COMMENTARY

Protein kinase profiling

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Dr. Pelech is one of the co-discoverers of the MAP kinase family of cell signaling proteins and an author of over 180 scientific publications. He has been a reviewer for over 28 scientific journals and 28 international granting agencies.

Over the last decade, there has been an explosion of interest in protein kinases as drug targets. Currently, about 30% of pharma R&D activity is focused on these enzymes. Eight small molecule kinase inhibitors are on the market, and at least 80 more are advancing through human clinical trials. Upwards of 500 additional kinase inhibitors are lying in the wings in the discovery and preclinical stages. Within the next decade, most of the newly FDA-approved pharmaceutical drugs might target kinases. The resulting arsenal of selective kinase inhibitors could offer unprecedented medical treatments for so many of the major diseases that confound us today, such as cancer, diabetes, Alzheimer's and many of the 400 other human diseases that have linkages to cell signaling defects.

About 2% of the human genome encodes at least 515 protein kinases. They play key roles in cellular regulation, and they may catalyze the reversible phosphorylation of more than 10,000 proteins at greater than 500,000 sites. It is no wonder then that defective signaling through malfunctioning kinases can have such a profound effect on human health.

Remarkably, barely two dozen, non-proprietary protein kinases serve as the intended targets of all of the approved kinase inhibitors and those in clinical trials. For some targets, such as ErbB2 and p38 MAP kinase, more than a dozen different companies have competing drug candidates in clinical trials. As much as 95% of the protein kinases are still largely neglected by industry, except perhaps for deployment in counter-screens to check the specificity of leading drug candidates.

In vitro screening for protein kinase inhibitors benefits a target-driven drug discovery approach. However, our knowledge of the normal and pathophysiological functions of the vast majority of the protein kinases and their regulation is extremely deficient.

An alternative strategy to *in vitro* protein kinase screening is to track the expression and phosphorylation of protein kinases and their substrates in lysates from cell lines exposed to compounds in their culture media. This can also be performed with tissue extracts from animals treated with drug leads *in vivo*. This is much more revealing than *in vitro* kinase profiling, since the drugs are tested in a physiological setting in living cells. Furthermore, this can yield a wealth of information about the more obscure protein kinases that could advance them as attractive drug targets.

We have developed two methodologies at Kinexus to track protein kinase expression and phosphorylation in cell and tissue extracts using the best of 3000 commercial antibodies that we have validated in-house. One of these, which is called Kinetworks™, is based on multi-immunoblotting with up to 40 antibodies simultaneously. Over the last 7 years, we have used the Kinetworks™ technique to analyze over 10,000 diverse cell and tissue lysates. Much of this data is available to the scientific community through our on-line Internet databank KiNET (www.kinexus.ca/kinet.htm). Our findings have uncovered profound differences in kinase protein levels based on species, tissue, gender, age and disease status. By contrast, from gene expression analyses, we determined that 90 percent of the human protein kinases genes are ubiquitously expressed at appreciable mRNA levels in over 30 diverse tissues. It is clear that the correlation between mRNA and protein levels for kinases in human tissues is extremely poor and the mRNA data can be very misleading.

Most protein kinases are regulated by direct phosphorylation, so assessment of the specific phosphorylation states of these kinases can provide an indirect measure of their activity status. Again, the thousands of Kinetworks™ analyses that we have performed have often revealed an inverse correlation between the amount of active and total forms of a given protein. In retrospect this is not surprising, since cells probably maintain a reservoir of inactive proteins that are poised for rapid stimulation by phosphorylation. Once phosphorylated and activated, these proteins are also tagged for speedy degradation. In view of this, we believe that the phosphorylation status of proteins is likely to show the tightest correlations with the phenotypic changes in cells in response to extracellular stimuli and disease. With over 500,000 human phosphorylation sites, the phosphoproteome should be a rich source of biomarkers for disease diagnosis.

To track the expression and phosphorylation of protein kinases and their substrates in higher throughput and at lower cost, we recently developed Kinex™ antibody microarrays. These microarrays are printed with over 600 pan- and phosphosite-specific antibodies assembled from over 20 commercial suppliers. While these protein microarrays are extremely sensitive and reproducible, they can generate a high degree of false positives and negatives. This is largely due to the unpredicted cross-reactivity of antibodies and the occurrence of proteins in complexes. Nevertheless, these antibody microarrays have tremendous potential for discovery of biomarkers and utility for both patient and drug profiling.

Setting up the infrastructure to perform extensive *in vitro* or *in vivo* kinase profiling within most companies really does not make financial sense, never mind the lost opportunity that arises from the inordinate time it takes to establish such operations. The real need for "systems biology" approaches is becoming increasingly expounded. In reality, large scale proteomics analyses are inadequately supported by government and charitable granting agencies, so it is almost impossible for academic laboratories to carry out this kind of research. But the availability of cost effective commercial proteomics services still makes this feasible. The affordability of our services is reflected by the fact that we have provided our Kinetworks™ and Kinex™ analyses to over 650 academic labs in addition to over 150 companies world-wide.

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