BACKGROUND

The advent of precision oncology has led to a shift towards biomarker-driven clinical trial designs and molecular profiling of individual patients. Identification of patients with the target biomarker profile may be useful in guiding patient selection as an enrichment strategy for clinical trials. Targeted multiple reaction monitoring mass spectrometry (MRM-MS) analysis for multiplexed quantitation of biomarker proteins in FFPE tissue provides direct, more accurate and precise quantification over current ‘gold standard’ immunohistochemistry (IHC) methods. However, MRM-MS has not yet been broadly applied to clinical trials. In this study, we demonstrate the systematic development, optimization and analytical validation of quantitative, multiplexed MRM-MS assays for robust biomarker quantification in clinical FFPE tissues, including sample analysis under GCLP. Results from an MRM panel targeting 8 clinically relevant biomarker proteins will also be shown, including the measured HER2 levels in FFPE breast tumors classified by IHC as 0, 1+, 2+ or 3+.

METHODS

MRM-MS biomarker panels were developed and optimized for multiplexed quantitation of ≤12 proteins, in which unique peptides derived from each target protein were monitored as a surrogate measure of protein levels. Tumor regions from FFPE tissue sections were dissected using laser capture or macrodissection, solubilized, digested with trypsin to generate peptides for analysis, spiked with fixed levels of stable isotope labeled (SIL) peptide standards, and analyzed by MRM-MS. Analytical validation was performed per NCI CPTAC guidelines, including response curves, assay repeatability, selectivity, stability, and reproducibility of endogenous detection. Clinical performance was assessed using commercially sourced FFPE-tumor tissues, including a cohort of breast tumor tissues with a wide range of HER2 expression.

RESULTS

Assay performance results were protein/peptide dependent, with sensitivity in the low pg/µg total protein range. For HER2, assay linearity was demonstrated over 2.5 to 3 orders of magnitude, with a precision and accuracy of <15% over 3 independent runs. In sample analysis, the MRM-MS was sufficiently sensitive to detect HER2 in 1 µg total protein from FFPE breast tumor classified by IHC as negative (0).

CONCLUSIONS

GCLP-compliant quantitative multiplexed large-scale clinical analysis of protein biomarkers by MRM-MS in FFPE tissue is feasible and enables precise and accurate quantitation of proteins when IHC methods are unsuitable or unavailable. Data can be used for patient stratification, optimization of treatment outcomes, drug resistance prediction, and to support clinical development of novel therapeutics.

REFERENCES


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THOUSANDS OF ANTIGENS ARE RECOGNIZED IN MICE VIA ENDOGENOUS ANTIBODIES AFTER BEING CURED OF A B78 MELANOMA VIA IMMUNOTHERAPY

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BACKGROUND

Antibodies can play an important role in innate and adaptive immune responses against cancer. Using a high-density peptide array, we assessed potential protein-targets for antibodies detected in mice cured of melanoma through a combined immunotherapy regimen. Our goal was to determine the linear peptide sequences recognized by anti-tumor antibodies produced in mice cured of melanoma following immunotherapy.

METHODS

Mice with GD2-expressing syngeneic B78 melanoma were treated with a combination immunotherapy (local radiation therapy + intratumoral anti-GD2 mAb linked to IL2) capable of inducing an ‘in situ vaccine’ effect (ISV), enabling mice to be cured of their tumors with long-term immune memory. Naive and immune sera were collected from these mice. Using flow cytometry, immune sera showed strong antibody-binding against B16 (parental cell line of B78 without GD2 expression). These sera were then used on a Nimble Therapeutics’ peptide-array (either whole proteome or a curated list of ~650 proteins) to determine specific antibody-binding sites, and data were analyzed using a dynamic programming method that scans adjacent peptides to determine whether a peptide is bound by antibodies. Proteins were selected if peptides were bound using immune sera but not bound with the sera from naïve or non-responding tumor-bearing mice.

RESULTS

Multiple proteins were selectively identified by immune sera that were not detected by sera from naïve or non-responding tumor-bearing mice. When focusing on the whole mouse proteome data, thousands of peptides were targeted by 2 or more mice and exhibited strong antibody binding only by immune sera. We also identified a few proteins that elicited an immune response in the naïve mouse sera that showed a significantly stronger signal in the immune sera of the same mice indicating that the cancer and/or the received therapy strengthened the immune response to these proteins.

CONCLUSIONS

We are able to detect selective antibody binding to immune sera. However, we are continuing to refine our analytical methods and are further investigating the identified proteins. These peptides may potentially serve as targets for antibody-based or cellular therapies. In addition, we are examining whether some of the identified tumor-specific endogenous antibodies might be used as biomarkers to predict response to our ISV regimen and potentially other immunotherapy treatments.

REFERENCE


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