Can the Diagnostic and Prognostic Value of Prostate Specific Antigen be Improved by Harnessing the Specificity of Mass Spectrometry?

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Background and Significance

In the untiring search to identify and quantify cancer biomarkers, the field of proteomics has emerged with great promise.[1] Liquid chromatography coupled to mass spectrometry (LC-MS) has certainly become a vital tool in these experiments. However, the limits-of-detection (LOD) of existing mass spectrometry platforms mandate the integration of devices such as the air amplifier and other strategies to improve the overall sensitivity in order to become clinically relevant. It is important to note that the specificity attained using mass spectrometry is unparalleled and thus provides the stimulus for the efforts to improve the sensitivity. The concentration of the clinically relevant peptides and proteins in early disease stage tends to be below that detectable with current LC-MS technology. Only when a patient is consumed with cancer will biomarkers be in high enough abundance, when the prognosis for a recovery at this disease stage is markedly decreased. An aerodynamic device described herein as the air amplifier will improve the early detection and quantification of low abundance cancer biomarkers by lowering the LOD of LC-MS/MS methods. The development of a technology, through high precision engineering, enabling a robust analytical strategy as applied to a relevant biological concern (i.e. prostate cancer) is described in a manner that can ultimately improve diagnoses and disease treatment options.

Previous reports of protein cleavage isotope dilution mass spectrometry demonstrate the potential for quantitative studies.[2-4] Prostate specific antigen (PSA) has emerged as a significant biomarker in the diagnosis strategy of prostate cancer.[5-8] It is used in a complementary manner with other tests including digital rectal exams (DRE) and invasive tissue biopsies. However it has been demonstrated that elevated PSA accompanies other prostatic conditions including benign prostatic hyperplasia and prostatitis. The immunologically detectable forms of PSA include complexes with α_1-chymotrypsin and α_2-chymotrypsin in addition to other binding proteins. By utilizing mass spectrometry, different protein forms can be identified in hopes to distinguish various prostatic conditions.

Instrumentation

Mass spectrometry is an extremely sensitive and specific technique that can be used to analyze a broad range of compounds over a wide dynamic range

of molecular weight and concentration.[9, 10] These factors have led to increasing interest in coupling liquid separation techniques directly to mass spectrometry for effective analysis of complex mixtures (e.g. serum). Electrospray ionization (ESI) facilitates the direct coupling of liquid separation methods (*e.g.*, HPLC, chromatofocusing, capillary electrophoresis) to the mass analyzer. ESI conditions can be manipulated to enhance detection limits ranging from very low (< 100 Da), to extremely high (>100 MDa)[11], molecular weight compounds. The utilization of mass spectrometry for cancer biomarker analysis extends back to the 1970's[12], and recent reports show great promise for its use as a diagnostic tool.[13-17]

In a conventional electrospray ionization only a minute fraction of the ion generated are sampled by the mass spectrometer. This is inherently due to sample losses occurring in the subsequent stages of ion transmission following sample ionization. Many factors contribute to the low transmission efficiency including the generation of ions by multiple Coulombic explosions that occur at the onset of a droplet on the ESI needle tip. Substantial sample loss in mass spectrometry analyses transpires in the atmospheric pressure region between the electrospray emitter tip and the capillary inlet.[18] It is estimated that only one out of 10^3-10^4 ions generated are sampled by the mass spectrometer.[19-23]

The air amplifier has proven useful in addressing this issue by increasing the observed signal by an order of magnitude.[24-29] The air amplifier's function is accomplished by throttling a thin annular jet of compressed gas into a venturi nozzle passage through a small gap. The jet expands to supersonic conditions upon its exit, creating a low-pressure region that enables air entrainment into the venturi nozzle. The annular jet is typically directed toward the exit of the venturi tube via the Coanda effect. In the Coanda effect, a jet moving over a curved surface can remain attached to the surface as long as a balance between the sub-ambient pressure in the jet sheet and the centrifugal force occurs. The entrained air can be accelerated in the axial and radial directions by contouring the venturi tube. Previous studies have considered air amplification as a means of focusing ions generated through an electrospray process into the inlet of a mass spectrometer. Ion abundance increases of a factor of ten or more and increases in the theoretical mass resolving power were observed in several tests, indicating that the air-amplifier concept is potentially useful as a low-cost alternative to other focusing strategies such as ion funnels. However, significant user experience and case-specific tuning of the air-amplifier parameters (gap width, placement of the emitter and mass-spectrometer inlet) were required to achieve optimal results. Recent work in our laboratory has extended the use of the air amplifier to LC-MS by heating the inlet nitrogen and the body of the air amplifier.

Experimental

Recently the air amplifier has been integrated with liquid chromatography and demonstrated improved ion abundance. This enables increased identifications resulting in greater proteome coverage. In a targeted proteomics experiment, the air amplifier is implemented on a triple quadrupole mass

spectrometer. Selected reaction monitoring is routinely used for selective and quantitative tandem MS. These experiments are described in further detail below.

The diagnostic relevance of the air amplifier is studied in a discovery-based proteomics experiment. In the discovery experiment, a digest of prostate specific antigen (PSA) will under go LC-MS analysis. Using this system will allow for quantitative measures including improvements in ionization time, number of MS/MS per unit time in each LC-MS/MS run, and the number of proteins identified using the air amplifier. Concurrently, an analysis of an 80 mg/mL human serum albumin (HSA) digest will be performed to determine the actual improvement in limits of detection. Biological samples are highly complex as the number of peptides within a digested serum sample is ~10^{5-6} with a concentration range of ~10^{9}. Current LC-MS sensitivity is a limiting factor of candidate biomarkers that fall below the assay LOD by approximately 1000-fold. With the improved air amplifier design enhancing signal 10-fold, and combining this with other methods for decreasing LOD, clinical diagnostics will hold much better promise for disease diagnosis and treatment options. Limits of detection with the air amplifier will be determined with the HSA digest spiked with decreasing amounts of the PSA digest followed by LC/MS analysis. A calibration curve from lowest to highest concentration of the HSA digest will be used to evaluate the limits of detection as a function of the response across the LC gradient with the air amplifier ON and OFF.

An integral portion of the biomarker discovery experiments will employ selected reaction monitoring (SRM). This method allows both detection and quantification of specific molecules. A mock serum sample (HSA digest) will be spiked with PSA and then digested. Stable-isotope labeled tryptic fragments will be added at known concentrations and serve as the internal standard. As a result, the LOD for targeted proteomics (SRM) can be determined for both the air amplifier ON and OFF and compared with previous results by Muddiman and coworkers that had a detection limit of 4.5 mg/L of PSA in serum.[4] With the improved air amplifier design, we anticipate an improvement in ion abundance of >10 fold.

Conclusions

The methodology developed for the detection and quantification of PSA will be widely applicable to other disease systems (e.g. breast cancer, cardiovascular disease). This will be accomplished by establishing disease-specific biomarkers with their respective internal standards for use in the SRM proteomics analysis. Non-specific point of care testing will continue to have its place in clinical diagnostics however the specific information obtained from the mass spectrometry approach will be highly useful for ongoing treatment plans.

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