug combination) approaches [10,16]. KAR was also applied to determine the kinase dependencies in 151 leukemia patient samples, revealing candidate kinases which are potential therapeutic targets in leukemia for further pharmacological and biological studies [16].

Computational tools to connect kinases with therapeutics

Once the kinase dependencies in a specific cancer context have been identified, the next challenge is to predict what drugs would be useful in targeting these kinases. Kinase inhibitor connectivity map (K-Map) is a novel and user-friendly web-based program that systematically connects a set of query kinases to kinase inhibitors based on quantitative profiles of the kinase inhibitor activities [17,18]. The K-Map is inspired by the 'connectivity map' concept [19], where gene expression changes are used as the 'universal language' to connect between biological systems, genes and drugs. Instead of gene expression signatures, comprehensive kinase activity profiles are used as the 'language' for connecting kinases and small molecules in K-Map to reveal the complex interactions between kinases and inhibitors. As an example, essential kinases-mediating resistance to the EGFR inhibitor gefitinib in an EGFR mutant non-small-cell lung cancer (NSCLC) cell line were identified from a kinome RNAi screen. By querying these essential kinases to K-Map, the tyrosine kinase inhibitor bosutinib was predicted as a more effective drug than gefitinib in killing EGFR mutant NSCLC cells. *In vitro* experiments validated that bosutinib alone is a more effective agent than gefitinib, and that the combination of bosutinib and gefitinib demonstrated synergistic effect in EGFR mutant NSCLC cells [18]. This example

illustrates the utility of K-Map in connecting kinases with kinase inhibitors and identifying drug candidates for combinations. The power of such integrative bioinformatics approaches is highlighted in a recent study where KAR was integrated with K-Map to dissect the kinase dependencies in triple-negative breast cancer cell lines. The predictions from these computational tools were validated by published literature and follow-up *in vitro* experiments [20].

Conclusion

As we build on years of basic scientific discovery, recent advances in the fields of cancer genomics and medicinal chemistry are now converging to revolutionize cancer treatment. The computational tools described here have the potential to harness the power of big data in biomedical sciences and provide new strategies to delineate kinase dependencies in cancer cells. Together with other high-throughput screening

methods, these computational tools will accelerate the discovery of driver kinase targets and identification of rational drug combinations for personalized medicine.

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