PROFILING A HUMAN: THE ROLE OF ANTIBODIES

Francesca Lake explores the role of antibodies in the ongoing efforts looking to build an atlas of the human body and its diseases

It's become widely recognized that a plethora of 'things' feed into a person's overall health – the classical view of a one-size-fits-all approach to a disease has more or less been side-lined. What's more, with drugs continuing to fail to reach the clinic, research teams have turned back to the drawing board, finding our understanding of basic human biology to be wanting.

Some exciting endeavors now exist that are hoping to provide a better blueprint for human health and disease, and antibodies play a crucial role in many of these approaches. Here we take a look at some of the ongoing projects seeing researchers attempt to improve our understanding of human biology and diseases, utilizing techniques that involve antibodies.

One major effort seeking to improve our understanding of the human body is the Human Protein Atlas (HPA). Set up in 2003 with the aim of mapping all human proteins through the integration of antibody-based imaging, mass spectrometry-based proteomics, transcriptomics and systems biology, this delightfully open-access endeavor launched three new sub-atlases recently, covering the blood, brain and metabolism.

"We use antibodies to screen tissues, organs and cells using immunohistochemistry and confocal microscopy," Matthias Uhlen, Program Director of the HPA and Professor of Microbiology at KTH in Stockholm, Sweden, told *BioTechniques*.

The HPA is a huge undertaking: *"We generate more than 700 images per antibody,"* noted Uhlen. *"We now have more than 10 million images, each annotated by a certified pathologist."*

ATLASES FOR THE BRAIN

The results from the HPA's brain atlas told us a lot about the differences and similarities between mouse, pig and human brains – integral information given the continued need for animal models.

Comprising approximately 1900 brain samples and covering 27 brain regions, the atlas shows that the cerebellum is the most distinct region, and highlighted interesting differences in neurotransmitter systems. It also demonstrated that different cell types within the brain share specialized proteins with peripheral organs, an intriguing finding (Figure 1) [1].

All of the immunohistochemistry and immunofluorescence images produced by the brain atlas are manually annotated and given a reliability score, which includes considerations such as the reproducibility of staining in different cell lines and comparable experimental evidence in the literature, among others – a massive and commendable effort, and one which should shore up results given the known problems with antibody work and reproducibility historically.

Another brain-based atlas effort is being undertaken by the NeuroScience Associates (NSA) – a neurohistology service – which recently joined forces with the artificial intelligence (AI) start-up Aiforia (Helsinki, Finland) to build a whole-brain reference dataset for research into amyotrophic lateral sclerosis (ALS). Thomas Westerling (Aiforia) highlighted to *BioTechniques* that one of the reasons for recent disappointments in neurodegenerative disease research projects has been the lack of a comprehensive atlas. With the NSA able to perform neuro-histology of whole-hemisphere pieces of the brain, Aiforia hopes to apply its AI to process the resultant images, providing hope for a field that has seen much failure [2].

Another effort looking to go back to the drawing board with the brain – specifically seeking to better understand synaptic proteins – is applying a newly developed method. Researchers at MIT and the Broad Institute of Harvard and MIT (MA, USA) have used fluorescence nucleic acid probes to label and image a seemingly unlimited number of synaptic proteins at high resolution [3]. *"Multiplexed imaging is important because there's so much variability between synapses ▶*

Immunohistochemical staining of a human brain showing the location of HOMER3 in Purkinje cells of the cerebellum.

Credit: The Human Protein Atlas, CC-BY-SA.

and cells, even within the same brain,” explained Mark Bathe (MIT). “You really need to look simultaneously at proteins in the sample to understand what subpopulations of different synapses look like, discover new types of synapses, and understand how genetic variations impact them.”

While conventional fluorescence microscopy only enables the imaging of four proteins at a time, this new method – based on DNA PAINT and itself called PRISM – imaged 12 proteins in a sample containing thousands of synapses. Their results could help understand how the brain’s structure is affected by knock-out of genes associated with disorders such as autism and schizophrenia (Figure 2).

PROFILING FOR IMMUNITY

The HPA has also turned its attention to the COVID-19 pandemic, with surprising results. Albeit a pre-print and thus not having been peer-reviewed at the time of writing, Hikmet *et al.* utilized the HPA’s tissue atlas to reveal results suggesting that ACE2, a protein touted as being key for coronavirus penetration of cells in the respiratory tract, is in fact mainly localized to nonrespiratory areas of the body including the intestinal tract and gallbladder – its expression in the human respiratory system was limited [4]. If those results stand up to peer review, this casts doubt on vaccine efforts looking to target the protein. The HPA is also involved in efforts to develop a serological assay.

Another interesting effort – and one reversing attention back onto understanding antibodies themselves – comes from MiLaboratory, a Californian start-up that probes the adaptive immune system. It’s clear that we still do not fully understand our immune system and its constituent parts – only in 2019 did Soto *et al.* publish their work providing insight into how the immune system is able to be so diverse given its small number of genes [5].

“If you knew your immune response, you could tell very early on what kind of clinical interventions you need and what to expect for progression,” explained Yuri Nikolsky (CEO of MiLaboratory). MiLaboratory was recently involved in work looking to develop a yellow fever vaccine, which required high-throughput sequencing of T-cell receptor repertoires to understand the large diversity of T-cell clones and their dynamics and role in response to immunization – understanding that will be key to development of an effective vaccine [6].

Their profiling tech is particularly intriguing for the current pandemic, given it is designed to work in a small cohort of patients. “One patient at a time is what you normally have to deal with when you have something sudden, like an epidemic – you don’t have time or the samples for a clinical trial; there is no prior art, and you need to make a decision based on what you can analyze,” Nikolsky noted.

BUILDING A COMPREHENSIVE TUMOR ATLAS

The Human Tumor Atlas Network (HTAN), a part of the National Cancer Institute’s Cancer Moonshot Initiative, has recently announced its plans to generate 3D atlases of cancer transitions – that is, tumor initiation, expansion, metastasis and resistance – in a bid to improve understanding of tumor biology and thus the detection, treatment and prevention of cancers [7]. This effort will also utilize antibody-based profiling, building on the CODEX (co-detection by indexing) approach developed in 2018, which iteratively visualizes antibody-binding events [8]. The authors of that method generated a dataset covering around 700,000 cells from normal and diseased spleens, covering segmentation, quantification and spatial data. They also predicted that their results laid “the foundation for a pan-cellular reference database defining cellular types not only by identities of proteins expressed, but also by definitions for specific cell-to-cell interactions” – something the HTAN hopes to go some way to achieving.

REVISITING THE REPRODUCIBILITY QUESTION

While this new understanding of human biology should go some way to pushing science forward, antibody-based research has in the past been plagued by problems. Last year we covered antibodies and their reproducibility crisis in detail [9]. At that point, we reported on work from the HPA that had suggested that of 5000 commercial antibodies tested, over 50% were not useful for the proposed application, which is somewhat damning.

While this does not bode well, we also reported on various efforts looking to ameliorate the problem. Since then this story has unfurled further, with one study showing that of 16 antibodies advertised by companies as specific for C90RF72, a protein product of a gene involved in ALS, only one could accurately be used for immunofluorescence. Some of those that failed had been used in studies [10], casting doubt on those results as well as any subsequent research building on them.

However, things continue to improve – that study, for example, also provided a streamlined process to antibody validation. The HPA goes to large lengths to validate its 55,000 in-house-generated antibodies, as well as the commercial antibodies it uses, at high resource cost. “Validation is difficult since this has to be done in an application and sample preparation-specific manner,” Uhlen noted. “The fact that the HPA applications involve the analysis of denatured

proteins (for immunohistochemistry and immunocytochemistry) means that antibodies recognizing several epitopes (polyclonal) are preferred not to be dependent on a single epitope that might disappear during the denaturation of the protein." For them, the validation success rate is around 30%, and lowest for monoclonal antibodies. Many commercial suppliers are also now putting a high emphasis on validation.

CHALLENGING DATA ANALYSIS

These large-scale efforts to understand human biology also come hand-in-hand with large-scale efforts needed to store and analyze the resulting data. The HPA, for example, generates data in-house, but also takes in data from other efforts, resulting in petabytes of total data. This requires a lot of resource spend to handle, particularly in terms of IT infrastructure. As a result of this evolution, over the whole life science spectrum, data are becoming cheaper to generate yet cost and time to results are increasing. While manual annotation is still key for efforts like the HPA, bioinformatics and AI companies such as MiLaboratory and Aiforia are becoming increasingly valuable, as they take on the burden of some of those data-handling efforts.

AI in particular is likely to become increasingly used in imaging approaches. "We're definitely just scratching the surface of what is possible to extract in images," noted Anne Carpenter (Broad Institute of Harvard and MIT), who developed the software CellProfiler. She has high hopes for the future: "We are in the middle of this transition where within a few years biologists are going to be able to drag-and-drop their images into a web app and the software will automatically recognize a variety of structures within the cell without any training or tweaking or understanding how the image processing is working. If we can train models that are generalizable enough, we should be able to get to the point where image analysis becomes quite a lot easier for the biologists."

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Confocal LNA-PRISM images of rat hippocampal neuronal synapses [3].

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