



drug discovery Channel: Article

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Systems-Based Approach Gaining Ground Hype Turns into Opportunity as Systems Biology Begins to Bear Fruit

Angelo DePalma, Ph.D.

Biologys status as the engine of drug discovery is hardly ever questioned today, but it was not always so. For the first century of the modern pharmaceutical age, chemists were expected to make educated guesses, often with little input from biology, at which structures to make and how to optimize them. Todays target-based drug discovery methods illustrate how far we have traveled.

Most pharmaceutical companies are mining the big targets like kinases and GCPRs that were discovered and validated by biologists. Meanwhile the search for new disease-relevant targets continues. Target-based drug discovery provides not just a biological rationale but a molecular basis for designing specific properties into drug structures.

Still, the relationship between candidate drugs and biological targets remains unpredictable. While the pharmaceutical industry is by no means ready to abandon target-based methods, the next "best thing" in drug discovery, systems biology, is already producing fascinating results.

Medicinenet.com defines systems biology as "the study of biological systems taking into account the interactions of the key elements such as DNA, RNA, proteins, and cells with respect to one another." A systems-based approach to drug discovery does not examine drug-target interactions exclusively and sometimes, not at all. Rather, it seeks to uncover new therapeutic molecules and mechanisms by determining the net effects of treatment on biomarkers, genes, and proteins related to biological pathways.

Understanding Drug Response

“Genomics and bioinformatics have always focused on target discovery,” said Ian Humphrey-Smith, CEO of the Biosystems Informatics Institute (BII, www.biiuk.com). “Until we are able to reduce adverse effects and enhance target selectivity, we are not adding value.”

BII takes a three-pronged approach to systems biology that incorporates bioinformatics screening, near-target kinetic modeling, and molecular modeling of protein-protein interactions. Also critical, says Humphrey-Smith, are the interactions among alternate pathways and positive/negative feedback loops. “Unless you understand these accurately, you are not understanding the drug response.”

At a recent conference at the New York Academy of Sciences (www.nyas.org), four speakers presented their versions of the pathway approach. Yan Feng, Ph.D., a scientist at the Novartis Institute for Biomedical Research (www.nibr.novartis.com), presented data on cell cycle inhibitors, generated through a pathway approach.

Dr. Feng first identified genes that control various stages of the cell cycle through a genome-wide siRNA knockdown. Most knockdowns affect one major slice of the cell cycle and one or two others to a lesser extent. Some crossover also exists among the siRNAs, as some work on more than one gene. Cells were sorted and identified using an imaging cytometer, which classified cells according to cell cycle status through such parameters as DNA content, nuclear size, or levels of PH3 (a marker for mitosis).

In much the same way, Dr. Feng was able to identify compounds from a small library that activate or knock down genes associated with individual cell cycle stages.

Also, two speakers offered results from experiments to design a drug screen, based on patterns of activated or inactivated genes or proteins.

Paul Young, Ph.D., vp of research at Avalon Pharmaceuticals (www.avalonrx.com), demonstrated that disrupting specific targets or cellular pathways with siRNAs causes reproducible changes in the gene expression profile of treated cells. Events downstream of knocked-down genes, as well as the gene itself, are affected.

Dr. Young selected 5-20 genes whose changes were most stable and from them constructed a panel or barcode that serves as a molecular signature specific to the disrupted pathway or target. Panels appear like zig-zag graphs with some genes showing lower activity and others higher activity.

Compounds tested against this panel of genes in a cell-based assay fall into two basic categories: those that more or less match the gene activity pattern, or barcode, of the siRNA knockdown and those that do not. The former constitute the hits for that screening experiment. What is exciting about this approach is it assumes nothing about the specific target or targets on which the compound acts, which is almost certainly a protein or receptor. Such barcodes can uncover new drugs as new disease-related pathways and targets and even pick up off-target activity.

Ellen Berg, Ph.D., CSO at BioSeek (www.bioseekinc.com), offered an approach similar to Dr. Young's, but with proteins as the readout instead of genes.

BioSeeks BioMAP discovery system, of which the protein signature panel is a part, employs cell-based assays in which cells are stimulated by multiple inputs and measured for multiple readouts. The signatures consist of measuring levels of clinically relevant biomarkers, such as receptors, cytokines, chemokines, enzymes, and lipid mediators, whose concentrations change when a drug is administered. Subsequent experiments utilize those same signature panels to screen new molecules. Those that produce the same signature panel are considered hits.

Dr. Berg described an experiment in which eight signature proteins were measured in the presence and absence of antitumor necrosis factor-alpha, or anti-TNF-alpha, a drug used to treat rheumatoid arthritis. Drugs that act through the same mechanism as anti-TNF-alpha must produce the same protein signature as the drug and are likely to be good starting points for a new drug.

According to Dr. Berg, Bioseek scientists can detect and distinguish hundreds of distinct mechanisms of action for every important therapeutic category using similar protein expression profiles. In addition, the profiles can help uncover activity against specific subtypes of, say, inflammatory disease. Again, all this is possible without knowledge of a specific chemical mechanism.

Peter Krutzik, a graduate student working in the lab of www.stanford.edu discussed a cytometry technique, phospho-specific flow cytometry, or phospho flow, which was pioneered by the Nolan group. As its name implies, phospho flow is a flow method that primarily targets phosphorylated proteins. The group says the technique is

rapid, scalable, and highly multiplexing, taking on up to 10 different cellular characteristics from a heterogeneous cell population, for example from peripheral blood.

Krutzik presented his work on the Jak-Stat signaling cascade, defects in which are implicated in systemic lupus erythematosus. Using phospho flow, Krutzik identified compounds active against the Jak-Stat pathway activity from a natural product library and demonstrated a dose-response effect among the hits.

The IT Factor

Software and information management has become a critical component and in some cases, a bottleneck for systems biology work. Data from one type of experiment often disappoints, so biologists increasingly need to analyze multiple, complimentary data sets simultaneously, says Kevin Meldrum, director for genomics marketing at Agilent Technologies (www.agilent.com). Recognizing the importance of information technology, in 2005 Agilent acquired Silicon Genetics.

“Our customers are interested in looking at a system within the context of a disease or model organism and trying to understand how the pieces fit together,” said Meldrum. For example, Agilent customers will compare data for DNA deletions, translocations, or insertions to more comprehensive gene-chip DNA or RNA expression data. Or using Agilent’s ChIP-on-chip technology, biologists can examine interactions among regulatory proteins, such as transcription factors, and compare them to DNA expression in the same cells.

“Instead of taking a single point of view for a biological problem, its now possible to look from multiple aspects and put the pieces together to obtain insight into factors that trigger gene expression,” said Meldrum. “Its an exciting time to be in genomics, as multiple techniques are emerging that complement gene expression, and which together present a more holistic picture of complex biological systems.”

Another firm GeneGO (www.genego.com), founded in 2000, provides software for high-throughput biological pathway experiments. The company offers MetaCore, an integrated software suite for functional analysis of microarrays, SAGE, proteomics, and other experimental data. MetaCore is based on a curated database of human protein-protein and protein-DNA interactions, transcriptional factors, signaling, metabolism, and bioactive molecules. GeneGos MetaDrug product predicts drug metabolism and toxicity of new compounds in the context of the complete biological system.

About one-third of GeneGos customers are academic researchers, the rest from pharm/biotech, said COO Andrej Bugrim, Ph.D., who noted exponential growth of interest in systems approaches from a small number of visionary academics, eight years ago, to mainstream industrial biologists today. “Its definitely now more mainstream,” Dr. Bugrim said, and “becoming more so all the time as tools like microarrays become less expensive.” The next few years, he believes, will be marked by greater integration of high-throughput tools and standardization of complex workflows.

Modeling and Simulation

Another area where IT plays a role is in modeling and simulation of complex, interrelated systems. Gene Network Sciences (GNS; www.gnsbiotech.com) recently collaborated with Johnson & Johnson (J&J; www.jnj.com) on a project to unravel the mechanism of a preclinical-stage cancer drug. J&J wanted to know which pathways and targets the drug affected and how those pathways drive the endpoint of cell proliferation. GNS fed molecular profiling data into its network inference engine and uncovered thousands of possible models for how the drug affects the clinical endpoint.

GNS scientists then forward-simulated the drugs activity through a whole-genome knockdown. Eventually they discovered not only the molecular mechanisms driving proliferation but also new biomarkers that will allow J&J to stratify patients entering clinical trials.

“The only way to improve the dismal success rates of current drug development is to understand the biology better,” said Colin Hill, CEO of GNS.

Because it is so new, systems biology is still in its honeymoon phase with biologists, particularly those involved in drug discovery. To many biologists, however, the systems approach represents the natural convergence of parallel, high-throughput biological methods, glued together by ever more powerful information technology. As technology advances further, one hopes that hype will evolve into opportunity. “Systems biology is something people have talked about for a long time but no-one knew how it would take shape,” Hill said. “Now its beginning to live up to its promise.”

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