DDNEWS
SPECIAL REPORT

Cancer Metabolomics

INTO THE METABOLIC MAELSTROM

Metabolomics shifts from map-making to therapeutic strategizing

BY RANDALL C WILLIS

HIS HEAD TILTED BACK, his mouth agape with awe, the white-coated explorer simply could not take in the vastness of the ancient map that spread before him, taking up most of the wall with its multihued bulk. Even his young eyes strained to make out the delicate details of the dashed and solid lines that spewed in all directions, marked here and there by a strange nomenclature of letters and pictographs.

The knowledge of his ancestors lay before him, wisdom that he had already spent a lifetime trying to understand, knowing that he could spend the rest of his life in this pursuit and never add anything of substance to that store of information.

With patience and determination, he finally located his place in the universe, a confusing spasm of vectors and whorls to the Western side of the cartographer's nightmare. So peripheral was his search that part of his known universe was missing, a ragged tear a reminder of the fragility of paper and the limitations of yellowed Scotch tape.

Following the faint blue dashes emanating from his corner, he saw the immensity of what lay before him both in space and over the years to come. He also knew that his was but a static view, that the lines and nexi were in constant flux, vibrating with activity.

He slowly shook his head. He was not prepared. His undergraduate biochemistry degree had barely scraped the surface of the metabolic maelstrom that played out before his eyes.

He knew he would come back to the Boehringer Mannheim (now Roche) *Biochemical Pathways* chart again and again.

CREDIT: SCIEX

Sciex recently launched its Routine Biotransform Solution, designed to automate small-molecule metabolism and biologics catabolism studies by leveraging intuitive metabolism data processing with automated structural interpretation, advanced processing options and templates for analysis of antibody-drug conjugate catabolism.

Maelstrom measured

First published in 1965, the *Biochemical Pathways* chart developed by Gerhard Michal highlighted the sheer vastness of what was understood about cellular metabolism more than 50 years ago. The story the chart described and the science that evolved after it formed the basis of drug discovery and disease pathology efforts of the decades since.

A decade or so ago, however, the landscape shifted as researchers began to view metabolism less as something that simply happened in the background—providing energy and the building blocks of cells, tissues and organs—and more as something to be systematically reviewed both in healthy and diseased cells to better understand pathophysiology. The era of systems biology gave rise to fields such as glycomics, lipidomics, proteomics and more broadly, metabolomics.

"I think there is a realization, particularly in the last two to three years, that metabolism is really no longer viewed as something that happens in the cell to generate energy and building blocks, but something that is central and core at orchestrating many of the fundamental roles of the cell and the decisions and functions of the cells, such as differentiation and proliferation and cell death and many other different functions in the cell," offers Ian Hayes, commercial director at Luxcel Biosciences, a company developing resources to directly measure a variety of metabolic parameters in high-throughput cell culture.

"A lot of the work that was done on the various 'omics platforms did lay a foundation to say that metabolism was important in some of these aspects, but it didn't have the breadth to allow people to investi-

gate it in all the ways that it could be relevant," adds James Hynes, Luxcel's head of R&D. "I think what's been happening recently is there has been a convergence of some fields."

Because of research findings now almost a century old, cancer has been a particularly strong focus of metabolomics probing, with theories to explain metabolic shifts triggering uncontrolled cell growth, differentiation and spread that rose and fell like the metabolite levels themselves.

"For many years of research on cancer metabolism, there have been a lot of false starts," says Tim Pardee of Wake Forest Baptist Health and collaborator with Rafael Pharmaceuticals (formerly Cornerstone Pharmaceuticals). "[Ott] Warburg himself originally hypothesized that the mitochondria in tumor cells were damaged,

CREDIT: RAFAEL PHARMACEUTICALS

"In pancreatic cancer, we focused on one of the standards of care, modified FOLFIRINOX," says Sanjeev Luther, COO of Rafael Pharmaceuticals. "The other standard of care is gemcitabine and Abraxane combination. We're starting a new Phase 1 program shortly with CPI-613 plus GemAb combo. We're also starting a Phase 1 program in sarcoma in lung."

and that obviously turned out to be false."

Similarly, he continues, there was a suggestion that the metabolic pathways that run through the mitochondria—the cellular energy center—are of minimal importance. A growing body of evidence suggests that this is also not true.

"As the field has evolved, we've come to realize that there is a lot of heterogeneity in tumor metabolism and that tumor metabolism can have several key roles that have been underappreciated in the past," Pardee explains.

"It's only recently that people have really come to appreciate that the regulatory mechanisms for these enzymes really are differentially expressed between normal tissues and tumor tissues," he says. And it is that differential expression that offers an opportunity to find a therapeutic window for interventions specifically targeting cancer metabolism, such as those being developed at Rafael Pharmaceuticals (more below).

Part of what has allowed such windows to open has been significant improvements in the technologies central to metabolomics research—the workhorse LC- and GC-MS platforms, data analysis resources, sample preparation tools—as well as an expansion of mix-and-measure liquid assays that offer a clearer picture of metabolic dynamics.

"We're now calling it precision metabolomics because 'metabolomics' has come to mean a lot of different things," clarifies John Ryals, CEO of Metabolon. "It's a very precise technology. It covers a huge breadth of molecules."

He offers the example of blood, where in a single sample, they can monitor and name about 2,000 molecules. And the fact that these molecules are maintained homeostatically, he says, highlights their importance.

"That has allowed us to now start moving into precision medicine, which is kind of replacing the systems biology nomenclature a bit," he enthuses. "So, we can take an individual and take their blood sample and we can get a good reading on their general health, we can see if they have different kinds of chronic disease."

Measuring so many molecules in a variety of samples accurately enough to offer meaningful insights is a significant challenge.

"Obtaining reliable, reproducible data is critical in any analytical experiment," says Amanda Souza, metabolomics program manager in chromatography and mass spectrometry at Thermo Fisher Scientific. "While robust analytical methods can be achieved, it's important to consider all aspects of the experiment, including sample handling and preparation."

Consistency plays a major role in metabolomics analyses, she presses. Thus, care should be given to how samples are obtained, stored and further processed.

"Slight deviations in the workup can be reflected in the data, such as

METABOLIC CONTINUED ON PAGE 22

METABOLIC

CONTINUED FROM PAGE 21

if one or two tissue samples are left at room temperature longer than the remaining samples," she continues. "Moreover, a robust technique for thorough tissue homogenization to fully expose intracellular and extracellular matrix is a good approach."

On more than one occasion, Souza says, an interesting observation turned out to be the result of inconsistent sample handling or preparation. A surprising finding turns out to be not so surprising.

"A well-known example of this is the use of different anticoagulant agents in blood collection tubes where each agent impacts the metabolic profile differently," she recalls. "It's important to remind ourselves that the use of analytical quality control helps to improve data quality by being able to detect and account for these nuances."

According to Ryals, sample processing was a significant focus for Metabolon and has become a subject of pride.

"The processes on how we handle samples, I think we've really engineered that to the point where it is hard to see that we will be able to get much more out of it," he waxes. "It is already far better than we ever thought we would get to."

And instrumentation advances have allowed researchers to move beyond untargeted, survey-like metabolomics studies to more targeted approaches.

"A non-targeted or unknown approach captures a wider space of chemical entities," explains Souza. "A greater number of metabolites are measured but here, identification tools are needed to determine the putative identity of these metabolites, and then further confirmed with purified standards."

She suggests that this approach is useful in discovery experiments because in many cases what metabolites will be a telltale sign to the investigation is unknown.

"In this case, profiling as much of the metabolome provides a broader scope of the phenotype in the search for unknowns," she presses. "Acquiring data in an unknown fashion also provides the ability to retrospectively mine the data, which is helpful in answering future questions without reprocessing samples."

In a targeted approached, however, metabolites of interest are determined ahead of the analysis.

"These metabolites may be specific to a biochemical pathway or an array of compounds reflecting different chemical classes," Souza says. "A targeted approach can be relative or absolute quantification, depending on the question being answered."

These methods are often carried out in a high-throughput routine manner, she continues, and analysis is typically straightforward because you know what to look for.

"More people are asking me about untargeted analysis for metabolomics," adds Souza. "High-resolution mass spectrometers enable us to generate the necessary data for this approach. In turn, having comprehensive analysis software to process this type of data is in demand. Identification tools for determining unknown species and statistical capabilities for differential analysis are of great interest."

And as with the processing of samples, the processing of data can be a critical point of failure if not handled correctly.

"When you get to the data handling side, one of the things we have spent most of our time is developing software analysis of this data," says Ryals.

"The onboard computing power of the instruments has become a lot better," he admits. "We used to crash the machines because we filled up their cache with data. Now, we don't do that. They've built in enough computing power."

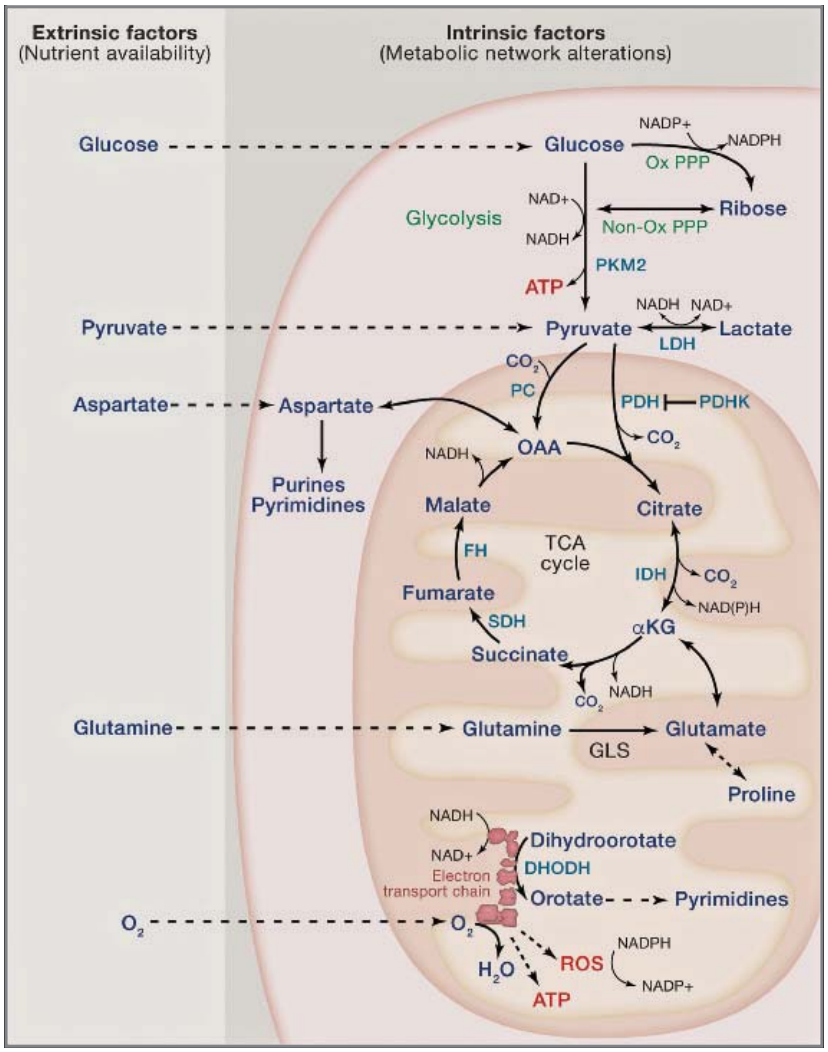
"But at the same time, it made the data processing of the finished data a lot more challenging," he counters. "There's a lot of software on those instruments that are doing things that do not help you out, and in fact, they make the data lower-quality. So, we've had to go in and bypass many of the onboard instrumentation mechanisms to make that quality higher."

"We kept up with those developments by continuing to develop our software and our mathematics so that we could deal with this incredibly complex and noisy data set that comes off of these instruments," he adds.

In March, Sciex announced the launch of its Routine Biotransform Solution, designed to automate small-molecule metabolism and biologics catabolism studies by leveraging intuitive metabolism data processing with automated structural interpretation, advanced processing options and templates for analysis of antibody-drug conjugate catabolism. The solution links LC, QTOF-MS and the company's MetabolitePilot Software 2.0.

"Until now, customers have not had many options for software processing around biologics catabolism studies," explained Sciex's Farzana Azam, senior director of the Pharmaceutical/CRO Business, in the announcement. "Additionally, the data processing and interpretation has often been manual and laborious."

"When combined with SWATH Acquisition, researchers can do single-injection analysis and get comprehensive coverage of their sample," she continued. "This enables them to be confident they are not missing any low-level metabolites/catabolites that may be important."



INSIDE OUT. Always in flux, cellular metabolism is impacted by both internal and external factors that should be accommodated in any models. (Adapted from Vander Heiden and DeBerardinis. *Cell* 2017;168:657-666.)

Enhancing metabolic models

"There are lots of very expert metabolomics people who will do very detailed work," says Luxcel's Hynes. "And in instances where you've got core facilities and you've got access to that as a research group, it is an incredibly powerful tool."

"But in areas where—and it's been growing—metabolism is just one part of a bigger story, researchers just don't have the time or the bandwidth to go off and become expert on 15 or 16 different platforms," he explains. "They need convenient ways of assessing critical central parameters of their cell models."

According to his colleague Hayes, one problem researchers faced with the onset of metabolomics, genomics and such disciplines was that to a large extent, these technologies were siloed.

"You had specialist groups that were working on metabolism, and specialist groups that were working on metabolomics, as they still do now with mass spec instrumentation, but there were not available assays to put in the hands of your regular cell biologists who want to look at metabolism in the context of their particular area of interest," Hayes explains.

"Along with other companies—we're not the only one in this space—Luxcel has really brought assays into the cell biology labs in a way where they can do bite-sized, meaningful metabolism studies in their own model of choice, without having to be specialists in mass

"Metabolism is just one part of a bigger story; researchers just don't have the time or the bandwidth to go off and become expert on 15 or 16 different platforms. They need convenient ways of assessing critical central parameters of their cell models."

–James Hynes, head of R&D for Luxcel Biosciences

spec or specialists using dedicated analytical instruments."

To do this, Luxcel developed a series of assays designed to work in microtiter dishes on standard plate readers.

"The importance of an optical assay, a fluorescence assay, is that they are really amenable to broad accessibility," Hayes explains. "The assay works on a fluorescence plate reader, which is the workhorse of most pharma and academic cell culture labs."

He estimates that there are currently about 20,000 plate readers sitting on lab benches worldwide.

The company's goal is to offer cell biologists assays to measure almost any metabolic parameter and to smooth the process of correlating findings from one parameter with those of another.

"We have easy, mix-and-measure assays for mitochondrial respiration, glycolysis, looking at mitochondrial respiration and glycolysis under a stress environment," Hayes lists. "We have assays that measure fatty acid oxidation, and we have assays that also measure intracellular oxygen concentration. And we're launching a whole suite of assays that are multiparametric, real-time, live-cell kinetic assays that work on these fluorescent microtiter plate readers."

And the democratization that is occurring at the bench has its parallels at the bioinformatics stations, as well.

"At post-data gathering stages, we have been able to develop software and biological understanding of how these pathways are working together," says Metabolon's Ryals. "As we start now automating and putting that into software, it starts getting very powerful."

"Data visualization tools that we've developed are really enabling investigators to get a much better understanding of what they're looking at without having to spend months in the library or months online trying to understand some biochemical pathways," he presses, stating bluntly that if you want to have impact on the researchers, you can't expect that they're going to go get another degree in computer science. "It's got to be easy."

Beyond simply expanding the repertoire of approaches to cataloguing and monitoring the dynamics of metabolic shifts in cancer, the extension of these resources into the cell biology sphere is also allowing researchers to better understand how well their *in-vitro* cellular models match known impacts of disease and therapeutic intervention *in vivo* (see also sidebar, "Shifting culture-al norms").

As part of its recently announced MetaCell project, funded under the European Commission's Fast Track to Innovation initiative, Luxcel is working with BMG Labtech and Oxford University's Adrian Harris to demonstrate a central role for cancer metabolism and the importance of physiologically relevant models in cancer.

"Drug failure and poor drug discovery in oncology, as in other areas, is really to a large extent due to a lack of correlation between laboratory models and disease, and that's a principle driver of the enormous cost of drug development at the moment," says Hayes.

"One of the challenges previously was developing what were deemed to be effective therapeutic interventions on an *in-vitro* model that didn't reflect what was happening *in vivo*," echoes Hynes. "Obviously, the metabolic pathways that are active within these cells can have a significant impact on the efficacy of therapeutic interventions, particularly when that therapeutic intervention is focusing specifically on metabolism."

"There is an incredibly adaptable and plastic metabolic network, where these cells, as conditions within the tumor alter, start to metabolize other substrates; so, where lactate becomes important, glutamine becomes important, even more recently acetate becomes important to maintain supply of things like acetyl-coA," he enthuses. "And all of this has come together in a way that people are now focusing on metabolism—particularly the catabolic processes that have become obviously important—as druggable targets for therapeutic interventions."

Targeting metabolism

Given the fundamental nature of cell metabolism, and particularly when you reach into the central engines of cell biology like glycolysis and the tricarboxylic acid (TCA) or Krebs cycle, it might be unthinkable that researchers could directly target these processes in cancer cells without triggering widespread issues with all other tissues in the body. As it stands now, targeting most cell surface receptors and signalling pathways triggers untold side effects.

To go even deeper in biology would seem foolhardy, and yet companies like Rafael Pharmaceuticals and Agios Pharmaceuticals are doing just that.

According to Rafael's Pardee, the main impetus of the company was due to its scientific founder Paul Bingham, who came to appreciate perhaps earlier than most that cancer metabolism was something that had really been underexplored in terms of a target for cancer therapies.

"I think it was mostly because people were really unsure that there would be any kind of therapeutic window," he says. "To this day, when we talk to folks about our lead compound that inhibits the TCA cycle, people are always concerned about safety in such an approach."

What Bingham recognized early on, says Pardee, was that if you picked a component of a pathway that was sufficiently differentially regulated, you could target that process in a safe and efficacious manner.

In the case of Agios, that target was isocitrate dehydrogenase (IDH), an enzyme involved in sugar metabolism through the TCA cycle. The company has three products currently in clinical trials that target different forms of IDH.

Working with Celgene, Agios is pursuing an inhibitor (enasidenib) of a mutant form of IDH2 for the treatment of both newly diagnosed and relapsed or refractory acute myeloid leukemia (AML). Similarly, its ivosidenib targets mutant IDH1 and is in early clinical studies in patients with newly diagnosed or relapsed/refractory AML, as well as some solid tumors. In very early clinical analysis, AG-881 is a dual inhibitor of both IDH1 and IDH2 mutants that Agios is testing against advanced solid tumors, again in collaboration with Celgene.

Agios also has several molecules in the target validation and compound optimization phases, and in April, the company signed an exclusive licensing agreement with Aurigene Discovery Technologies to research, develop and commercialize small-molecule inhibitors of an undisclosed cancer metabolism target.

For its part, Rafael Pharmaceuticals is also targeting the TCA cycle—but using its Altered Energy Metabolism Directed (AEMD) platform—and has homed in on analogues of lipoic acid, a co-factor for several enzymes that regulate entry points to the cycle.

As Pardee explains, Bingham recognized that several of these enzymes are regulated by the relative ratios of three forms of lipoic acid: its reduced form with two free sulfhydryl groups, an acylated form and its oxidized form. For example, when the acylated form predominates, pyruvate dehydrogenase (PDH) kinases take that as a signal that there is too much carbon flux through PDH and respond by phosphorylating and thereby inactivating PDH.

This response is precisely the mode of action of Rafael's lead product CPI-613. But more than that, the drug also performs, as the company describes it, as a cocktail of one because it also acts to inhibit the activity of another TCA cycle enzyme α–ketoglutarate dehydrogenase (KGDH) by a completely different mechanism.

"There is definitely heterogeneity in cancer metabolism," Pardee states. "There are a number of different tumor types that have shown that even within a particular tumor, you can have cancers which are more glycolytic and some which are more dependent on glutamine, for example. What this drug does is simultaneously shuts off carbon entry to the TCA cycle from either glucose or glutamine."

But beyond its doubled opportunities for efficacy, the dual mechanism of action offers a potential secondary benefit.

"What I think is another underappreciated aspect of cancer metabolism is that tumors are really highly dynamic metabolically and in a number of ways," Pardee continues. "So, if you had an inhibitor that solely inhibited entry of glucose carbons, for example, it would be highly likely that you could get evolved resistance around glutamine carbons. But by simultaneously inhibiting both, I think we're really inhibiting the ability of the tumor cells to develop resistance to CPI-613."

In his experience to date, he continues, they have yet to be able to generate any signs of resistance to CPI-613 when cancer cells have been exposed to the drug.

In late May, Rafael announced positive results from its Phase 1 study of CPI-613 in combination with FOLFIRINOX (oxaliplatin, irinotecan, 5-fluorouracil) in patients with metastatic pancreatic cancer, publishing the results in *Lancet Oncology*.

"When we look at the 18 evaluable patients who were treated in this Phase 1 study in combination with modified FOLFIRINOX, a chemotherapy regimen that by itself has a complete remission (CR) rate of less than 1 percent, and to see three of those 18 patients with CR for a 17 percent CR rate is incredibly exciting," recounts Pardee. "And then there were an additional eight patients who got radiographic responses by RECIST criteria, so an objective response rate of 61 percent."

"So more than half of the patients who were treated with that combination had an objective radiographic response, which is just not anything that has been

METABOLIC CONTINUED ON PAGE 24

CREDIT: METABOLON

The technologies being used by Metabolon's cancer metabolism researchers are now being called precision metabolomics because, as CEO John Ryals notes, "metabolomics has come to mean a lot of different things. It's a very precise technology. It covers a huge breadth of molecules."

METABOLIC

CONTINUED FROM PAGE 23

seen before to my knowledge in pancreatic cancer," he enthuses.

According to Rafael Chief Operating Officer Sanjeev Luther, the company is pursuing CPI-613 not only in pancreatic cancer, but also in relapsed/refractory AML, relapsed Burkitt lymphoma, relapsed T cell lymphoma and myelodysplastic syndrome. They are also initiating new Phase 1 studies with another standard of care.

"In pancreatic cancer, we focused on one of the standards of care, modified FOLFIRINOX," Luther reminds. "The other standard of care is gemcitabine and Abraxane combination. We're starting a new Phase 1 program shortly with CPI-613 plus GemAb combo. We're also starting a Phase 1 program in sarcoma in lung."

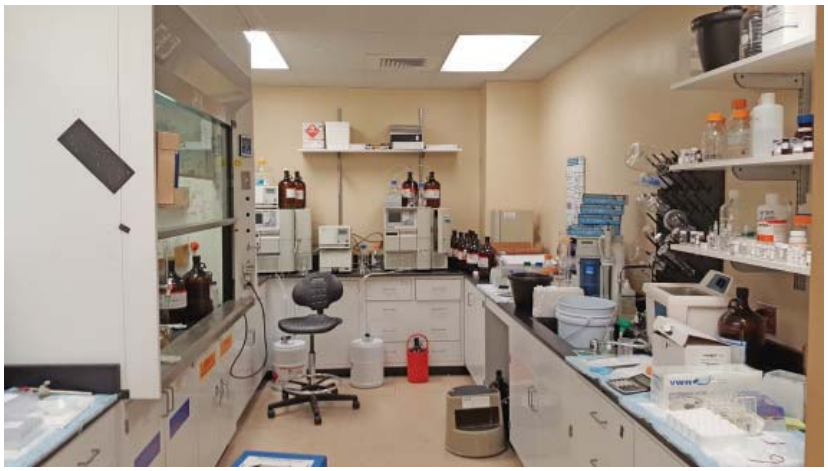
Although Rafael Pharmaceuticals and Agios are at the forefront of metabolic interventions, they are hardly alone in their interest in targeting metabolism or in using metabolomics to better understand how their current candidates impact metabolism.

"We work with maybe 500 clients a year," says Metabolon's Ryals. "Right now, I'd guess about 15 percent are cancer studies."

"Originally, it was just those companies just trying to target metabolism," he continues. "Now it's getting into the companies trying to understand what some of these things like checkpoint inhibitors are actually doing metabolically."

Such efforts are likely to become increasingly important as the complexity of metabolic influences becomes better understood, as was suggested by Dana Farber's Matthew Vander Heiden and UT Southwestern Medical Center's Ralph DeBerardinis, who wrote about cancer biology and metabolism earlier this year in *Cell*.

"Pathways downstream of oncogenes and tumor suppressors regulate cancer cell metabolism," the authors wrote. "However, the extent to which metabolic preferences are hard-wired by the tumor genotype is less clear because many non-genetic factors also influence tumor metabolism. As in all tissues, tumor metabolism is dictated by a variety of intrinsic and extrinsic factors."



CREDIT: RAFAEL PHARMACEUTICALS

The main impetus of Rafael Pharmaceuticals comes from scientific founder Paul Bingham, who came to appreciate earlier than many that cancer metabolism was underexplored as a target for cancer therapies.

The researchers suggested that intrinsic factors included things like genetic alterations, cell lineage/tissue of origin, histological subtype and tumor grade, while extrinsic factors ranged from access to nutrients and oxygen to attachment to extracellular matrix, and from interactions with stromal cells to exposure to chemotherapy and radiation.

Hearkening back to Ryals' comments about checkpoint inhibitors, the authors suggested that understanding the interactions, for example, between cancer and immune cell metabolism could be vitally important for combining metabolism-targeted therapies and immunotherapies.

"Nutrient availability and metabolism affect T cell and macrophage differentiation, raising the possibility that targeting tumor cell metabolism may either promote or prevent anticancer immune responses," they explained. "Cancer cells compete with T cells for glucose in tumors, and restricting T cell glucose metabolism causes lymphocyte exhaustion."

"Thus, therapies that decrease glucose use by cancer cells could make glucose available for T cells and enhance immune-effector functions to further limit tumor growth," they postulated. "However, if the same therapies inhibit T cell glucose metabolism, they may limit antitumor immunity."

Whatever the approach, it is clear that we have only really started to understand the full implications of the metabolic maps that decorated our lab walls in graduate school, and to go beyond viewing those pathways less as static biochemical roads and more as molecular weather maps of turbulent systems moving through our cells and tissues—systems that can turn a pleasant day into an oncological maelstrom. ■

EDITCONNECT: E061731

"I think there is a realization, particularly in the last two to three years, that metabolism is really no longer viewed as something that happens in the cell to generate energy and building blocks, but something that is central and core at orchestrating many of the fundamental roles of the cell."

–Ian Hayes, commercial director for Luxcel Biosciences

SHIFTING CULTURE-AL NORMS

As technologies for metabolite isolation and identification have improved over the last decade or so, there has been a significant re-evaluation of the paradigms once held true.

At the recent American Association for Cancer Research conference, for example, the Warburg Effect was a subject of repeated scrutiny, as researchers reflected on the assumptions made over several decades regarding metabolic realignment in cancer cells toward lactate production even in the presence of oxygen, a process described as aerobic glycolysis.

"Work that was published in 2016 by our partner in Oxford demonstrates that the effect is far from consistent across all different cancers, and that it is highly influenced by the high concentrations of glucose that is historically used in cell biology," says Ian Hayes, commercial director of Luxcel Biosciences.

It is not that Warburg misunderstood his findings, Hayes and his colleague James Hynes, head of R&D at Luxcel, are quick to point out, but rather that the effect may have had little to do with what happens within the body.

"There has been an explosion in people rushing to go and do 3D models and using specialist cells and doing very fancy experiments, but when you go back and look at the critical role that metabolism plays in cancer, you have to question fundamentally why most cell biologists do their studies in cell culture media that is at 25 mM glucose," continues Hayes, a level that is far in excess of what is found in blood or tissues.

Similarly, he says, researchers are doing their studies in an oxygen environment that they believe is around 21 percent (atmospheric oxygen); again, levels far higher than those encountered *in vivo* in tissues or in a tumor, where hypoxia can have a profound effect.

"These two parameters are profoundly important in terms of the metabolic phenotype, but they're often completely ignored in the scientist's rush to go out and get the latest 3D model," Hayes notes.

In a recent review published in *Cell*, Matthew Vander Heiden of Dana Farber Cancer Institute and Ralph DeBerardinis of UT Southwestern Medical Center highlighted the impact of such culture conditions on metabolism.

"How cancer cells generate aspartate in different contexts illustrates how such constraints can affect production of a key metabolite for nucleotide synthesis" they wrote. "Cells in culture, where oxygen is in excess, produce aspartate from glutamine via the TCA cycle, a pathway that involves multiple oxidation steps. However, in lung tumors, where oxygen is less abundant, aspartate is produced from glucose via a series of reactions with fewer oxidation steps."

"One of the things that has been most interesting for us and most surprising when we talk to customers, is the fact that even when people are conscious of controlling environmental levels of oxygen in hypoxia workstations, they are controlling the environmental level of oxygen in the chamber," Hayes says.

But once cell culture is done with cells in a 96-well plate, he explains, there is a profound gradient between the atmospheric oxygen and the oxygen at the bottom of the plate where metabolically active cells constantly consume oxygen and drive down oxygen concentrations locally.

To help researchers monitor oxygen levels, Luxcel developed MitoXpress-Intra, a nanoparticle-based oxygen sensor that measures the oxygen concentration inside and in very close proximity to the cells in culture.

The goal, explains Hynes, is to have an accurate disease-specific model such that scientists can measure metabolic parameters like glycolytic activity, mitochondrial membrane potential, ATP levels and reactive oxygen species (ROS) generation under physiologically relevant conditions. Such relevance is vital for drug discovery to succeed.

"The next phase of cancer metabolism research will need to address increasingly complex questions about how intrinsic and extrinsic influences integrate to create exploitable metabolic phenotypes in cancer," echoed Vander Heiden and DeBerardinis. "This will require consideration of the metabolic preferences hard-wired into cancer cells by tissue of origin, interactions between benign and malignant cells within the microenvironment and influences of the diet and microbiome on the host."

"Cell culture systems must be improved to better reflect the metabolic limitations in tumors, and these studies will be propelled by improvements in quantitative assessment of metabolic fluxes in different contexts." ■