in such an integrated omics service. Although the university is home to the Metabolomics Core Facility, at which metabolomics analyses on samples could be conducted, "We were always having this challenge of seeing how everything fits together," says Jones. "This is really the strength of [an integrated] approach."

Made to measure

One group that is taking advantage of General Metabolics' platform is another company in Cambridge, Massachusetts, called Mitobridge, which looks at mitochondrial dysfunction and hopes to find mitochondrially based drug targets.

"We've been working with [General Metabolics] to see what our compounds are doing in animal and cell models to find biomarkers that modulate mitochondrial metabolism," says Eric Bell, a translational biologist at Mitobridge. "It helps our efforts for drug discovery, as it could help us funnel out good or bad compounds."

Whereas General Metabolics and Mitobridge are still new, other groups have already started to see the benefit of using an integrated omics approach when trying to better understand cellular function. In 2012, cancer researchers published a study that demonstrated the advantages of using integrated proteomic, transcriptomic and network analysis to find molecular changes in breast cancer tumors (*Mol. Cell Proteomics* **11**, M111.014910, 2012).

"Our goal was to search for new biomarkers," says Dennis Sgroi, a cancer pathologist at the Massachusetts General Hospital in Boston and a senior author of the paper. "By combining transcriptomics and proteomics, we wanted to find a biomarker that's more powerful than either one [analysis] would allow." The study was intended as a proof of concept, and so instead of concrete biomarkers, the results pointed to the intersection of several pathways, such as stress response and tumor metabolism, where the researchers could begin to look for biomarkers that show up in multiple pathways.

"By doing both [types of omics], you're going to get a broader feel of what's going on," Sgroi says. "You're casting a wider net when you're doing both, and it gives you a good sense of functionality."

At the Weizmann Institute in Rehovot, Israel, Eran Segal uses integrated omics for his research on the microbiome. Although there are many regulatory changes in the microbial and host messenger RNA, as shown by transcriptomics, only a fraction of these changes actually results in something meaningful for the disease being studied, such as diabetes or Crohn's disease. "When integrating multiple regulatory levels"—where 'regulatory levels' are the multiple omics"intersecting the changes [in these levels] allows us to filter out the many real, but less relevant, changes and zoom in on the relevant ones," Segal, a computational biologist who integrates metabolomics, proteomics and transcriptomics in his work, told *Nature Medicine*.

If integrated omics offers the opportunity to declutter the various processes and pathways in a cell to arrive at the most relevant and useful markers, it perhaps comes as no surprise that personalized medicine is also tapping into the approach's promise. The NCI DREAM drug sensitivity challenge is one such example, in which testing tumor samples for drug sensitivity can yield clinically actionable results. Inspired by this application, Pal is launching a company—tentatively named First Ascent Biomedical—that will primarily use transcriptomic data along with proteomic data to predict the sensitivity of patient tumor samples to chemotherapy drugs. Although his follow-up analysis showed improvement once it integrated five data types, Pal says that the cost of running multiple analyses is limiting the company to only two data types.

"We will rely on functional data from testing more than 60 drugs, as well as transcriptomic and, to some extent, proteomic data," says Pal. Using this will help the company to create a computational model that will help to predict how tumors would respond to many of the currently available drugs. "Mutation data alone doesn't offer good information."

Shraddha Chakradhar

Cannabinoid receptor with an 'identity crisis' gets a second look

Five years ago, neuroscientist Ken Mackie of Indiana University in Bloomington called cannabinoid receptor 2 (CB2) "a cannabinoid receptor with an identity crisis"[1]. Throughout the previous two decades, researchers had reported conflicting results about the location of the receptor in the body, arguing in particular over its role in the central nervous system. The confusion dampened enthusiasm for CB2 as a drug target, even though preclinical work had shown it to have promise for illnesses ranging from liver fibrosis[2] to Alzheimer's disease[3]. Now, new research tools are clearing up the mysteries and recasting CB2 as a receptor with vast therapeutic potential.

"CB2 apparently is part of a protective system," says Raphael Mechoulam, who led a team that identified tetrahydrocannabinol (THC) as the psychoactive compound in cannabis in 1964 and continues his research at the Hebrew University of Jerusalem in Israel. "Mice in which the CB2 is blocked are okay—as long as they are not sick. But once they are sick, then the CB2 is extremely important in a whole variety of diseases." Mechoulam, who is widely considered the father of cannabinoid research, has developed several compounds that selectively target CB2, including one now licensed to Hoffmann-LaRoche.

THC, the most widely known compound found in cannabis, has two major effects in the body: when it binds to CB2 receptors, it suppresses inflammation, and when it binds to cannabinoid receptor 1 (CB1), it induces a mental high. That second effect limits THC's usefulness as a medicine because psychotropic effects are undesirable. But compounds that bind to CB2 but not to CB1 could potentially treat illness without giving people a buzz.

Trials and tribulations

A few years ago, researchers were growing frustrated with CB2. Companies such as Pharmos, Shionogi, Eli Lilly and GlaxoSmithKline ran clinical trials with CB2-targeting drugs, mostly to treat pain from wisdom-tooth removal or osteoarthritis. In the trials with published results, the drugs proved harmless but did not achieve the desired efficacy[4,5]. Meanwhile, researchers struggled to locate CB2 in the body. Initially, they had found CB2 mostly in the spleen and in circulating immune cells, leading them to call it the "peripheral cannabinoid receptor"[6]. But by using antibodies that bind to CB2, some scientists have reported finding the receptor in other tissue types in rodents, including neurons in the brain.

It was also unclear whether using antibodies as a tool for mapping the CB2 receptor in the body produced reliable results. Further studies showed that it could not be wholly relied on; in mice engineered to lack functional CB2, the CB2 antibodies bound to other proteins instead[8]. This made it difficult to interpret results from immunostaining experiments[9]. Another problem was that mice engineered to lack functional CB2 had had only a portion of the gene for CB2 removed. The remaining part

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Budding targets: The CB2 cell receptor responds to compounds found in cannabis.

of the gene was often transcribed into scraps of mRNA, which could potentially have been translated into partial CB2 proteins. Such proteins would not function as receptors, says Mackie, but they might bind to antibodies or interact with other receptors in unpredictable ways.

"It became very clear at that stage that we didn't really have very good tools for studying the CB2 receptor," says Cecilia Hillard, a neuroscientist at the Medical College of Wisconsin in Milwaukee. "Every time we felt like we had a handle on it, it was slippery, and we couldn't measure what we thought we were measuring again."

The problems with the tools were exacerbated by the properties of the receptor itself. Although researchers still debate the part that CB2 plays in the central nervous system, most agree that healthy animals express very little CB2, and what they do produce is mostly in their peripheral immune cells. During injury, infection or inflammatory conditions, immune cells are activated and CB2 expression ramps up in the unhealthy tissues. This pattern could explain why patients generally suffer few side effects from CB2 agonists, says Hillard, although immune suppression from CB2 agonists could be a problem in some situations. Because CB2 agonists have little or no effect on healthy animals, however, it is hard to compare different drugs or to predict how they will affect an animal with an injury, illness or inflammatory condition, says Pal Pacher, a physician-scientist at the National Institute on Alcohol Abuse and Alcoholism in Rockville, Maryland.

CB2, take two

Hillard's team has developed a new transgenic mouse model that might help to solve such mysteries. The mouse, which the team described at the 2014 meeting of the International Cannabis Research Society (ICRS) in Baveno, Italy, produces green fluorescent proteins whenever it produces CB2. Instead of using antibodies to detect CB2, researchers can simply observe which cells fluoresce. The mouse is also an inducible CB2 knockout, allowing researchers to strip CB2 from one cell type at a time.

Other approaches include the development of new CB2-binding molecules. For example, Hoffmann-LaRoche is working on labeled ligands for positron emission tomography (PET) imaging, which could reveal CB2 receptors in live mice and humans[10]. At the June 2015 ICRS meeting in Wolfville, Nova Scotia, the Hoffmann-LaRoche team also described a new class of highly specific CB2 agonists.

Most of the older agonists showed some preference for CB2, but at high doses, they also triggered CB1 activation—and the effects of CB1 can counteract those of CB2, Pacher says. CB1 is much more abundant, so even drugs with a 20- to 50-fold preference for CB2 can be overwhelmed by the effects of CB1. Pacher thinks that this could partially explain why earlier clinical trials failed.

The newer agonists are selective enough that they will not bind to CB1. Some show up to a 40,000-fold preference for CB2, according to Pacher. But Mackie says that even these compounds would probably have been useless in past clinical trials because the trials looked at the wrong illnesses. "Choosing a human study group for convenience, which is kind of how osteoarthritis and third molar extraction got chosen, isn't necessarily matching with what the preclinical results suggest may be a good patient population to study," he says.

The most promising indications, says Mackie, are human conditions that closely mimic rat and mouse models in which CB2 agonists proved helpful. These include neuropathic pain[11] and fibrosis of the kidneys[12] and of the liver[13]. Researchers are also pursuing CB2 agonists for neurodegenerative illnesses such as multiple sclerosis[14], and some think that the drugs could help with cocaine addiction[15]. Different CB2 agonists can trigger different pathways in the cell, so researchers might be able to target particular illnesses by matching them with the right CB2-targeting drug, says Mackie.

Although only CB1 and CB2 are widely accepted as cannabinoid receptors, cannabinoid compounds also interact with other receptors, some of which could be valuable drug targets. Researchers have proposed classifying two such receptors, GPR55 and GPR18, as cannabinoid receptors in their own right[16]. Compounds that block GPR55 could some day help patients with diverse ailments, including cancer and chronic pain, says Mary Abood, a cell biologist at Temple University in Philadelphia. But research on GPR55 and GPR18 is in its early stages, with nothing yet approaching the clinical-trial stage.

Uncertainties still surround CB2, but researchers are now armed to solve them, thanks to Hillard's transgenic mouse and the wealth of new CB2 agonists and other modulators. "These will be very good tools in the future. Tools which could help to move this field ahead," Pacher says. "For a while I think it was sitting still, but I think now it will move."

Nala Rogers

1. Atwood, B.K. & Mackie, K. *Br. J. Pharmacol.* **160**, 467–479 (2010).
2. Lotersztajn, S. *et al. Br. J. Pharmacol.* **153**, 286–289 (2008).
3. Benito, C. *et al. J. Neurosci.* **23**, 11136–11141 (2003).
4. Ostenfeld, T. *et al. Clin. J. Pain* **8**, 668–676 (2011).
5. Pereira, A. *et al. Clin. Pharmacol. Ther.* **93**, S1 (2013).
6. Atwood, B.K. & Mackie, K. Br. *J. Pharmacol.* **160**, 467–479 (2010).
7. Dhopeshwarkar, A. & Mackie, K. *Mol. Pharmacol.* **86**, 430–437 (2014).
8. Baek, J.H., Darlington, C.L., Smith, P.F. & Ashton, J.C. *J. Neurosci. Methods* **216**, 87–95 (2013).
9. Marchalant, Y., Brownjohn, P.W., Bonnet, A., Kleffmann, T. & Ashton, J.C. *J. Histochem. Cytochem.* **62**, 395–404 (2014).
10. Slavik, R. *et al. J. Med. Chem.* **8**, 4266–4277 (2015).
11. Gutierrez, T., Crystal, J.D., Zvonok, A.M., Makriyannis, A. & Hohmann, A.G. *Pain*, **152**, 1976–1987 (2011).
12. Barutta, F. *et al. Diabetes* **60**, 2386–2396 (2011).
13. Horváth, B. *et al. Br. J. Pharmacol.* **165**, 2462–2478 (2012).
14. Malfitano, A.M. *et al. PLoS ONE* **8**, e62511 (2013).
15. Zhang, H.Y. *et al. Proc. Natl. Acad. Sci. USA* **111**, E5007–E5015 (2014).
16. Console-Bram, L., Marcu, J. & Abood, M.E. *Prog. Neuro-Psychopharmacol.* **38**, 4–15 (2012).