METABOLOMICS a playbook for functional genomics

If we have learned anything from scientific research in the last 20 years, it is that finding cures to complex diseases is difficult. Despite the promise of the genomic revolution, disease progression and patient outcomes are still not easily predicted by genetic factors alone.

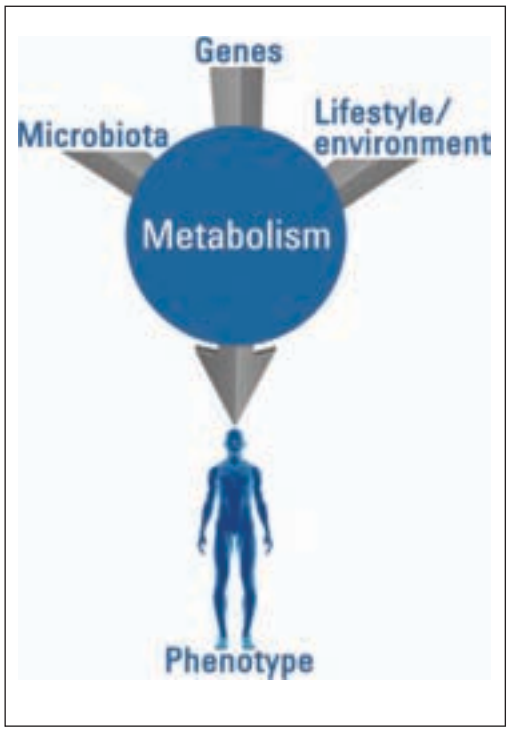
By Dr David Ferrick

Part of the explanation for this gap is that age-related diseases are heavily influenced by changes in cellular metabolism. For example, cancer, the quintessential genetic disease, is being redefined by metabolic abnormalities, endowing tumours with the ability to outcompete normal cells in the tumour microenvironment. Adding fuel to the fire, there is early evidence that directing the metabolic programming of immune cells may improve the efficacy and durability of immunotherapies that are completely agnostic to cancer's genetic drivers. Measuring metabolism, either functionally or using metabolomics, provides insight into the dynamics of cellular energetics.

We should aim to reconcile these seemingly discordant approaches to understanding disease, and apply this knowledge to early medical intervention before complications and irreversible damage have occurred. Perhaps imagine that the intersection of a person's genes and metabolism are like a game of cards. In life, the hand you are dealt is represented by the genome you inherit. Metabolism is how you then choose to play those cards. Meaning you can control your nutrition choices, lifestyle or environmental conditions. This analogy would suggest that although you may be dealt a bad genome card, if you 'play your cards right' by living a healthy lifestyle, you could potentially alter the outcome and *vice-versa*. In essence, genetics provides the boundaries of 'what can happen', and metabolism shows us 'what is happening.' The savvy healthcare provider should therefore endeavour to understand both the genetic and metabolic drivers of a disease to really see the full picture (**Figure 1**).

These new and provocative scientific perspectives on metabolism are being driven by enabling advances in metabolomics – the study of metabolites that are transformed during metabolism and visualised as functional maps of cellular processes. Metabolites sit uniquely at the functional end of the genome and the front-end of our environment. They can be assembled into profiles and collated phenotypes that provide a functional readout for interpreting biological behaviour, in the context of both genetic and environmental factors. Metabolomics uses sophisticated analytical chemistry techniques, combined with software that provides computational methods, to reconstruct cellular pathways of

Figure 1



interest. In the hands of skilled scientists, these tools are revealing changes in metabolic pathways that are providing new insights into disease progression.

Metabolomics is a rapidly-growing field and some analysts believe the market for metabolomics tools will be worth more than a billion dollars by 2020. The scientific impact will be most likely realised in research and product development, specifically diagnostic and drug discovery applications. Primary areas of application today are in oncology, followed by central nervous and cardiovascular disorders. Of note are the nascent opportunities for huge growth in immunology and immunotherapies. Virtually every area of life science research will benefit in some way from increased knowledge of the metabolic networks that underlie their relevant cellular processes.

Metabolomics, like its counterparts in genomics, transcriptomics and proteomics, has the same inherent issues of complexity, instrumentation and informatics. For this reason it will also be challenging to avoid some of the pitfalls and learning experiences of the other 'omic' approaches. To this point, it has been encouraging to witness significant advances in pivotal areas that are driving adoption and standardisation in the last decade.

At present, the majority of metabolomics analyses are performed with mass spectrometers (MS) because of their broad dynamic range, reproducible quantitative accuracy and ability to assess complex bio fluids. Due to most samples having large numbers of diverse metabolites with nearly identical sizes, separation with gas chromatography, liquid chromatography or capillary electrophoresis is routinely performed prior to MS analysis.

Although the majority of instrumentation available today still requires significant training, in general these technologies are faster, more sensitive, more reliable, and more automated than ever before. The same can be said of the methods used on these instruments. More kits that include methods, standards and reagents for the most routine protocols is also an opportunity. Kits would greatly lower the barrier to entry, improve data quality and increase reproducibility.

Progress in metabolomics was initially driven by biomedical research, leading to new understandings, particularly for the etiology of age-related diseases. But the question that remains at the centre of many translational and clinical programmes is whether metabolomics can also change the trajectory of healthcare delivery. We will not have to wait too long for answers, as the research community is becoming more aggressive in their attempts to convert metabolic discoveries into diagnostics and therapeutics. Increasingly, metabolic networks and metabolite associations are being enlisted to identify biomarkers for diagnosis and validate targets for drug discovery. It is likely that this will be a focus and proving ground over the next decade.

Our ability to drive metabolomic measurements into the mainstream of science will be a key success factor. This will be challenging due to the multitude of pathways that overlap and influence each other, in both the forward and reverse directions. Indeed, a current map of all cell metabolism pathways looks somewhat like a bowl of spaghetti, with more than 8,700 reactions and 16,000 metabolites (**Figure 2**). Technique improvements are expected in separation science, mass analysis and informatics. Through informatics, metabolomics results will eventually connect to functional novel cell-based assays, which will directly link metabolomic, metabolic flux and bioenergetic data, respectively, to cellular pathways and biological mechanisms. These advancements are filling the gap in our ability to directly measure cell biology, physiology and medicine.

The ability to characterise more metabolites, enabled by the recent improvements in mass spectrometry instrumentation, has created a huge demand for software that can process, analyse and visualise large data sets. Several public and commercial software resources are now available that

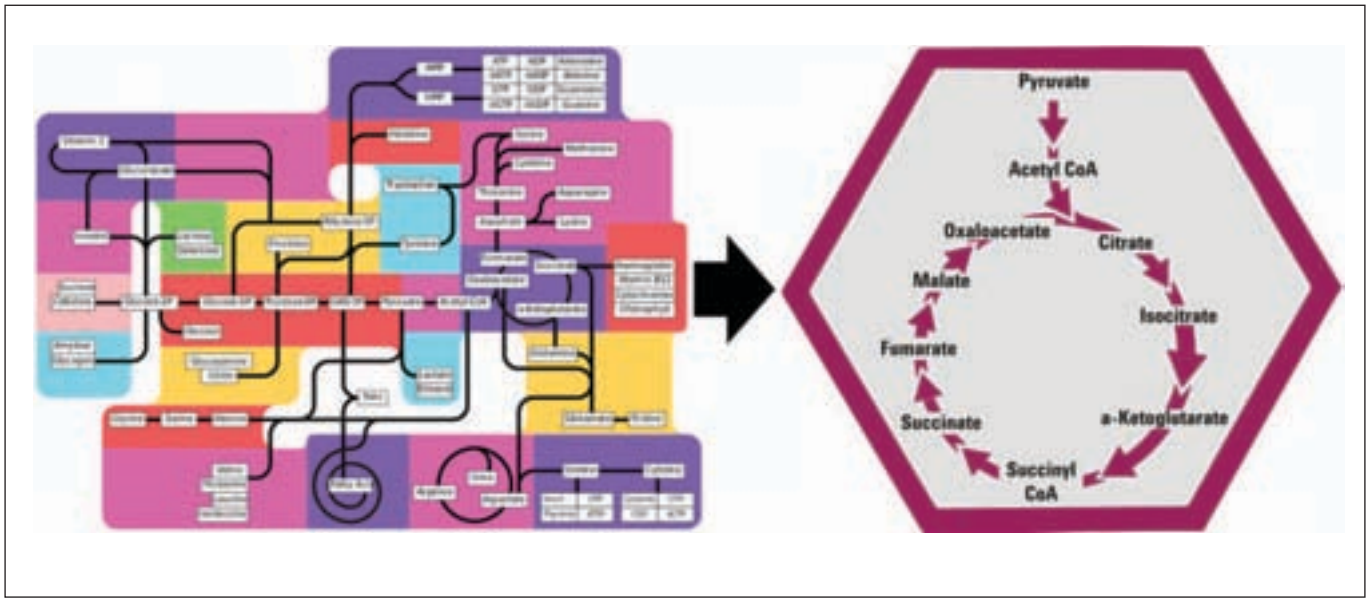


Figure 2

can identify and quantify much larger numbers of metabolites with greater reliability. Widespread adoption is being facilitated by the development of comprehensive analytical tools that standardise the manner in which data is visualised and interpreted, as well as improved protocols for sample handling and quality control. Commercial vendors are playing an increasingly important role in the overall development and maturation of these tools, enabling greater mainstream acceptance and use.

With mainstream adoption will come greater understanding of the two basic categories of metabolomics; untargeted (discovery), and targeted. Untargeted metabolomics is a method of identifying the differences between the metabolomes (full metabolite complement of an organism) of two sample groups, both known and unknown. Untargeted metabolomics can be useful to 'fish' for novel clues about a physiological condition or a unique pathological process. There are three basic steps to untargeted metabolomics: 1) Profiling, or differential expression analysis that reveals metabolites of interest which differ in abundance between the two sample groups; 2) Identification of the metabolites' chemical structure using spectral libraries and databases; 3) Interpretation, which can be the most challenging step, as this involves the process of associating the identified metabolites to distinct biological processes and/or properties, the end game. Targeted metabolomics, on the other hand, is a hypothesis-driven approach most often aimed at novel associations between known metabolites in well-defined pathways. With targeted metabolomics, the metabolites under study are already known and meaningful to the researcher. Sample comparison is based solely on these known compounds. This method has become very popular for identifying novel biomarkers and metabolic pathways within the context of specific physiological and, more importantly, disease states. As such, this type of analysis is becoming more commonplace in basic research and drug discovery.

Augmenting targeted metabolomics is a relatively new and exciting area of metabolic research called 'metabolic flux analysis'. One can actually trace specific metabolites to generate a dynamic roadmap by modifying them via the replacement of an element such as carbon, with a heavier stable isotope of that element carbon 13, ^{13}C (99% of all carbon exists as carbon 12). Thus, the route and eventual fate of a metabolite can be followed as the labelled carbons are processed through the metabolic pathways and become part of new metabolites. This is a powerful approach for effectively building dynamic maps that can illustrate how the flow of metabolites initiate and maintain both beneficial and disease phenotypes. Literally hundreds of living blueprints now exist for proliferating tumours, differentiating stem cells, activating immune cells and degenerating neurons; and the list grows larger each day. These central and critical blueprints of normal and disease processes highlight the need for rapid quantification of the activity of a given pathway; and with the latest tools available today researchers can now watch the switching that occurs between these pathways, in almost real time.

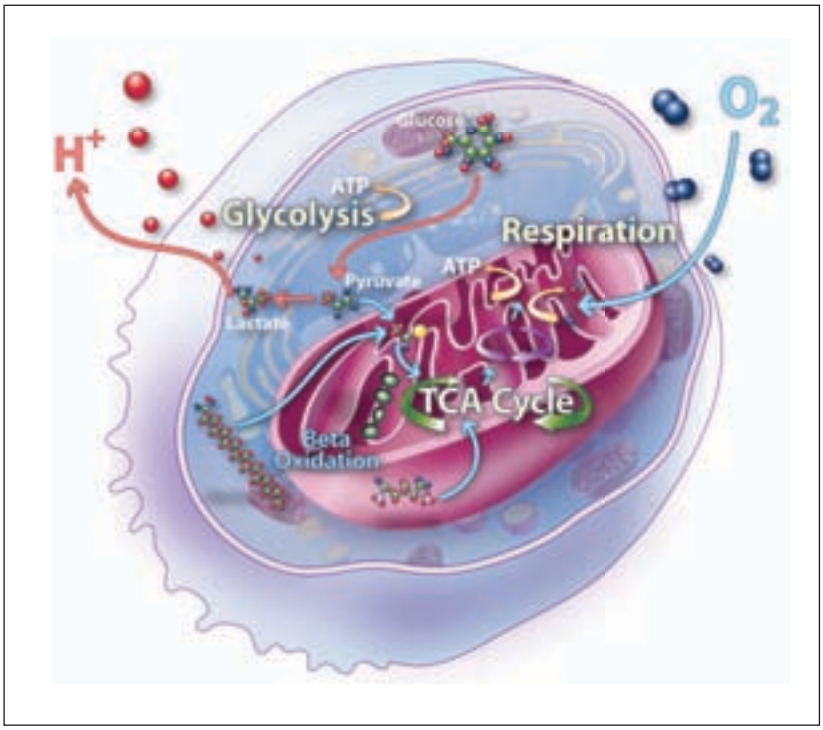


Figure 3

As most metabolic pathways are very efficient, with large dynamic ranges, when a cell is forced to use a less-efficient pathway it does so quite effectively, but potentially at a cost. This cost could represent the earliest stages of disease. With the tools just described we can now map these changes. In other words, because it takes a lot of stress to cause metabolic pathways to change, falter and fail, this may explain why it can take decades for cancer to establish, the first neurons to die in degenerative diseases, or complications to develop from obesity. Furthermore, scientists are now beginning to understand the mechanisms, whereby metabolic fitness can be directly influenced by environmental factors such as nutrition, exercise, smoking, sedentariness, exposure to pollution and so on.

Where to start a metabolomic analysis, and even more so identify which changes matter, can be daunting due to the large size, degeneracy and complexity of the metabolic system. This challenge can be overcome by knowing where your cells are within the metabolic landscape, a big picture view, as defined by mitochondrial respiration and glycolysis. This view of the metabolic landscape will enable the researcher to identify a point of interest, and more importantly in which direction their research should go. With this information researchers can then target the metabolomic and/or flux analysis towards a select number of pathways that are *a priori* shown to effect the biological state of a living cell.

Facilitating this type of approach has been made easier due to a modernised method to measuring energy metabolism in living cells. In fact, over the last 10 years, more than 2,500 publications have emerged that describe a method that essentially provides researchers with a metabolic compass. By measuring the rate of extracellular fluxes of oxygen consumed by cells, one can selectively measure aspects of mitochondrial energy metabolism in real time. Likewise, by measuring the rate of export of protons from the cell into the media, one can measure glycolytic processes with equal accuracy and ease. These two major metabolic networks – mitochondrial respiration and anaerobic glycolysis – produce energy, as well as transiting a large fraction of carbons in the cell for both catabolic and anabolic processes (**Figure 3**).

By establishing which of the two major energy pathways a cell prefers, and the substrates they are utilising and expelling into the media, researchers can then narrow down the search before metabolomic analyses, and identify which metabolic pathways are important for the cell to establish or maintain a particular biological state. Additionally, these approaches provide a higher-level quantification of the total flux in the mitochondrial and glycolytic networks, which can then be used to help balance flux equations to determine the appropriate movement of carbons in the dynamic blue prints of metabolic circuits just discussed.

In summary, metabolism can no longer be considered as a discipline unto itself, detached from the considerations of genetic programmes, protein networks and pathways of living cells, tissues and organisms. With significant improvements in the technologies available to measure and harness the ubiquitous nature of metabolic processes, there should no longer be 'black box' areas in biology, especially those influenced significantly by environmental factors. As powerful as genetically altered animals and *in vitro* manipulated cell models have been for basic and applied research, they can bypass and silence many critical environmental factors as they would otherwise present in our daily lives. Incorporating tools such as metabolomics and live cell bioenergetics to assess chronic environmental aspects of age-related diseases will enable us to improve our laboratory models for the most debilitating, costly and lethal diseases of the modern world: cancer, obesity/diabetes, neurodegeneration and cardiovascular disease. Perhaps not surprisingly, evolution supports

this notion as higher levels of cellular regulation, and the complex organisation of multicellular organisms, appeared much later than the fundamentals of energy metabolism. As the tools to measure metabolic behaviour in biological systems become more commonplace, we will continue to re-evaluate some of the more dogmatic components of the post-genome era, and create a more balanced appreciation of the integrated nature of biology. DDW

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