International effort mapping a pathway to generate renewable antibodies

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In the first study of its kind to date, an international group of scientists including Drs. Tony Pawson and Karen Colwill at Mount Sinai Hospital and Drs. Dev Sidhu and Aled Edwards at the University of Toronto have highlighted the power of renewable antibody technologies to create precise reagents for use in biomedical research -- a methodology that will help accelerate scientists' understanding of all the proteins coded in the human genome.

The study was published online today in the leading biomedical journal Nature Methods.

Antibodies are essential tools in biomedicine, used both for discovery and therapy. However, many commercially available antibodies for research are of inadequate quality and supply, which hinders scientists from identifying new causes of and potential therapies for diseases including cancer. The problem is compounded because the genomic revolution has dramatically increased the demand for renewable, high-quality affinity reagents to the many newly discovered and uncharacterized proteins.

“The use of renewable antibodies hasn’t been fully exploited,” said Dr. Tony Pawson, Distinguished Investigator at the Samuel Lunenfeld Research Institute of Mount Sinai Hospital and study co-author. “Our study shows we can rapidly make reagents that are highly specific to a large number of proteins, which will facilitate a more precise examination of the proteins and pathways involved in various complex

illnesses."

"The potential of the human genome project has not yet been fully realized because we haven't had the full set of necessary tools," echoed Dr. Sidhu, Principal Investigator at the University of Toronto's Donnelly Centre for Cellular & Biomolecular Research, and Director of the Toronto Recombinant Antibody Centre (TRAC). "The team has now generated a roadmap to dramatically increase the number and quality of these tools, and this is an important advance."

Hybridoma and recombinant display technologies are two general methods to develop renewable antibodies. Drs. Pawson and Colwill at the Lunenfeld, in collaboration with the Structural Genomics Consortium and other researchers in Canada, the United States, Sweden, Australia, Germany and England, conducted a systematic analysis of these methods and their suitability for large-scale applications.

The Structural Genomics Consortium produced 20 protein interaction modules (SH2 domains) from a variety of proteins, which they distributed to researchers in five laboratories globally for antibody generation using different methods. One group generated monoclonal antibodies by hybridoma technology using a high-throughput, robotic approach. The other groups generated recombinant Fab or single-chain Fv (scFv) reagents using a technology termed phage display.

Thousands of different antibodies were evaluated for potency and specificity at the antibody-generating laboratories. The antibodies were then further characterized at the TRAC prior to testing the highest performing ones at the Lunenfeld in formats of highest utility to biologists: immunoprecipitation, immunoblotting and immunofluorescence assays. Criteria were established to prioritize the most promising examples from each step and all the assays combined set a high standard for antibody validation.

When evaluated, each technology produced hundreds of antibodies suitable for biological assays. Importantly, results showed that recombinant antibodies were comparable to the more established hybridoma technology, and imply that future endeavours to create renewable antibodies should consider both approaches.

“We showed that high-affinity, high-specificity renewable antibodies generated by different technologies can be produced quickly and can prove complimentary,” said Dr. Colwill, the lead author and a staff scientist in Dr. Pawson’s lab at the Lunenfeld. “We believe this work serves as a foundation and template for future larger-scale studies to create renewable protein binders.”

Provided by Mount Sinai Hospital