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Preview

"Going KiNativ": Probing the Native Kinome

Lee M. Graves[1,*] and David W. Litchfield[2,*]
[1]Department of Pharmacology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-7365, USA
[2]Department of Biochemistry and Department of Oncology, Schulich School of Medicine and Dentistry, University of Western Ontario, London, ON N6A 5C1, Canada
*Correspondence: lmg@med.unc.edu (L.M.G.), litchfi@uwo.ca (D.W.L.)
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In this issue, Patricelli et al. (2011) describe an in situ chemoproteomics approach (KiNativ™) for profiling the kinome and kinome response to specific kinase inhibitors that enables characterization of inhibitor interactions with endogenously expressed kinases in native conditions.

Protein kinases are involved in the regulation of a broad range of cellular processes and are shown to have intimate relationships with the pathogenesis of many human diseases. Accordingly, kinase inhibitors have emerged both as indispensible tools for elucidation of the biological functions of kinases and as therapeutic agents for molecular-targeted therapy (Dar and Shokat, 2010). In fact, based on the promise of drugs such as Gleevec/imatinib, which neutralizes BCR-Abl for the treatment of chronic myelogenous leukemia, and PLX-4720, which targets the V600E mutant of B-Raf that occurs frequently in melanoma, there continues to be intense interest in the discovery of new kinase inhibitors. Despite some successes, the development of novel inhibitors and the identification of their bonafide cellular targets remain major challenges. A major obstacle relates to the shared architecture and catalytic mechanism for the 500 or so protein kinases that are encoded within the human genome. In this respect, many inhibitors that are currently available are directed against the catalytic or ATP site of kinases, raising the prospect of off-target interactions with the many other kinases or other ATP-binding proteins that are present in cells. Accordingly, the design of selective kinase inhibitors typically requires detailed knowledge of the kinase target, including structural information and understanding of its regulation, as well as information regarding its oligomerization state and binding partner interactions (Dar and Shokat, 2010). An additional complication is highlighted by the recent, somewhat paradoxical observations that, in some instances, ATP-competitive kinase inhibitors can act as activators of the pathways that they were intended to inhibit. This is the case for the Raf kinase inhibitors (PLX4720, GDC-0879) that do effectively inhibit Raf signaling in cells expressing B-V600E Raf mutants (Hatzivassiliou et al., 2010; Poulikakos et al., 2010). By comparison, in cells expressing wild-type B-Raf through a mechanism that involves inhibitor-induced dimerization between B-Raf and C-Raf (Raf-1), these inhibitors actually "prime" the activation of these complexes, leading to enhanced ERK/MAPK signaling in cells. Similar observations have been made with Akt and other AGC family kinases (Frye and Johnson, 2009). Overall, these findings demonstrate that kinase inhibitors can act in a cell context-dependent manner.

Over the past decade, many powerful technologies have been developed to characterize kinase inhibitor selectivity and identify kinase inhibitor targets. These approaches have involved the immobilization of kinase inhibitors or ATP onto solid supports to enable the affinity capture of kinases. For example, in the studies described by Karaman et al. (2008), competition experiments performed with selected kinase inhibitors defined the relative binding affinities and specific kinase interaction profiles for these inhibitors. While these studies revealed considerable detail regarding the selectivity and specificity of ATP-competitive kinase inhibitors (including dasatinib, lapatinib, and imatinib), these studies were highly dependent on the use of recombinant expressed kinase domains. By comparison, other approaches have involved screening for interactions with endogenous kinases using immobilized selective or pan kinase inhibitors (kinobeads) and quantitative mass spectrometry (Godl et al., 2003; Bantscheff et al., 2007; Sharma et al., 2009). This highly effective approach has provided considerable insight into the effects of inhibitors on the kinome of multiple disease models. A complementary method (KiNativ) employs highly reactive biotinlyated acyl phosphate derivatives of ATP and ADP as an affinity tagging approach to capture large numbers of native kinases from cells (Patricelli et al., 2007).

In the new study by Patricelli et al. (2011), the utility of the KiNativ approach has been extended to enable evaluation of the functionally relevant properties of kinase inhibitors on native kinases. In short, the authors developed a targeted mass spectrometry approach to interrogate the acyl phosphate-tagged kinome from cell lysates. Using a list of selected peptide kinase fragment ions and linear ion trap mass spectrometry, the authors report enhanced detection and quantification of more than 150 probe-labeled kinases from a chosen proteome. Thus, these studies represent one of the most comprehensive, direct analyses of ATP-binding sites of the native kinome performed to date. Furthermore, by performing the acylation reactions in the presence of kinase inhibitors, it was possible to obtain apparent binding constants for inhibitors with specific kinases.

Of significance in this study, the authors provide evidence that the response to kinase inhibitors depends on both the cellular context and the native properties of the endogenous kinase. This is most clearly demonstrated with epidermal growth factor receptor (EGFR), mitogen-activated protein kinase kinase 5 (MAP2K5), and Raf kinases. For EGFR, the authors show that the potency of erlotinib was highly dependent on the context of the receptor (i.e., intact versus

solubilized membranes). Only with the intact membranes did the potency of erlotinib match the cellular efficacy of this inhibitor. These studies also revealed some unexpected observations. Profiling dasatinib lead to the discovery of MAP2K5 as a novel target, something previous drug screens had failed to do, suggesting the native conformation of MAP2K5 reflects the true binding interactions with this inhibitor. Lastly, the analysis of Raf kinase inhibitors further illustrates the advantage of the KiNativ approach in inhibitor analysis. The authors profiled Raf kinase inhibitors for binding to recombinant or native B-Raf, inhibition of phospho-ERK1/2, and cell proliferation. Importantly, large differences in the inhibitor binding and cellular efficacy were detected when recombinant B-Raf was analyzed, whereas the results with native B-Raf more closely resembled the response in cells. Moreover, the observation that ATP probe labeling increased in A-Raf and Raf-1 in response to Raf inhibitor (PLX4720 and others) further suggests that, consistent with the paradoxical activation of Raf-1 by these compounds, inhibitor-induced conformational changes were detected by this method.

Overall, there are many notable advantages to the KiNativ approach. The most significant of these is the ability to profile kinome responses of native endogenous kinases and to obtain information regarding the true binding properties of kinase inhibitors in a cellular context. In addition, KiNativ may enable identification of changes that increase or decrease the affinity of the active site for the biotinylated probes. As suggested with PLX4720 treatment of A-Raf, this may require the optimal positioning of active site residues to facilitate probe labeling. Furthermore, it is likely that other unexpected inhibitor targets such as MAP2K5 will be identified when assayed in their native conformations.

One potential disadvantage of this method is that it requires sophisticated methods of mass spectrometry as well as knowledge of the protein/peptide sequence and desired ion fragments for analysis. The acyl phosphate probe must also react with one or more of the conserved lysines in the catalytic pocket of the kinase. In contrast, affinity capture of kinases using immobilized inhibitors (Godl et al., 2003; Bantscheff et al., 2007; Sharma et al., 2009) is more adaptable to standard methods of mass spectrometry. The method described here also requires the preparation of cell lysates and is dependent on the efficacy of the ATP/ADP probe chemical reactions that requires the removal of endogenous ATP. These treatments may disrupt normal protein-protein interactions and allow proteolysis, dephosphorylation or other modifications to occur. In light of these limitations, it is unlikely that any one method will be sufficient for all kinase-inhibitor analyses. With that said, the authors have successfully applied this technology to broadly analyze large numbers of kinases in parallel and demonstrated the efficacy of this unique approach to gain new insight into kinase inhibitor specificity.

In conclusion, by demonstrating that for several inhibitors the binding profiles closely corresponded to their cellular activity, it is evident that KiNativ represents a significant advance over kinome and kinase inhibitor profiling that relies exclusively on the analysis of purified components. Given the utility of kinase inhibitors as research tools and promising agents for molecular-targeted therapy, the opportunity to characterize their interactions with native endogenous kinases undoubtedly represents a significant step forward.

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