IGF1 Biomarker Assay Validation

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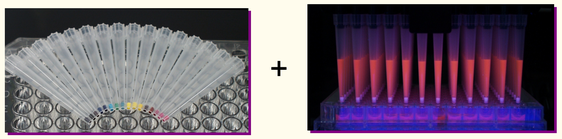
Introduction

As proteomic initiatives make headway in biomarker discovery and general protein cataloguing, it becomes imperative to be able to repeatedly screen for the same target proteins from thousands of individuals for clinical investigation and biomarker validation. Even though clincal proteomics technologies have matured over the past decade, conventional approaches are still not capable of routinely performing such analyses and have therefore been subjugated by classical immunoassay platforms for such population screening. However, these methodologies are inherently blind to the structural diversity found within any human population that are caused by genetic and/or posttranslational modifications, which may ultimately influence biomarker behavior. Such problems can be resolved through novel affinity-based mass spectrometry approaches. Shown here is the development and validation of such a mass spectrometric immunoassay (MSIA)[1], targeting the human biomarker insulin like growth factor 1

MSIA Approach

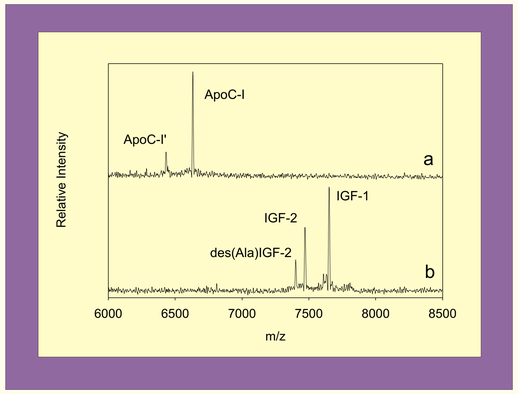
Affinity pipettes + HT processing



Three-dimensional Volumetric Arrays

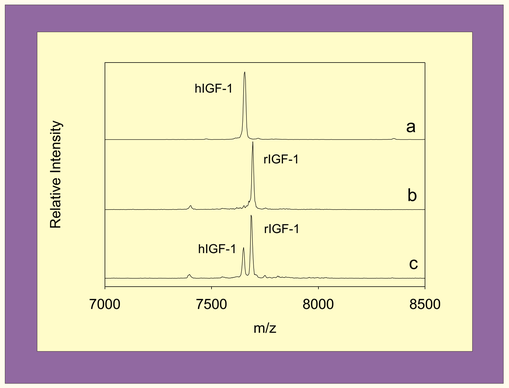
- Customized affinity pipettes used for target protein capture, enrichment and purification from crude biological fluids for subsequent MALDI-TOF MS analysis.
- Combined with HT robotics for parallel sample processing[2-4].
- Advantage over similar clinical proteomics approaches is the design as a three-dimensional volumetric array, which is ideal for low concentration protein analysis.

Assay Development



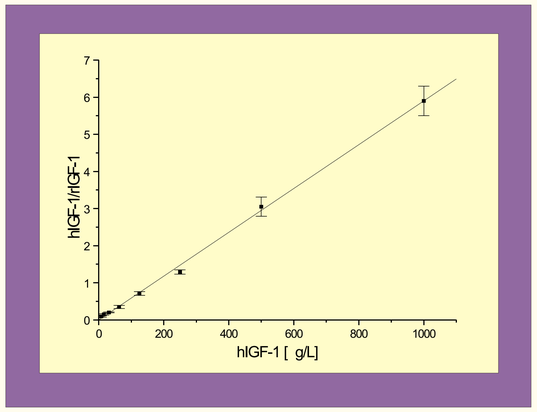
- MSIA-Tips derivatized with anti-human IGF1 polyclonal antibodies.
- Due to the nature of the insulin like growth factors to form complexes with binding proteins in plasma, a disruption step is required to liberate IGF1 prior to affinity capture.
- Non-specifically retained proteins are removed through the application of aqueous and organic rinses resulting in purified protein for MS interrogation.
- Developed assay has a lower limit of detection of ~15 pM.

Internal Reference Selection



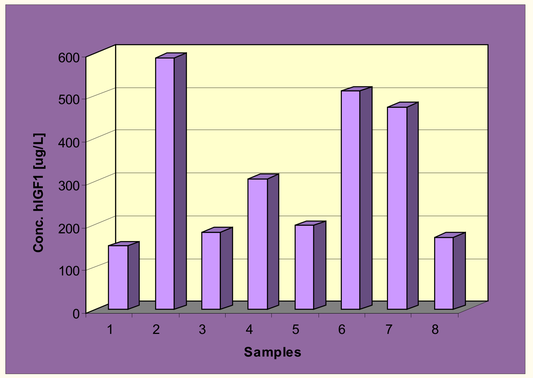
- Internal reference standard (IRS) was selected to normalize the human IGF1 MALDI-TOF MS response.
- Rat IGF1 was identified as an ideal IRS due to difference in molecular weight from the human form as well as affinity for the anti-human IGF1 polyclonal antibody.

Assay Response Validation



- Assay response validation was performed via the construction of a standard working curve.
- Constant amounts of rat IGF1 were combined with a serial dilution of purified human IGF1 standard.
- Samples were treated and analyzed using the newly developed assay protocols.
- Observed a linear response (linear least squares fit R^2 = 0.998 & SEE = 0.080) with a (essentially) zero intercept (hIGF-1/rIGF-1 = 0.0059 [hIGF-1 (in mg/L)] 0.0022) covering the concentration range of 7.8 ug/L to 1,000 ug/L.

Application



- Resulting standard working curve used to validate the assay was then used to address human plasma samples.
- Samples were prepared in the same fashion as those used in the standard working curve, but used human plasma instead of purified human IGF1 solutions.
- Plasma IGF-1 concentration was determined to range between 149 to 589 mg/L for the individuals tested.

Summary of Results

This work demonstrates the development and validation of a novel clinical proteomics approach in which specific protein targets can be expeditiously and selectively isolated from a complex biological fluid for MS characterization. This approach, with its HT capacity is ideal for second-generation proteomics studies in which novel biomarkers need to be validated through large population screenings. The ability to perform absolute protein quantification is another facet of this approach that is not readily achievable with other clinical proteomics methodologies. The development, validation and application of mass spectrometric immunoassays, as shown here, are proving advantageous to proteomics and the identification and development of biomarkers for clinical application.

Selected References

1) Nelson, R.W., et al., *Mass-Spectrometric Immunoassay. Anal. Chem., 1995.* **67(7): p. 1153-1158.**
2) Niederkofler, E.E., et al., *Determination of beta-2 microglobulin levels in plasma using a high-throughput mass spectrometric immunoassay system. Anal. Chem.., 2001.* **73(3): p. 3294-9.**
3) Kiernan, U.A., et al., *High-Throughput Protein Characterization Using Mass Spectrometric Immunoassay. Anal. Biochem., 2002.* **301(1): p. 49-56.**
4) Nedelkov, D., et al., *High-Throughput Comprehensive Analysis of Human Plasma Proteins: A Step toward Population Proteomics. Anal Chem, 2004.* **76(6): p. 1733-7.4)**