Post-translational mAbs

By Kai-Jye Lou, Senior Writer

Generating mAbs that target post-translational modifications such as phosphorylations could open up a host of new targets for cancer and other diseases associated with aberrant protein modifications, but the low immunogenicity of the modified sites has thus far stymied the use of conventional mAb-generation platforms.

University of California, San Francisco researchers have now developed a structure-guided phage display platform capable of producing mAbs that target post-translational modifications.¹ Next, the team will have to show that the mAbs target endogenous proteins *in vivo*.

Prior efforts to target post-translational modifications with mAbs usually involved animal immunization and the establishment of mAb-producing hybridomas. This approach is low throughput, takes months and has rarely yielded clones that produced mAbs that target phosphorylated amino acid residues.^{2,3}

In addition, hybridomas often do not provide a limitless source for a mAb of interest because they can experience genetic drift. These cells thus require regular monitoring and testing to ensure they still produce the original mAbs.

Phage display-based approaches can rapidly generate mAbs but are even less efficient at generating mAbs that target post-translational modifications than animal immunization-based approaches.⁴

“Current phage display methods are not efficient for generating antibodies against peptides and phospho-peptides,” said James Wells, chair of the Department of Pharmaceutical Chemistry and a professor in the Department of Pharmaceutical Chemistry and Department of Cellular and Molecular Pharmacology at UCSF. “This led us to explore the use of alternative antibody scaffolds with which to build our phage display libraries.”

The UCSF group carried out a computational search of 60 known antibody structures to look for regions that might mimic a phosphate-binding pocket. The researchers identified one such pocket on a mouse Fab and built it into the complementarity-determining region (CDR) of a humanized Fab display scaffold.

Next, the team applied phage display mutagenesis to the parent scaffold to generate libraries of mAb scaffolds that specifically bound

to phospho-serine, phospho-threonine or phospho-tyrosine.

The researchers sequenced the CDR regions of the mAb scaffolds and used *in vitro* assays and structural analysis to identify the best ones for each phospho-amino acid residue. They then used the lead phospho-serine and phospho-threonine scaffolds to build two phage display libraries of single-chain Fv mAbs.

Serine and threonine together account for nearly 100% of known phosphorylation sites, whereas tyrosine accounts for less than 0.05%.⁵

ELISA-based selection of the phage libraries identified 51 mAbs that selectively bound to phosphorylated peptides containing phospho-serine or phospho-threonine over nonphosphorylated counterparts, with affinities ranging from 42 to 5,000 nM. The peptides were 13–19 amino acids in length and derived from biologically relevant targets including protein kinase B (PKB; PKBA; AKT; AKT1).

Follow-up western blot analysis showed that a sample subset of the mAbs bound to the corresponding phosphorylated protein.

Results were published in *Nature Biotechnology*.

“Using an antibody scaffold that contains a natural phosphate-binding motif as a starting template, followed by library construction and panning selection, provides a robust approach in generating difficult-to-find antibodies,” said Herren Wu, VP of antibody discovery and protein engineering at the MedImmune LLC unit of AstraZeneca plc. “It is a powerful approach, and one can envision that it can be applied to generating specific antibodies to other post-translational modifications.”

“The study provides a direct example of how structural biology could be used to guide antibody design,” added Sachdev Sidhu, an investigator at the Ontario Institute for Cancer Research and a professor at the University of Toronto. “The findings should spur additional interest to generate more antibody structures, and the approach could provide insights on the structures that will be needed to generate mAb libraries against various post-translational modifications.”

Although the approach yielded highly specific mAbs to phosphorylated peptides, Andrew Bradbury told SciBX that he now wants to see additional studies to determine whether phospho-specific antibodies generated with this approach will bind to endogenous phosphorylated proteins and without off-target binding to other proteins. Bradbury is a group leader in the biosciences division at the Los Alamos National Laboratory.

He cautioned that just because an antibody binds a peptide fragment of a protein with a post-translational modification does not mean it will bind the full-length, endogenous protein with the same modification.

Rare mAbs, reduced effort

The UCSF group's strategy could bolster the efficiency of generating high-quality, phospho-specific mAbs and possibly mAbs against other post-translational modifications while reducing the time and resources needed.

“Our method is done entirely *in vitro* and allows us to generate mAbs with high specificity and affinity for phospho-amino acids within a span of two weeks. Traditional hybridoma-based methods take at least two months just to isolate the clones you want.”

—James Koerber,
University of California,
San Francisco

“The speed of this approach is substantially faster than others, and the success rate of finding multiple unique antibodies with decent affinity is much higher,” Wu told *SciBX*. “It would also be relatively easy to adopt this approach in laboratories that have existing capability and capacity in structural biology and combinatorial library generation and screening.”

“Our method is done entirely *in vitro* and allows us to generate mAbs with high specificity and affinity for phospho–amino acids within a span of two weeks,” said James Koerber, the paper’s lead author and a **Life Sciences**

Research Foundation postdoctoral fellow at UCSF. “Traditional hybridoma-based methods take at least two months just to isolate the clones you want. You will then need to do additional screening and scale-up production, which takes additional time and resources.”

“Since we are building recognition for phospho–amino acids into our initial step, we don’t need to carry out the additional screening and counterselection steps required by traditional methods,” added Wells.

Because antibody generation is entirely *in vitro*, the approach is also amenable to automation.

Koerber said that the sequencing and structural analysis steps give the group a better idea of how to design antibodies specific for post-translational modifications, including insights into what types of post-translational modifications could be targeted and which amino acid residues to mutate in the antibody scaffolds.

Sidhu added that the strategy shows that it is possible to bootstrap the way to a desired structural model.

“The group didn’t generate antibody libraries using an existing scaffold but instead used their knowledge of existing antibody structures and structural modeling to rationally design new scaffolds that they then used to generate their libraries,” he told *SciBX*.

Broadening horizons

A key next step will be to determine how broadly applicable the strategy is.

“I have no doubt that this approach will generate a high degree of interest in industry and academia as being able to rapidly generate high-quality mAbs specific for post-translational modifications. It can greatly facilitate the study of disease mechanisms and cell biology.”

—Herren Wu, MedImmune LLC

“I have no doubt that this approach will generate a high degree of interest in industry and academia as being able to rapidly generate high-quality mAbs specific for post-translational modifications. It can greatly facilitate the study of disease mechanisms and cell biology,” said Wu. “It would be great to see how broadly this approach can be expanded to work on other post-translational modifications, such as acetylation and ubiquitination.”

Koerber said that the group now is trying to automate the approach for generating phospho-specific mAbs.

Wells said that the team is looking into the potential development of mAbs against other post-translational modifications such as acetylation, methylation, sulfation and proteolysis.

UCSF has filed for a provisional patent covering the technology, which is available for licensing.

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