DEVIL IN THE DETAILS

No matter what you call it, metabolic profiling is crucial to drug discovery and health.

BY RANDALL C. WILLIS AND DAVID FILMORE

"Genomics and proteomics remain incredibly rich sources of data, but their complexity and relative inaccessibility have prevented them from reaching their full potential as sources of drug targets," says Alan Higgins, senior director of investigational medicine for Paradigm Genetics (*www.paradigmgenetics.com*), explaining the challenge drug development specialists face. "In addition, changes in genes and proteins do not always reflect changes in biological function, so it is difficult to identify mechanisms and establish cause and effect using these techniques alone."

By comparison, he suggests, metabolic profiling provides more functional information at the biochemical level that researchers can use to see what pathways and mechanisms are altered by specific interventions or diseases. Thus, company scientists are putting a lot of time and resources into developing new tools and techniques to identify metabolic markers that will explain disease pathogenesis and serve as biochemical signposts during preclinical and clinical drug screening.

Approaching metabolism

Researchers performing metabolic profiling are looking for changes in metabolite levels in response to either an internal change, such as the onset and progression of disease, or an environmental perturbation, such as diet, toxins, or drugs. Clinicians and scientists have performed metabolic profiling for decades to diagnose disease and monitor its progression. For example, people with diabetes determine their need for insulin by watching for changes in blood-glucose levels.

In some cases, scientists have taken a global approach to metabolite characterization, looking for significant changes in individual molecules among a myriad of compounds in the sample. Other researchers, however, are taking a more targeted approach, hoping to focus their analytical efforts on the metabolic components of an individual biochemical pathway, such as lipid metabolism. More often than not, however, the approach is dictated by the profiling technology used, and there seems to be no limit to how companies approach the situation.

"Metabolic profiling, along with gene-expression profiling and quantitative histomorphometry, is a vital element of our proprietary systems biology technology platform, which we regard as our 'global positioning system' for triangulating mechanisms of disease and drug actions," Higgins explains (Figure 1). "Our measurements are mainly based on LC/MS, but we also use GC/MS to ensure broad coverage of different biochemical classes."

Using these methods, Paradigm Genetics is trying to identify biomarkers and therapeutic targets for disorders resulting from liver injury and metabolic diseases, such as diabetes and obesity. The company is also working with partners to establish a therapeutic product pipeline.

Another technology gaining traction in metabolite profiling is NMR spectrometry, with companies like Metabometrix (*www.metabometrix.com*) and Triad Therapeutics (*www.triadthera.com*) leading the way. These systems are often hyphenated to LC or solid-phase extraction systems to reduce sample complexity. But according to Werner Maas, vice president of Bruker BioSpin (*www.bruker-biospin.com*), the NMR spectra of biofluids can yield large numbers of resonances, and it might be impossible to assign each resonance to a specific metabolite without using multidimensional spectroscopic methods (*1*).

"A different approach is to apply statistical methods to describe the commonalities and differences of a large sampling of spectra," Maas explains. "The aim is to find principal components or characteristics such that, when plotted, all 'normal' samples obtained from a pool of healthy subjects group together or cluster. An abnormal or diseased subject would fall outside this classification and become an outlier."

Using statistical software packages with NMR, researchers can identify potential biomarkers for everything from disease pathology to drug efficacy and toxicological profiles.

Metabolon (*www.metabolon.com*) also relies heavily on the application of statistical analysis, but it uses software to deconvolute data derived using high-end MS (Figure 2).

"With our modified hardware and software, we are able to identify many of the molecules in the metabolome," explains John Ryals, Metabolon CEO and founding CEO of Paradigm Genetics. "The mandate of Metabolon is to develop the most advanced metabolomics technology and use it to solve problems in drug discovery and development."

The company has focused its efforts on identifying biomarkers associated with neurodegenerative disorders, with the goal of developing its own therapeutic product pipeline, but it is also actively seeking partnering opportunities.

Profiling in action

Rather than focus on specific pathways or biological mechanisms, some companies have chosen to attack metabolic screening challenges using a systemwide approach. For example, researchers at

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6.3.4.7
1-(5'-Phosphoribosyl)-
5-formamido-4-
imidazole carboxamide
3.6.1.20
4.3.2.2
Urea cycle
NH3
IMP
1.1.1.205
2.7.4
3.6.1.5
1.7.1.7
6.3.5.2
3.1.3.5
2.7.1.73
3.6.1.19
2.4.2.8
2.4.2.22
Inosine
3.5.4.4
2.4.2.8
3.2.2.12
XTP
3.6.1.17
2.4.2.1
Hypoxanthine
Xanthine
Urea
1.1.1.154
Histidine
Inosine

the biotechnology company Beyond Genomics (*www.beyondgenomics.com*) have developed a Molecular Phenotyping platform that allows them to profile complex biological samples by measuring and identifying transcripts, proteins, and metabolites. They then use their BioSystematics informatics platform to identify correlations and connections between the molecular components, and coordinate this data with clinical information. Using these systems, the scientists hope to improve drug safety and efficacy analysis, as well as better understand disease pathophysiology.

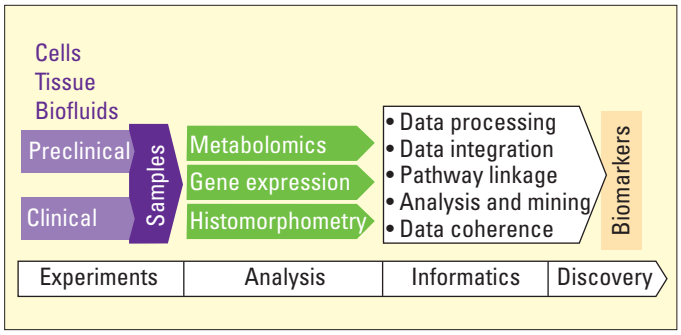


Figure 1. Three-part harmony. By combining the results of metabolomics, gene expression, and histomorphometry studies, researchers at Paradigm Genetics hope to identify biomarkers of disease and drug mechanisms of action. (Courtesy of Paradigm Genetics.)

In February, Beyond Genomics entered into a pilot project with AstraZeneca to use the biotech company's platforms and expertise in predictive toxicology. By discovering specific biomarker signatures associated with adverse events, the companies hope to identify unforeseen mechanisms of drug-induced toxicity. "By partnering with AstraZeneca in the important area of toxicology, we can demonstrate that Beyond Genomics' systems biology approach delivers critical knowledge that can be applied to reduce costs and advance drugs with improved safety profiles," explains Muz Mansuri, executive chairman.

Perhaps the greatest strength of these metabolic profiling methods, however, comes from their ability to identify previously unknown correlations between unexpected metabolic partners, either in disease or in response to chemical perturbation. In May, Shawn Ritchie, a researcher at metabolomic specialist Phenomenome Discoveries (*www.phenomenome.com*), described the preliminary results of a research project with GlaxoSmithKline (GSK) at the Targeting Metabolic Syndrome Conference in Boston. In this study, the researchers examined the metabolic response of rat muscle cells to treatment with a peroxisome proliferator-activated receptor (PPAR)-delta agonist being developed at GSK.

Nuclear receptors primarily involved in lipid and carbohydrate metabolism, PPARs have caught the attention of several drug development companies with efforts in fields such as obesity and cardiovascular disease. The PPAR-gamma agonists Avandia (rosiglitazone), from GSK, and Takeda Pharmaceuticals' Actos (piaglitazone) have been marketed for treating Type II diabetes, while several other PPAR agonists are in clinical trials or are pending FDA approval.

In the recent study, however, the researchers used Phenomenome's *DISCOVA*metrics platform to perform nontargeted metabolome analysis of drug dosage and time effects on rat muscle metabolism. They found they could accurately predict specific fatty acids in triglycerides and phospholipids from skeletal muscle even though global free fatty acid signatures did not appear to change with drug treatment.

"It is important to note that the most valuable observations in this study would have been overlooked using a targeted method of analysis," Ritchie says. "This strongly reemphasizes the importance of nontargeted methods for studying the metabolome, and, in particular, intact complex lipids."

Supply-side activity

As the importance of metabolic profiling continues to grow, instrumentation companies are tailoring their products (and marketing approaches) to the molecular and biochemical characteristics of the target metabolite families. For example, Waters (*www.waters.com*) has developed the Acuity system of ultraperformance liquid chromatography (UPLC). As the name implies, the new system shows improved resolution and detection sensitivity and, when linked to the company's orthogonal acceleration time-of-flight (TOF) mass spectrometer and informatics system, a higher degree of selectivity for more reliable metabolite identification in complex mixtures. The company has been working with scientists from AstraZeneca, GSK, Imperial College, and the Scripps Research Institute to identify drug toxicity markers in plasma and urine samples taken from animal models in early drug development.

Similarly, at the recent American Society for Mass Spectrometry conference in Nashville, Bruker Daltonics (*www.bdal.com*) introduced its new ultrOTOF-Q system, an orthogonal TOF mass spectrometer that incorporates the company's Focus technology. Offering improved peak resolution over a broad mass range, the new system is particularly useful for obtaining structural information on molecules in the low *m/z* ranges typical of metabolites. The company also introduced the Metabolic Profiler platform it developed in collaboration with Bruker BioSpin. By allowing researchers to run samples through both electrospray ionization-TOF MS and one-dimen-

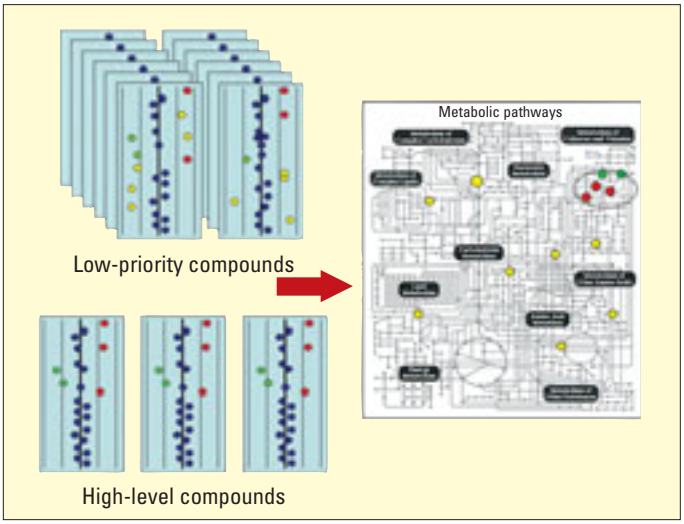


Figure 2. Metabolism à la mode. Using high-end MS and informatics tools to perform metabolic profiling, researchers at Metabolon can prioritize compounds on the basis of their ability to cause desired biochemical changes. (Courtesy of Metabolon.)

sional flow NMR, and analyze them using a single informatics package, the new systems will facilitate the identification and characterization of metabolites as surrogate markers for drug efficacy and toxicity.

One problem, however, with using NMR or MS to characterize the full spectrum of molecules in a given metabolome is that these instruments can be rather expensive, and some researchers find it difficult to get time on the machines to perform otherwise routine analyses. To address this problem, scientists at electrochemical detection specialist ESA, Inc. (*www.esainc.com*), developed the Metabolomics System, an instrument and software package designed for metabolite-profiling studies. The system incorporates the company's CoulArray multichannel electrochemical detector, which, according to company marketing manager Darwin Asa, allows researchers to focus on redox-active metabolites in a sample and ignore numerous "uninteresting" waste or housekeeping metabolites, such as urea or glucose, which might interfere with analysis (Figure 3).

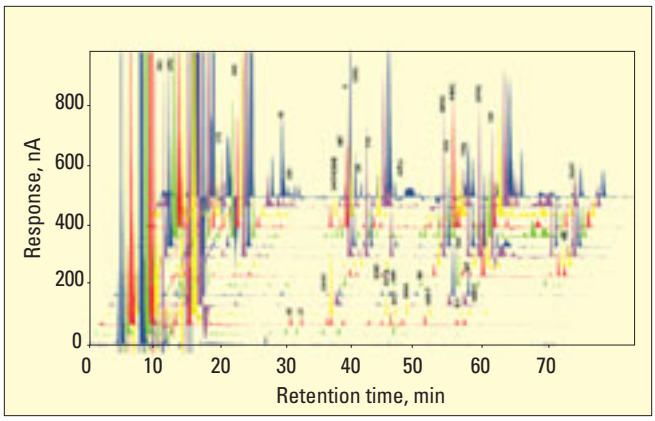
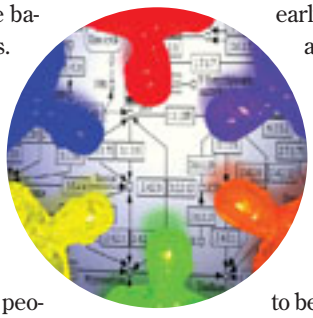


Figure 3. Profiles in courage. Using their Metabolomics System, ESA researchers can profile hundreds of redox-active compounds. (Courtesy of ESA.)

"Even if molecules are not resolved chromatographically, we can often resolve them electrochemically on the basis of their differing redox potentials," Asa explains. "Using the CoulArray to identify a key metabolite for identification allows researchers to focus the analytical power of their mass or NMR spectrometer on identifying the important biomarkers, instead of spending large amounts of time and effort trying to identify everything that might be potentially important."



As happens so often in science, whereas many people agree about the importance and potential of metabolic profiling, there is some disagreement about what to call the technology.

The name game

"The definition or nomenclature game is confusing," explains Metabolon's Ryals. "I have heard the technology called biochemical profiling, metabolic profiling, metabolomics, and metabonomics, but it is all the same thing."

Ryals, who prefers "metabolomics," defines the technology as the measure of small (<2000 Da) nonproteinaceous molecules in samples, regardless of the samples' source. The molecular-weight cutoff is largely a pragmatic one, he argues, because essentially all monomeric molecules above this range are proteins.

According to Ryals, the choice of "the L word" follows the logic of the naming conventions of the other "-omics" technologies, namely:

Gene → Genome → Genomics,
Protein → Proteome → Proteomics,
Metabolite → Metabolome → Metabolomics.

Not everyone sees the issue as simply as Ryals, however.

Although Jeremy Nicholson, head of biological chemistry at Imperial College, London, and founder of Metabometrix, agrees with Ryals' premise that metabolomics is an extension of the metabolome, he believes the technology involves the analysis of individual cells under static conditions. By comparison, he argues, metabonomics takes a more global or systems biology approach.

"In metabonomics, static cellular and biofluid concentrations of endogenous metabolites are evaluated," he explained in a recent review with colleagues from Imperial College (*2*), "as well as full time courses of metabolite fluctuations, exogenous species, and molecules that arise from chemical rather than enzymatic processing (metabonates)."

Using this definition, he argues, metabolomics is a subset of metabonomics.

"I used to think that when a science area degenerated—or evolved—into one of nomenclature battles, it was time to get out," Ryals relates. "However, this one seems to be happening very early. All I can say is that 'metabolomics' is generally accepted by most scientists and not attributable to anyone. 'Metabonomics' is used by Nicholson and his colleagues, and he claims to have invented the term. So, one school is very egocentric, and the other is reasonably pure."

Others, however, seem to take a more pragmatic approach to the controversy.

"The whole -lomics versus -nomics debate seems to be much ado about nothing," ESA's Asa says. "You could even argue that some of the debate is being driven by forces unrelated to scientific study. As we have been promoting our products, we have tended to use the -lomics and -nomics terms interchangeably."

Paradigm Genetics' Higgins agrees. "Metabolomics and metabonomics are essentially the same thing, although there are some that choose the latter to describe NMR-based methods," he explains. "We prefer not to get into those arguments, so we tend to prefer the term 'biochemical profiling.'"

Regardless, as a logical extension of the genomics and proteomics revolutions, metabolite profiling is likely to present researchers and clinicians with a better understanding of drug mechanisms and disease pathology. To (badly) paraphrase the Bard, "What's in a name? That which we call a quantitative analytical technology would still measure metabolites."

References

(1) Maas, W. *Today's Chemist at Work* **2004,** *13* (1), 21–24.
(2) Lindon, J. C.; Holmes, E.; Nicholson, J. K. *Anal. Chem.* **2003,** *75,* 385A–391A. ■