A promiscuous kinase inhibitor reveals secrets to cancer cell survival

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Deregulated kinase signaling networks drive the growth and survival of many cancer cells. However, the genetic complexity and rapidly evolving nature of most cancer cells create challenges when identifying the most relevant kinases to inhibit to achieve optimal therapeutic benefits. A new strategy that takes advantage of a well-characterized multitargeted kinase inhibitor describes a nongenetic approach to tease out key kinases that promote proliferation of specific cancer cell types.

Disruption to protein kinase signaling as a result of genetic mutations and altered protein expression or regulation is a hallmark feature of many cancer cells (1). As such, kinases and the development of specific kinase inhibitors have become a significant focus of anti-cancer treatment strategies, buoyed by successes in cancer treatment, such as the tyrosine kinase inhibitor imantinib (Gleevec®) in the treatment of chronic myelogenous leukemia (CML),[2] where ~95% of the patients harbor the constitutively active BCR-Abl tyrosine kinase fusion protein (2). However, there are few cancer types like CML, where the cancer cells appear to be sustained by a single deregulated kinase and where inhibition of the cancer-fueling kinase has dramatic biological and clinical effects. Instead, the genetic complexity of most cancer cells, particularly so in advanced metastatic cancer, presents a challenge for achieving therapeutic benefits using potent and highly selective kinase inhibitors. In addition, the inevitable emergence of drug resistance is a problem that occurs with most kinase inhibitors (3). As a result, the idea of a one drug–one target approach has received much scrutiny. How then, can we rationally identify the multiple targets and multitargeted compounds needed to effectively combat cancer? Rao *et al.* (4) present a new nongenetic strategy to identify what combination of kinases must be simultaneously inhibited to achieve a sustained anti-proliferative and death-inducing effect on tumor cells.

Previous thinking held that more promiscuously acting drugs would likely be less beneficial in treating a clinical situation and potentially more toxic to normal tissue. But recent work has demonstrated that effects with pleiotropic drugs depend on the disease and the drug's pharmacological properties. Indeed, the identification of drugs that interact with multiple targets and have polypharmacological features may actually offer therapeutic benefits for complex diseases with a diverse array of genetic alterations, such as cancer. Well-known examples of common drugs with polypharmacology and therapeutic benefits include aspirin and metformin. Aspirin (acetyl salicyclic acid) may have as many as 23 different targets that account for its analgesic, anti-pyretic, and anti-coagulating properties (5). Similarly, metformin, which is one of the most frequently prescribed drugs for controlling blood glucose levels in diabetics, acts on a variety of metabolic proteins in the liver and intestines (6). Metformin is also recognized to have anti-cancer activity through inhibition of the PI3K/Akt/mTOR kinase signaling pathway and may reduce the incidence of cancer in diabetics (7). A number of kinase inhibitors with polypharmacology features have therapeutic benefits in treating various cancers and include sorafenib, dasatinib, regorafenib, and bosutinib (8).

A major challenge then for the cancer-targeting field is to identify the key kinase signaling pathways that sustain proliferation and survival of specific cancer cell types and then to find a drug or combination of drugs that can inhibit those pathways. A variety of approaches can address this challenge, including systemic analysis of drug combinations and genetic knockdowns or knockouts. However, these strategies can present challenges when attempting to evaluate or manipulate multiple targets. Rao *et al.* (4) provide another strategy that uses a multikinase inhibitor with established targets and mechanisms of action to predict more effective drug combinations or motivate the development of new multitargeted kinase inhibitors. The approach uses a broad-spectrum kinase inhibitor called SM1-71 that acts by targeting the ATP-binding site and contains an acrylamide moiety that forms covalent adducts with cysteine residues. Thus, this compound has both reversible and irreversible mechanisms of interacting with kinases. An accompanying publication by the authors (9) established the pharmacology of SM1-71, showing that more than 20 kinases were inhibited by SM1-71 at nanomolar to low micromolar potency, many of which were regulators of cell growth and proliferation.

The inhibitory effects of SM1-71, a control compound with the acrylamide removed, or more specific kinase inhibitors were tested in a variety of cancer cell lines that harbored a diverse set of genetic mutations. Importantly, the effects of SM1-71 and established kinase inhibitors on cell proliferation factored in a growth rate (GR_{50}) correction that took into

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[2] The abbreviations used are: CML, chronic myelogenous leukemia; PI3K, phosphatidylinositol 3-kinase.

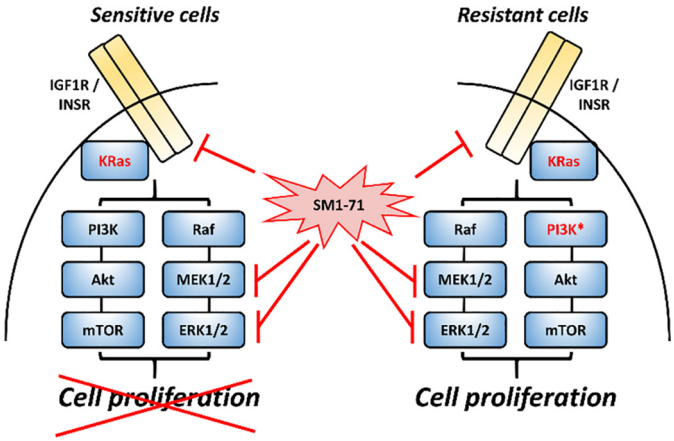

Figure 1. Using polypharmacology probes to assess cancer cell line–dependent kinase signaling. Summary of proposed signaling mechanisms involved in the cell-dependent responses to the polypharmacologic probe SM1-71. Adapted from Fig. 3 by Rao *et al.* SM1-71–sensitive H23 cells on the *left* contain a $KRAS^{G12C}$ mutation and are effectively inhibited by targeting IGF1R/INSR and MEK1/2. SM1-71–resistant H460 cells on the *right* contain $KRAS^{Q61K}$ and $PI3KCA^{E545K}$ mutations. The use of a PI3K inhibitor may enhance sensitivity to kinase inhibitor combinations in these cells.

account the doubling time of each cell line to determine whether the treatment caused partial cell growth inhibition, cytostasis, or cell death (10). Eight of the 11 cell lines tested were sensitive to SM1-71, which turned out to be more potent than the individual treatments with selective kinase inhibitors of ERK1/2, MEK1/2, PI3K, ALK, or EGFR and provided support for the polypharmacology approach. To determine why SM1-71 was more effective, the authors focused on the H23 lung cancer cell line containing an activating $KRAS^{G12C}$ mutation. They demonstrated that analysis of differential phosphorylation downstream of KRAS in the presence of SM1-71 or the control compound pointed to AKT as a critical readout in SM1-71's mode of action. Testing SM1-71 against an array of kinases capable of acting on AKT allowed the authors to narrow down the key kinases to the insulin-like growth factor-1 receptor/insulin receptor tyrosine kinases (IGF1R/INSR) and MEK1/2 protein kinases as the primary drivers of this cancer cell line. Thus, combining specific inhibitors of these kinases or downstream effector kinases can effectively inhibit these type of cancer cells. This strategy is amenable to testing in other cancer cell lines or with other compounds with polypharmacological properties.

As an additional benefit of this approach, the authors were able to reveal mechanisms that contribute to drug resistance in different cell types. Fig. 1 summarizes a proposed model highlighting lung cancer cells that are sensitive (H23 cells) or resistant (H460 cells) to polypharmacological agents like SM1-71. Both cell lines contain activating KRAS mutations, but the H460 cells also contain an activating mutation in $PI3K^{E545K}$ that may confer resistance to SM1-71. Thus, incorporating PI3K inhibitors may enhance the elimination of these cells. Interestingly, HCT116 colorectal carcinoma cells and H1975 non-small-cell lung cancer cells are sensitive to SM1-71 and, like the H460 cells, contain activating PI3K mutations. However, the mutations in PI3K in the HCT116 and H1975 cells are located on different amino acids, which suggests that the type of activating mutation is important for drug sensitivity or that mutated PI3K is not an oncogenic driver in these cells.

The studies establish a role for the use of polypharmacological drugs to define factors and pathways that fuel cancer growth and survival and that might be targeted therapeutically to achieve potent anti-cancer effects. The multitargeted chemical reagents can quickly narrow down the key targets and save resources that would be used in evaluating more selective kinase inhibitors in combinatorial screens. It will be interesting to determine whether the results of the current studies using 2D cell cultures will hold true when applied to 3D spheroid cell models and *in vivo* studies. Emerging evidence indicates that cells grown in 2D cultures have limitations in mimicking *in vivo* conditions and subsequent cellular response to anti-cancer drugs. Assuming that the approach can be generalized with additional models and drugs, the work from Rao *et al.* highlights a potentially powerful new strategy to help researchers understand and target the kinases that are essential for the survival of genetically complex cancer cells.

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