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APPLICATION NOTE

Using High Throughput SPR to Assess the Binding Kinetics, Affinities, and Epitope Coverage of Antibody Libraries

Abstract

The generation of antibodies to source the discovery of therapeutics is highly commoditized and there is a trend towards producing fully human antibodies by *in vitro* and *in vivo* methods to reduce the risk of immunogenicity. The OmniChicken™ (Ligand Pharmaceuticals, Inc.) is a new transgenic animal platform that offers some unique advantages in the discovery of therapeutic antibodies. Upon immunization with human antigens, chickens produce antibodies that recognize a broader range of epitopes than those from mammalian hosts, due to the phylogenetic distance between humans and birds. Transgenic chickens expressing a human antibody repertoire harness the innate ability of wild-type chickens to yield a comprehensive immune response to human targets. Key observations underpinning the development of this exciting antibody platform were made possible through the use of detailed binding studies performed by high throughput surface plasmon resonance (SPR), using the Carterra® LSA™. The expanded epitope coverage includes antibodies that are cross-reactive to orthologs of the target, which are highly sought-after to enable preclinical toxicology and Mechanism of Action (MOA) studies in rodent and other species. Additionally, the antibody repertoire includes high affinity antibodies. The potential for identifying antibodies that target novel epitopes with high affinity is of great benefit to drug discovery in the quest for the next blockbuster antibody therapeutic.

Introduction

Monoclonal antibody therapeutics comprise one of the fastest growing and crucial classes of pharmaceutical agents on the market today. With over 60 antibody drugs currently approved for use and more than 550 in clinical development[1], the use of antibodies in the clinic for the treatment of cancer, autoimmune diseases, and other chronic conditions is escalating. In response, drug discovery scientists are searching for new technologies and methods to advance antibody production and improve screening and manufacturing capabilities.

The development of transgenic animal platforms to source the discovery of therapeutic antibodies ensures they are produced as similar to native human antibodies as possible, in an attempt to reduce the risk of immunogenicity in patients. Transgenic mice, rats, rabbits, and cows have all been developed for therapeutic antibody production. However, these animals' immune responses to human proteins are limited as they are all mammalian species and tolerate mammalian-conserved epitopes.

To expand the range of possible epitopes, Ching *et al* recently developed a transgenic chicken – the OmniChicken™ – that provides an attractive alternative for therapeutic antibody production[2]. The OmniChicken carries humanized immunoglobulin genes so that it can be used to discover novel, high affinity human antibodies. Also, because chickens are phylogenetically distant from humans, it is possible for them to produce antibodies that recognize unique epitopes not accessible in mice or other mammalian transgenic platforms.

Here we describe how high throughput epitope binning assays using the Carterra® LSA™ high throughput SPR platform demonstrates the expanded epitope coverage possible with the OmniChicken. Results showed that the antibody repertoire targets novel epitopes to provide further therapeutic potential through different mechanisms of action (MOA), extending the possibilities for drug discovery and development. The wider epitope coverage also includes a prevalence of species cross-reactive antibodies, which are important for MOA and toxicology studies and obviate the need for surrogate antibodies. In addition, binding kinetic analyses using the Carterra LSA show that the OmniChicken can produce high affinity antibodies suitable for use as therapeutics.

What is high throughput SPR?

The Carterra LSA is an antibody screening and characterization tool that uses surface plasmon resonance (SPR) to measure hundreds of binding interactions in parallel in a real-time and label-free manner. It is particularly well-suited to determining binding kinetics and affinities of large antibody panels and epitope binning them. As such, it has recently facilitated a paradigm shift in the discovery of therapeutic antibodies, enabling the rapid and detailed characterization of large antibody libraries at the earliest stage of research (Figure 1). This helps to identify near-optimal leads requiring minimal engineering and therefore expedites the library-to-leads triage process and accelerates biotherapeutic discovery.

Using the Carterra LSA, it is possible to perform an epitope binning study on up to 384 antibodies per array, which could merge antibodies from different libraries and compare their epitope coverage. For example, when developing the OmniChicken, the Carterra LSA was used to examine the epitope coverage of antibodies from a transgenic chicken and benchmark it against an established set of standards produced from wild-type chickens[3]. The results showed that the antibodies produced by the OmniChicken have similar binding properties as those from wild-type chickens.

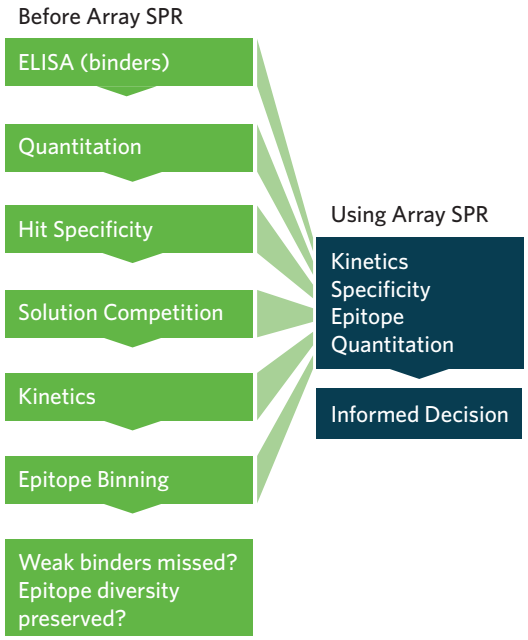

Figure 1: High throughput SPR revolutionizes antibody screening for drug discovery by shifting SPR upstream and allowing screening and characterization to be accomplished in the same step at the start of drug discovery.

In vitro and *in vivo* technologies for therapeutic antibody production

Since the first antibody was granted marketing approval as a therapeutic in 2002, monoclonal antibody therapy has become a vital tool in the treatment of numerous human diseases and conditions. As a result, pharmaceutical companies focus a great deal of resources on the discovery of new antibody therapeutics. This includes the development of new methods that help to produce antibodies as similar to native human antibodies as possible, in order to minimize immunogenicity in patients and generate good therapeutic agents.

Human therapeutic antibody production is possible through both *in vitro* and *in vivo* methods. Of those antibodies currently in the clinic, a number were produced using an in vitro technique termed complementarity-determining region (CDR) grafting. This humanization process takes antibodies produced in mice and uses genetic engineering to exchange parts of the murine sequence with the homologous human sequence. CDR grafting effectively produces a chimeric antibody to reduce immunogenicity and overcome therapeutic deficiencies of mouse monoclonal antibodies[4]. Since humanization of an antibody often results in a dramatic loss in binding affinity, it is routinely followed by affinity maturation to recover or improve upon the original affinity. The engineering steps of humanization and affinity maturation required to transform a murine (mouse or rat) antibody into a humanized antibody that is fit for the clinic are laborious and add several months to the development timeline.

Another *in vitro* system used extensively for antibody production is that of phage display on recombinant libraries. This technique involves the genetic engineering of bacteriophages to display human antibodies on their surface, which then undergo a selection process to find those antibodies that bind specific antigens. Although the antibodies produced using this method do not require humanization they often need further engineering to optimize binding affinity and developability characteristics[5].

Before phage display methods were developed, hybridoma (*in vivo*) methods were well-established and account for the majority of approved therapeutic antibodies on the market today. However, depending on the therapeutic indication, it can take about a decade to progress a lead from the clinic to the market using this method. Transgenic mice provide an *in vivo* immune system for the expression of fully human antibody therapeutics. Because these antibodies are produced in the intact immune system of an animal, the system takes advantage of inbuilt natural selection processes to produce antibodies with desirable properties, including high potency, specificity, manufacturability, solubility, and low immunogenicity[6]. As a result, further validation and optimization after initial discovery is not required to the same extent as for *in vitro*-derived antibodies.

An alternative to mammals: transgenic chickens

The range of species that have been genetically modified to produce human antibodies has expanded over recent years to include mice, rats, rabbits, and cows. However, these commonly used transgenic animal platforms are all mammalian species, which introduces some limitations. The main challenge is the difficulty in raising an immune response to - and therefore antibodies against - epitopes shared amongst mammals, as they will not be seen as foreign immunogens in a mammalian host.

To overcome this, Ching *et al* use birds as an alternative to mammals[2]. Birds, and in this case chickens, provide an attractive option for sourcing antibodies destined for therapeutics due to their phylogenetic distance from humans. Having diverged around 300 million years ago, proteins from chickens share less sequence homology with human proteins compared to those from mice or other mammals. Because of this, chickens recognize human protein antigens as foreign and are

more likely to raise antibodies against a broader surface of a particular human antigen. Indeed, epitope binning experiments using the Carterra LSA demonstrated that an engineered chicken can produce antibodies with expanded epitope coverage that recognize unique epitopes not accessible in mice.

A broad epitope coverage is highly advantageous in drug discovery as it increases the chance of accessing the functionally significant regions of a target. It is noteworthy that epitope selection is an empiric process because it is not yet possible to design an antibody *in silico* that targets a particular epitope precisely. It is also not possible to predict epitopes on target molecules. Targeting more epitopes improves the likelihood of identifying antibodies with unique characteristics that provide novel MOAs and therefore would make desirable therapeutics.

In addition, expanding the epitope coverage improves the chance of finding cross-reactive antibodies that recognize both the human and mouse orthologs of a target. Finding antibodies for conserved epitopes that are species cross-reactive facilitates the use of animal models of disease. This is highly desirable as it helps advance potential therapeutics from pre-clinical to clinical stage studies, decreasing drug discovery timelines. If a therapeutic antibody candidate binds the human target but does not bind the mouse version then it is not possible to test the toxicology or biology through MOA proof of concept studies in rodents. A surrogate antibody is therefore needed, which must be identified through a costly parallel campaign. Using a surrogate antibody is also severely limiting because it differs from the antibody destined for the clinic and therefore only probes the biological pathway rather than testing the antibody's therapeutic suitability.

The OmniChicken is a transgenic chicken that carries humanized immunoglobulin genes for the expression of antibodies with human variable regions and chicken constant regions. The complex genetic modifications that made this possible are outlined in the research paper from Ching *et al*[2]. These human antibody-expressing chickens exhibit normal B cell development and raise immune responses to conserved human proteins that may not be immunogenic in mice. Following expression, these antibodies are recovered and cloned as fully human recombinant antibodies for drug discovery.

Wildtype chickens have also been investigated previously[3] as an alternative for antibody production and discovery due to their phylogenetic separation from mammals. Chicken-derived antibodies can bind antigen with very high affinity and so have therapeutic potential themselves. However, the advantage of the transgenic OmniChicken over the wildtype is that fully human antibodies are produced. This removes the need for subsequent humanization processes and additional engineering, helping to expedite drug discovery and decrease costs.

Examining epitope coverage and kinetics

The Carterra LSA was used to assess the kinetic and epitopic diversity across the OmniChicken's antibody repertoire. Epitope binning experiments and kinetic studies made it possible to quickly examine the epitope coverage of each antibody library generated in the study.

Chickens were immunized with a model antigen, human progranulin (PGRN), which is a multi-domain protein that has previously been investigated in wildtype chickens[3]. Epitope binning experiments were then performed to examine the epitope coverage of the recovered antibody clones.

Following immunization with PGRN, a panel of more than 100 antibodies was investigated in detail with high throughput SPR and results compared to those from previous studies using wildtype chickens[3]. All seven of the PGRN domains were recognized by antibodies in the panel, with some recognizing the desirable human/mouse cross-reactive epitopes. This mirrored the broad epitope coverage seen in wildtype chickens (Figure 2), demonstrating that the OmniChicken is a great alternative for therapeutic antibody production.

In addition, kinetic analyses using the Carterra LSA showed a range of binding affinities from 0.11 to 200 nM.

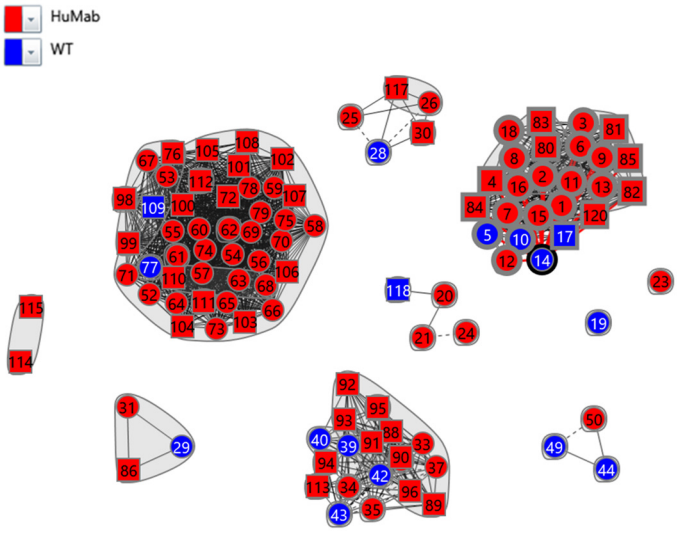

Figure 2: Network plot generated by Carterra's proprietary Epitope Data Analysis Software shows 90 OmniChicken antibody clones (red) and 16 wildtype chicken antibody standards (blue).

Summary

In this example, high throughput SPR experiments with the Carterra LSA show that generating antibodies in chickens produces antibody panels exhibiting broad epitope coverage, high target binding affinity, and species cross-reactivity. In the search for antibody candidates, this is a huge advantage, expanding the therapeutic potential and increasing the likelihood of targeting novel epitopes which may offer differentiated MOAs and associated IP opportunities.

Looking at the wider context of pharmaceutical development, the Carterra LSA offers drug discovery scientists the potential to perform powerful epitope binning experiments and detailed characterization of antibodies of interest at the earliest stage of the discovery process. Shifting the role of SPR upstream facilitates a streamlined and highly-informed lead selection process, which takes into account every antibody in a large antibody panel. Results from epitope binning and kinetic analyses can also be combined with data from orthogonal studies using Carterra's proprietary software to discover an antibody's unique therapeutic fingerprint and help in determining MOA.

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