Use of High Throughput SPR to Characterize Antibodies To Common Cancer Biomarkers

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Abstract

As a disease of disregulation, cancer is characterized both genetically and proteomically, requiring high quality tools to discern the disease’s difference between cell states. Among the reagents used to characterize biological systems at the protein level, antibodies are routinely applied, and engineered versions have proven useful for treating multiple therapeutic indications. We have generated a human polyclonal wide toolbox of polyclonal antibodies, identifying three antigenic 14mer sequences within each of the 200,000+ open reading frames at N-terminal, middle and C-terminal regions. Using a high throughput SPR system, Carterra® LSA™, we are able to define average on-rates, off-rates, and K_D’s for pAb’s generated to such widely used tools is critical for advancing translational opportunities into use in the clinic.

Methods

Two strategies were used to attach monoclonal and polyclonal antibodies to the surface of SPR chips. Due to the large difference in molecular mass between the ligands (~150 kDa antibodies) and analytes (~1,500 Da peptides), the ratio of the ligands on the surface to measured value of analyte is proportional to the ratio of molecular masses. Direct amino-coupling surface preparation. A high density 2H02M sensor chip was primed into 0.05M MES buffer pH 5.5 and the sensor chip was preconcentrated with 1-minute pulses of each of three solutions: 50 mM NaOH, 1M NaCl, and 10 mM Glycine pH 2.0. After preconcentration, the chip was activated with a 15-minute injection of a freshly prepared 1:1:1 (v/v/v) mixture of 0.4M EDC + 0.1M S-NHS + 0.1M MES buffer pH 5.5. The sodium azide protecting the polyclonal antibodies was removed by buffer exchange into 10mM Sodium Acetate pH 5.0 using Bio-Rad micro bio-spin 6 columns. The antibodies were placed on the chip using Carterra LSA multichannel printhead at several different concentrations – 50 µg/µL, 5 µg/µL, 0.5 µg/µL reaching maximum value of 10,000 RU after the 5-10 minutes application. The surface was quenched by 7-minute injection of 1M Ethanolamine pH 8.5, followed by short 0.05M MES buffer pH 5.5 injections to clean the prepared surface.

Protein A/G capture surface preparation. A protein A/G surface high density sensor chip RAGH02M was primed into 1xHBSTE buffer, and the antibodies were placed on the chip using the multichannel printhead achieving the maximum value of 10,000 RU, similar to the amino-coupling method. Cell culture supernatants were diluted in 1x HBSTE buffer with added 0.5 µg/ml bovine serum albumin (BSA) prior to multichannel printhead loading. Same filtered and desalted 1x HBSTE buffer with BSA was used for priming the LSA before the sequential addition of antibodies using the single flow cell for both surface preparations. Analysis of the results was performed using the Carterra LSA Kinetics application, which allows various types of referencing algorithms, X and Y alignment, spike removal and various mathematical models for data fitting and kinetics constants calculation.

Western Blot Characterization. 25 µg of the indicated protein whole cell or tissue extracts was loaded onto SDS-PAGE gels of varying percent concentrations, transferred to PVDF, and processed for immunoblotting with primary antibodies at the indicated dilutions and secondary detection using 1:10,000 anti-Rabbit IgG HRP, followed by washing and western blot detection and imaging.

Results

Figure 1: High-throughput Surface Plasmon Resonance. 1H2O or 2H2O antibodies can be screened in multiple arrays to provide affinity curves and epitope maps on the Carterra LSA platform.

Figure 2. Two strategies for coupling polyclonal antibodies. Two strategies (A) High Density 2H02M sensor chip primed into a 1xHBSTE buffer solution, and the antibodies were placed on the chip using the multichannel printhead achieving the maximum value of 10,000 RU, similar to the amino-coupling method. Cell culture supernatants were diluted in 1x HBSTE buffer with added 0.5 µg/ml bovine serum albumin (BSA) prior to multichannel printhead loading. Same filtered and desalted 1x HBSTE buffer with BSA was used for priming the LSA before the sequential addition of antibodies using the single flow cell for both surface preparations. Analysis of the results was performed using the Carterra LSA Kinetics application, which allows various types of referencing algorithms, X and Y alignment, spike removal and various mathematical models for data fitting and kinetics constants calculation.

Figure 3. Western Blot Characterization. 25 µg of the indicated protein whole cell or tissue extracts was loaded onto SDS-PAGE gels of varying percent concentrations, transferred to PVDF, and processed for immunoblotting with primary antibodies at the indicated dilutions and secondary detection using 1:10,000 anti-Rabbit IgG HRP, followed by washing and western blot detection and imaging.

Figure 4. Demonstration of antibody affinity on SPR and specificity in immunoassay performance.

Figure 5. Screening for a high affinity antibody. Four candidates from Figure 5, clones 39JB (A), 214JB (B), 42GP (C), and 62GP (D), were used for curve fit and kinetic analysis using LSA Kinetic Software.

Figure 6. Diverse Candidates Identified in Supernatant Screening.

SUMMARY

1. The high throughput 384 sample Carterra LSA SPR system was used to perform kinetic and specificity analysis on hundreds of antibodies related to cancer pathways and biomarkers.
2. SPR can be utilized for polyclonal antibody characterization using both larger antigens and 14-mer peptides.
3. Feasibility for detailed kinetic analysis of protein-protein interactions including on-rate, off-rate, and epitope differences was performed and supported by detection methods.
4. Western blot analysis of key cancer regulators using epitope specific antibodies correlate with SPR affinity and specificity analysis.
5. The high throughput platform can be used effectively to evaluate antibody candidates from culture supernatants for affinity measurements of both drug targets and biomarker proteins.
6. The system is capable of high throughput epitope binning of binders to facilitate development of antibody pairs for clinically relevant immunoassay development.