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(54) **Title**: METHODS AND MATERIALS FOR MONITORING MYELOMA USING QUANTITATIVE MASS SPETROMETRY

(57) **Abstract**: The subject invention concerns methods and materials for diagnosing, monitoring the progress, and/or providing a prognosis for multiple myeloma and other conditions associated with antibody production in a person or animal. The methods of the invention utilize mass spectrometry for quantitative monitoring and detection of antibody produced by the plasma cells. The methods of the invention can be utilized for diagnosis, monitoring, and/or prognosis of multiple myeloma, monoclonal gammopathy, and other immunological or hematological conditions and disorders. In addition to detecting and quantifying antibody in a sample, other biological markers, such as serum albumin and/or beta-2-microglobulin, can also be detected and quantified using the present invention, and in combination with detection and quantification of antibody. Thus, in one embodiment, both antibody and serum albumin and/or beta-2-microglobulin are detected and quantified using mass spectrometry and a diagnosis or prognosis made based on the results and levels detected.

DESCRIPTION

METHODS AND MATERIALS FOR MONITORING MYELOMA USING QUANTITATIVE MASS SPECTROMETRY

CROSS-REFERENCE TO RELATED APPLICATION

The present application claims the benefit of U.S. Provisional Application Serial No. 61/076,907, filed June 30, 2008, which is hereby incorporated by reference herein in its entirety, including any figures, tables, nucleic acid sequences, amino acid sequences, and drawings.

BACKGROUND OF THE INVENTION

Multiple myeloma (MM) is a cancer of the plasma cell, which primarily develops in the elderly population. The progression of the tumor is well understood, and it can be diagnosed by the presence of multiple myeloma cells in the bone marrow and monitored by the amount of antibody secretion from the clonal population of plasma cells. A premalignant condition known as monoclonal gammopathy of undetermined significance (MGUS) develops at certain rates in the U.S. population: 3% at age 50, 5% at age 70, and 7% by age 85; approximately 1% of MGUS patients progress to multiple myeloma on an annual basis (Kyle *et al.*, 2006). The molecular causes for progression from MGUS to MM are unknown. After the onset of the cancer, multiple myeloma patients suffer from several symptoms, including calcium dysregulation, renal failure, anemia, and bone lesions. A diagnosis of multiple myeloma is established using blood and urine tests. For advanced stage patients, complete skeletal surveys are also used to examine the damage caused by multiple myeloma in the bone marrow. Staging with serum calcium, creatinine, hemoglobin, and most importantly, the concentration of the "monoclonal serum protein" was established in 1975 by Durie and Salmon (Durie and Salmon, 1975). The International Staging System determined in 2005 uses those markers as well as serum albumin and β-2-microglobulin (Greipp *et al.*, 2005). The survival statistics indicate the importance of early detection and proper staging, and show the devastating impact of

multiple myeloma. Stage I patients have median survival times of 62 months, stage II 45 months, and stage III patient median survival is reduced to 29 months.

Despite the highly specific and easily detectable biomarkers, many challenges still exist for MM treatment. Several different treatment regimens are under investigation; these strategies have been the subject of numerous recent reviews (Fonseca and Stewart, 2007; Chanan-Khan and Lee, 2007; Thomas and Alexanian, 2007; Falco *et al.*, 2007). Novel therapeutic strategies include proteasome inhibition with agents like bortezomib (Voorhees and Orlowski, 2007; Manochakian *et al.*, 2007) and a combination of cancer cell targeting and immune modulation with thalidomide derivatives like Lenalidomide (Singhal and Mehta, 2007). While each of these agents can have some success against multiple myeloma cells, proteasome inhibitors are the only molecularly guided therapy to date: treatment is more effective for patients with myelomas that secrete high levels of monoclonal antibodies (Meister *et al.*, 2007). The use of the other agents is directed by the expected tolerance for side effects rather than molecular targeting. Regardless, these agents improve the patient outcome when compared to the current standard of care (Ma *et al.*, 2003), and drug combination strategies are currently in clinical trials (Srikanth *et al.*, 2008; Richardson *et al.*, 2007; Merchionne *et al.*, 2007). Proteomic research may contribute to guidance of existing and emerging therapies. Identification of novel targets including c-Jun and the Fanconi anemia pathway (Chen *et al.*, 2005) also offers opportunities to examine protein expression, binding partners, and post-translational modification. Furthermore, the bone marrow microenvironment is critical for progression of multiple myeloma and likely contributes to drug resistance; (Li and Dalton, 2006; Hazlehurst *et al.*, 2003; Dalton, 2003) this knowledge has led to preclinical models examining multiple myeloma in the context of the bone marrow microenvironment. Plausible targets in the bone marrow microenvironment include cytokine signaling, *e.g.* IL-6, (Chauhan *et al.*, 1997; Urashima *et al.*, 1997) and integrin mediated drug resistance (Damiano *et al.*, 1999). Proteome analysis may make a significant contribution here as well.

Patient monitoring strategies present significant challenges, particularly in the detection of MGUS patients most likely to develop multiple myeloma and ongoing assessment of relapse or recurrence in previously treated multiple myeloma patients. Many MM patients who have undergone treatment are repetitively checked at two week

or four week intervals, leading to high numbers of clinic visits and collection of large volumes of blood. Methods for patient sampling and detection of the monoclonal serum protein are presented from a process chemistry standpoint. Process chemists use extensive background knowledge of synthesis, analysis, and engineering to redesign industrial assembly lines or improve individual steps in manufacturing.

BRIEF SUMMARY OF THE INVENTION

The subject invention concerns methods and materials for diagnosing, monitoring the progress, and/or providing a prognosis for multiple myeloma and other conditions associated with antibody production in a person or animal. In one embodiment, quantitative mass spectrometry is used to monitor the amount of multiple myeloma cells in patients using serum samples. Each MM tumor secretes a specific (monoclonal) antibody; the amount of the tumor in the blood or bone marrow of a patient can be measured by the detection of this protein. Current methods use gel or capillary electrophoresis to monitor the relative amount and identify the type of the antibody that is secreted by the MM cells. The quantitative mass spectrometry techniques of the present invention combines these two measurements and can provide for absolute quantification for each of the antibody chains (A, D, E, G, and M, as well as kappa and lambda) in MM patients. Proteolytic peptides are used as surrogate biomarkers to measure the amount of the monoclonal antibody expressed in patients' sera. The methods of the present invention can be applied to MM patients, patients with the premalignant condition, monoclonal gammopathy of undetermined significance (MGUS), and other immune or blood disorders, such as Waldenstrom's macroglobinemia or HIV/AIDS. Additional diagnostic markers, including but not limited to serum albumin and beta-2-microglobulin, can also be quantified using the present invention.

In one embodiment of a method of the invention, monoclonal antibody proteins were excised from serum protein electrophoresis gels and digested with trypsin. Following trypsin digestion, the resulting isolated proteolytic peptides were sequenced with liquid chromatography coupled to tandem mass spectrometry. Using the results from several patient samples, specific peptides were selected to monitor each type of antibody (A, D, E, G, and M), as well as kappa and lambda light chain and other diagnostic molecules like serum albumin and beta-2-microglobulin (see Table 1). After

selecting peptides that were consistently detected in all patient samples, a quantitative assay was developed using liquid chromatography coupled to multiple reaction monitoring (LC-MRM) on a triple quadrupole mass spectrometer. After overnight digestion of patient serum, the peptides are analyzed in a 45-minute experiment separating them by reverse phase and filtering them by molecular weight and sequence specific fragment ions. Using these transitions (pairs of intact molecules and fragments), individual peptide molecules can be selectively quantified, even from a complex matrix like human blood serum. The methods of the present invention have been proven effective with control serum and patient samples. Absolute quantification is obtained by spiking in known amounts of synthetic peptide containing heavy isotope labels, *e.g.* ^{13}C and ^{15}N or by creating a mass shift by substituting an amino acid with one of a similar composition (such as Alanine for Glycine).

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A and 1B depict the antibody production for detected with conventional serum protein electrophoresis for use in multiple myeloma diagnosis and prognosis.

Figure 2 depicts the antibodies identified using immunofixation electrophoresis for use in multiple myeloma diagnosis and prognosis.

Figure 3 depicts the multiple myeloma diagnosis and prognosis method where monoclonal spike is an IgG with κ light chain. After LC-MS/MS, peptides, such as ALPAPIEK (SEQ ID NO:4) from IgG, can be selected for quantitative mass spectrometry assays.

Figure 4 depicts a schematic diagram of selected reaction monitoring used in multiple myeloma diagnosis and prognosis.

Figure 5 depicts the abilities of multiple myeloma diagnosis and prognosis, where single molecule can be detected by filtering the m/z values for peptide and specific fragments. Using the same serum sample shown in Figure 1B, the quantity and type of antibody are determined in a mass spectrometry assay; high levels of ALPAPIEK (SEQ ID NO:4) from IgG.

Figure 6 depicts an assay to confirm the multiple myeloma diagnosis and prognosis method DSTYSLSSTLTLSK (SEQ ID NO:28) from κ light chain were confirmed using Multiple Reaction Monitoring.

BRIEF DESCRIPTION OF THE SEQUENCES

SEQ ID NO:1 is a peptide sequence of the invention (IGHA1, 2).

SEQ ID NO:2 is a peptide sequence of the invention (IGHA1, 2).

SEQ ID NO:3 is a peptide sequence of the invention (IGHA1).

SEQ ID NO:4 is a peptide sequence of the invention (IGHG1, 3).

SEQ ID NO:5 is a peptide sequence of the invention (IGHG1, 2).

SEQ ID NO:6 is a peptide sequence of the invention (IGHG1, 2).

SEQ ID NO:7 is a peptide sequence of the invention (IGHG1).

SEQ ID NO:8 is a peptide sequence of the invention (IGHG2).

SEQ ID NO:9 is a peptide sequence of the invention (IGHG3).

SEQ ID NO:10 is a peptide sequence of the invention (IGHG3, 4).

SEQ ID NO:11 is a peptide sequence of the invention (IGHG4).

SEQ ID NO:12 is a peptide sequence of the invention (IGHM).

SEQ ID NO:13 is a peptide sequence of the invention (IGHM).

SEQ ID NO:14 is a peptide sequence of the invention (IGHM).

SEQ ID NO:15 is a peptide sequence of the invention (IGKC).

SEQ ID NO:16 is a peptide sequence of the invention (IGKC).

SEQ ID NO:17 is a peptide sequence of the invention (IGKC).

SEQ ID NO:18 is a peptide sequence of the invention (LAC).

SEQ ID NO:19 is a peptide sequence of the invention (LAC).

SEQ ID NO:20 is a peptide sequence of the invention (IGHE).

SEQ ID NO:21 is a peptide sequence of the invention (IGHE).

SEQ ID NO:22 is a peptide sequence of the invention (IGHD).

SEQ ID NO:23 is a peptide sequence of the invention (IGHD).

SEQ ID NO:24 is a peptide sequence of the invention (IGHD).

SEQ ID NO:25 is a peptide sequence of the invention (ALBU).

SEQ ID NO:26 is a peptide sequence of the invention (ALBU).

SEQ ID NO:27 is a peptide sequence of the invention (ALBU).

SEQ ID NO:28 is a peptide sequence of the invention.

SEQ ID NO:29 is a peptide sequence of the invention.

SEQ ID NO:30 is a peptide sequence of the invention.

DETAILED DESCRIPTION OF THE INVENTION

The subject invention concerns methods and materials for diagnosing, monitoring the progress, and/or providing a prognosis for multiple myeloma and other diseases or conditions associated with antibody production in a person or animal. In one embodiment, the disease or condition is one characterized by excessive antibody production, and in particular, excessive monoclonal antibody production. The methods of the invention utilize mass spectrometry for quantitative monitoring and detection of antibody produced by the plasma cells. The methods of the invention can be utilized for diagnosis, monitoring, and/or prognosis of multiple myeloma, monoclonal gammopathy, and other immunological or hematological conditions and disorders. In addition to detecting and quantifying antibody in a sample, other biological markers, such as serum albumin and/or beta-2-microglobulin, can also be detected and quantified using the present invention, and in combination with detection and quantification of antibody. Thus, in one embodiment, both antibody and serum albumin and/or beta-2-microglobulin are detected and quantified using mass spectrometry and a diagnosis or prognosis made based on the results and levels detected.

In one embodiment of a method of the present invention, a biological sample, such as a blood or serum sample, is treated to isolate the target protein therein. In one embodiment, the biological sample is subjected to size exclusion chromatography, gel electrophoresis, and/or affinity chromatography to isolate the target protein. In a specific embodiment, the target protein is an antibody. The target protein is then subjected to proteolytic fragmentation to create fragments of the target protein. In a specific embodiment, the target protein fragments are prepared by exposing the protein to trypsin for a sufficient period of time. Other means for fragmentation of a target protein are known in the art and can be used in the present methods. Optionally, the target protein can be denatured prior to fragmentation. In one embodiment, treatment of the target protein with urea and disulfide reduction and cysteine alkylation can be performed. Following fragmentation of the target protein into peptides, the peptides are subjected to mass spectrometry to identify and quantify the levels of the target protein. In one embodiment, following proteolytic fragmentation and prior to quantitative mass spectrometry, the peptide fragments are separated by reverse phase chromatography

and/or filtering by molecular weight. Using the results from several patient samples, specific peptides were selected to monitor each type of antibody (A, D, E, G, and M), as well as kappa and lambda light chain and other diagnostic molecules like serum albumin and beta-2-microglobulin (see Table 1). In a specific embodiment, the fragmentation peptides of the target protein are ALPAPIEK (SEQ ID NO:4) and/or DSTYSLSSTLTLSK (SEQ ID NO:28). Synthetic peptides having an amino acid substitution or synthetic stable isotope-labeled peptides (*e.g.*, comprising ^{2}H, ^{13}C, or ^{15}N atoms in the peptide molecule) having the same sequence as the fragmentation peptides can be used as internal standards during the mass spectrometry to provide for quantitation of the specific peptide fragments. The ratio of the peptide fragment to the isotope-labeled peptide standard can be used to calculate the quantity of the target protein. In one embodiment, the peptides are specific to heavy chains of human IgG, IgA, IgM, IgD, or IgE. In another embodiment, the peptides are specific to human kappa (κ) or lambda (λ) immunoglobulin light chains. In one embodiment, the internal standard peptides can have an amino acid sequence shown in any of SEQ ID NOs:1 to 30, or a fragment or variant thereof. In a specific embodiment, the synthetic internal standard peptide comprises the amino acid of SEQ ID NO:4 or SEQ ID NO:28, or a fragment or variant thereof. In one embodiment, the mass spectrometry methods comprise liquid chromatography coupled to multiple reaction monitoring (LC-MRM) using a triple quadrupole mass spectrometer.

The subject invention also concerns peptides of target proteins, such as immunoglobulin heavy chain, kappa light chain, lambda light chain, serum albumin, and beta-2-microglobulin, that can be used in the methods of the present invention. In one embodiment, a peptide corresponds to a proteolytic digestion fragment of a human IgG, IgA, IgM, IgD, or IgE heavy chain, or a human kappa or lambda immunoglobulin light chain. In one embodiment, a peptide of the invention comprises one or more stable heavy isotopes, such as ^{2}H, ^{13}C, or ^{15}N. In another embodiment, a peptide of the invention comprises one or more amino acid substitutions of similar composition (such as an alanine substituted for a glycine) from that of the sequence of target protein such that the subject peptide has a "mass shift" when compared to the corresponding peptide fragment of the target protein.

In a specific embodiment, a peptide of the invention comprises an amino acid sequence shown in any of SEQ ID NOs:1 to 30, or a fragment or variant thereof. In an

exemplified embodiment, a peptide of the invention has the amino acid sequence of SEQ ID NO:4 (for IgG heavy chain) or SEQ ID NO:28 (for kappa light chain).

Biological samples refer to a fluid or tissue composition obtained from a human or animal. Biological samples within the scope of the invention include, but are not limited to, whole blood, peripheral blood, blood plasma, bone marrow, spleen, serum, urine, tears, saliva, sputum, exhaled breath, nasal secretions, pharyngeal exudates, bronchoalveolar lavage, tracheal aspirations, interstitial fluid, lymph fluid, meningal fluid, amniotic fluid, glandular fluid, feces, perspiration, mucous, vaginal or urethral secretion, cerebrospinal fluid, and transdermal exudate. A biological sample also includes experimentally separated fractions of all of the preceding solutions or mixtures containing homogenized solid material, such as feces, tissues, and biopsy samples.

The methods of the present invention can be used with humans and other animals. The other animals contemplated within the scope of the invention include domesticated, agricultural, or zoo- or circus-maintained animals. Domesticated animals include, for example, dogs, cats, rabbits, ferrets, guinea pigs, hamsters, pigs, monkeys or other primates, and gerbils. Agricultural animals include, for example, horses, mules, donkeys, burros, cattle, cows, pigs, sheep, and alligators. Zoo- or circus-maintained animals include, for example, lions, tigers, bears, camels, giraffes, hippopotamuses, and rhinoceroses.

All patents, patent applications, provisional applications, and publications referred to or cited herein are incorporated by reference in their entirety, including all figures and tables, to the extent they are not inconsistent with the explicit teachings of this specification.

Following are examples that illustrate procedures for practicing the invention. These examples should not be construed as limiting. All percentages are by weight and all solvent mixture proportions are by volume unless otherwise noted.

Example 1

In multiple myeloma, because each plasma cell secretes a unique antibody, the replication of the tumor cell and the progression of disease can be monitored by measuring the serum concentration of the monoclonal antibody it produces. Initial

qualitative measurements are made using serum protein electrophoresis (SPEP) and dye visualization (see Figures 1A and 1B). Separation of the serum proteins is achieved, isolating albumin from four regions of globulins, termed alpha 1 (α1), alpha 2 (α2), beta (β), and gamma (γ), described by the differences in their migration relative to albumin. Normally, antibodies migrate into the γ region, but are low in intensity compared to albumin and are present only as diffuse bands (Figure 1A). The monoclonal antibodies produced in high concentration by multiple myeloma plasma cells can be visualized as a single narrow, discrete, dark band usually in the γ region of the gel (Figure 1B). Patients with abnormally high levels of protein in the gamma region can be diagnosed with multiple myeloma by identifying the type of monoclonal antibody using immunofixation electrophoresis (IFE), which is a separation similar to SPEP, but with specific detection for each antibody chain (Figure 2). Typical screens test for immunoglobulin G, A, and M heavy chains, as well as kappa (κ) and lambda (λ) light chains. Immunoglobulin D or E myelomas are very rare; when suspected, lanes of the standard IFE are replaced, enabling specific detection of IgD or IgE heavy chain proteins. In the example, the patient has a tumor that produces an IgG κ monoclonal antibody protein (Figure 2). The combination of these two tests establishes the relative amount and type of the antibody that is secreted by the multiple myeloma tumor cells. Gel-based techniques have recently been complemented by capillary array instruments that can analyze eight samples in parallel, greatly increasing the throughput and lowering the amount of sample preparation necessary (tubes of serum are simply loaded into the instrument, which automatically dilutes each sample in the buffer used for capillary electrophoresis). SPEP and IFE are performed separately.

The quantitative mass spectrometry methods of the invention can replace standard art methods with a single analysis. Protein bands from SPEP have been processed for protein identification using LC-MS/MS. Constant regions on an antibody are utilized in detection for quantitative monitoring; as shown in Figure 3, a peptide sequence ALPAPIEK (SEQ ID NO:4) is used to detect immunoglobulin G heavy chains. After generating peptides for monitoring each of the types of antibodies, a comprehensive method for antibody measurement was made. Briefly, minute volumes of serum (1 to 10 μl) were processed for detection of each of the antibody chains: G, A, M, D, E, κ, and λ. After protein denaturation with urea, disulfide reduction, and cysteine alkylation, trypsin

digestion was performed. The sample was then diluted and analyzed with liquid chromatography coupled to multiple reaction monitoring (LC-MRM) on a triple quadrupole mass spectrometer (Figure 4). Methods for quantitative mass spectrometry of other proteins, such as vitellogenin, thyroglobulin, C-reactive protein, and others, have been described in U.S. Patent Nos. 7,544,518 and 7,163,803; Published U.S. Patent Application Nos. 2009/0011447 and 2009/0148951; and publications by Anderson and Hunter (2006) and Kuhn *et al.* (2004).

In one embodiment, the mass spectrometer instrument selectively quantifies peptides by filtering the m/z of the intact species in the first quadrupole (Q1), fragments the molecules in the second quadrupole (Q2), and filters the m/z of a particular fragment in the third quadrupole (Q3). Each of these peptide and fragment pairs is known as a transition; the instrument measures each transition as part of a cycle, continuously moving from one to the next. For each peptide, multiple transitions are monitored; the coincidence detection of multiple fragments from the peptide increases the confidence in the assignment. Each target protein can be quantified using more than one peptide. While several rules for peptide selection have been put forward, selection of peptides in biological or clinical context frequently deviates from those guidelines. Examples of quantification with LC-MRM are shown in Figures 5 and 6 using the ALPAPIEK (SEQ ID NO:4) peptide from immunoglobulin G (IgG) heavy chain and DSTYSLSSTLTLSK (SEQ ID NO:28) from the κ light chain. The ion signals corresponding to the y_5 and y_6 ions of ALPAPIEK (SEQ ID NO:4) were detected at 22 minutes in Figure 5; y_4 were also monitored. The y_8, y_{11}, and y_{12} ion signals for DSTYSLSSTLTLSK (SEQ ID NO:28) were detected at 28 minutes, as shown in Figure 6. These ion signals were confirmed from the sample used for the SPEP and IFE, illustrated with the diagrams in Figures 1 and 2.

The quantitative mass spectrometry assay of the present invention is advantageous in animal models where limited amounts of blood serum can be obtained. The implementation of a single quantitative test provides advantages over the qualitative tests currently used to follow multiple myeloma patients. The speed and parallel processing that can be achieved with automated sample handling and MS detection will also significantly improve the throughput of patient samples. The adoption of the methods of the invention at a tertiary cancer center will enable surrounding primary care physicians

and hospitals to send samples to a centralized facility for processing and analysis. Point of care patient sampling can be performed with rapid turnaround of results to the treating physician (~1 day) even at a centralized facility.

Table 1.

Protein	Peptide Sequence	M/Z	Transitions					
IGHA1, 2	SAVQGPPER (SEQ ID NO:1)	470.747	4	498.268	5	555.289	6	683.348
IGHA1, 2	WLQGSQELPR (SEQ ID NO:2)	607.320	6	729.390	7	786.411	8	914.470
	WLQGSQELPR (SEQ ID NO:2)	610.820	6	736.390	7	793.411	8	921.470
IGHA1	TPLTATLSK (SEQ ID NO:3)	466.277	5	519.314	6	620.362	7	733.446
	TPLTATLSK (SEQ ID NO:3)	469.777	5	526.314	6	627.362	7	740.446
IGHG1, 3	ALPAPIEK (SEQ ID NO:4)	419.756	4	486.293	5	557.330	6	654.383
IGHG1, 2	EPQVYTLPPSR (SEQ ID NO:5)	643.841	4	456.257	6	670.389	7	883.452
	DPQVYTLPPSR (SEQ ID NO:6)	636.833	4	456.257	6	670.389	7	833.452
IGHG1	GPSVFPLAPSSK (SEQ ID NO:7)	593.828	8	846.473	9	945.541	10	1032.573
	GPSVFPLAPSSK (SEQ ID NO:7)	596.828	8	852.472	9	951.540	10	1038.573
IGHG2	GLPAPIEK (SEQ ID NO:8)	412.748	4	486.293	5	557.240	6	654.383
	GLPAPIEK (SEQ ID NO:8)	415.748	4	492.293	5	563.330	6	660.383
IGHG3	WYVDGVEVHNAK (SEQ ID NO:9)	708.850	6	697.363	9	968.480	11	1230.612
	WYVDGVEVHNAK (SEQ ID NO:9)	711.850	6	703.363	9	974.480	11	1236.611
IGHG3, 4	VVSVLTVLHQDWLNGK (SEQ ID NO:10)	904.507	5	617.341	10	1209.638	11	1310.686
IGHG4	SLSLSLGK (SEQ ID NO:11)	402.746	5	517.335	6	604.367	7	717.451
IGHM	DGFFGNPR (SEQ ID NO:12)	455.215	4	443.237	5	590.305	7	794.394
	DAFFGNPR (SEQ ID NO:13)	462.223	4	443.237	5	590.305	7	808.410
IGHM	QVGSGVTTDQVQAEAK (SEQ ID NO:14)	809.408	8	888.443	9	989.490	10	1090.538
IGKC	VDNALQSGNSQESVTEQDSK (SEQ ID NO:15)	1068.489	6	707.321	14	1495.651	8	893.421
IGKC	TVAAPSVFIFPPSDEQLK (SEQ ID NO:16)	973.518	8	913.463	9	1060.532	11	1320.684
	TVGAPSVFIFPPSDEQLK (SEQ ID NO:17)	966.510	8	913.463	9	1060.532	11	1320.684
LAC	AAPSVTLFPPSSEELQANK (SEQ ID NO:18)	993.513	10	1102.538	11	1199.591	12	1346.659
LAC	AGVETTTPSK (SEQ ID NO:19)	495.759	5	533.294	6	634.341	7	763.384
	AGVETTTPSK (SEQ ID NO:19)	498.759	5	539.294	6	640.341	7	769.384
IGHE	GSGFFVFSR (SEQ ID NO:20)	502.254	5	655.357	6	802.425	7	859.447

Protein	Peptide Sequence	M/Z	Transitions					
	GSAFFVFSR (SEQ ID NO:21)	509.262	5	655.357	6	802.425	7	873.462
IGHD	EPAAQAPVK (SEQ ID NO:22)	455.754	5	542.330	6	613.367	7	684.404
	EPAGQAPVK (SEQ ID NO:23)	448.746	5	542.330	6	599.351	7	670.388
IGHD	VRPGGVEEGLLER (SEQ ID NO:24)	678.362	7	845.437	10	1058.548	11	1159.596
ALBU	LVNEVTEFAK (SEQ ID NO:25)	575.312	6	694.378	7	823.420	8	937.463
	LVNDVTEFAK (SEQ ID NO:26)	568.303	6	694.378	7	809.404	8	923.447
ALBU	AEFAEVSK (SEQ ID NO:27)	440.725	5	533.294	6	680.362	7	809.404

Table 1 displays a list of monitored peptides of interest and their corresponding internal standards (where applicable) along with the Y-ion transitions used in MRM. The underlining designates an amino acid that can be labeled with a stable heavy isotope.

It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and the scope of the appended claims. In addition, any elements or limitations of any invention or embodiment thereof disclosed herein can be combined with any and/or all other elements or limitations (individually or in any combination) or any other invention or embodiment thereof disclosed herein, and all such combinations are contemplated with the scope of the invention without limitation thereto.

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CLAIMS

We claim:

1. An isolated peptide comprising an amino acid sequence shown in any of SEQ ID NO:1 to SEQ ID NO:30, or a fragment thereof.

2. The isolated peptide according to claim 1, wherein the peptide consists of an amino acid sequence shown in any of SEQ ID NO:1 to SEQ ID NO:30, or a fragment thereof.

3. The isolated peptide according to claim 1 or claim 2, wherein the peptide comprises a stable heavy isotope.

4. A method for diagnosing, monitoring the progress of, and/or providing a prognosis of a disorder or condition associated with antibody production in a person or animal, said method comprising treating a biological sample from the person or animal to isolate a target protein; fragmenting the target protein to create peptide fragments thereof; subjecting the peptide fragments of the target protein to quantitative mass spectrometry to identify and quantitate the amount of target protein in the biological sample; and correlating the identity and quantity of the target protein in the biological sample to a disorder or condition in the person or animal, whereby the disorder or condition can be diagnosed, monitored for progress, and/or a prognosis provided.

5. The method according to claim 4, wherein the target protein is an antibody.

6. The method according to claim 4, wherein the target protein is a serum albumin and/or a beta-2-microglobulin.

7. The method according to claim 5, wherein the antibody comprises human heavy chain of IgG, IgA, IgM, IgD, or IgE.

8. The method according to claim 5, wherein the antibody comprises human kappa light chain or human lambda light chain.

9. The method according to any of claims 4 to 8, wherein the disorder or condition is multiple myeloma or monoclonal gammopathy of undetermined significance (MGUS).

10. The method according to any of claims 4 to 8, wherein the target protein is identified and quantitated by spiking in a known amount of a specific labeled peptide corresponding to a fragment of a target protein during mass spectrometry, wherein the specific labeled peptide comprises a heavy isotope label or has an amino acid substitution to create a mass difference between the fragment of the target protein and the specific labeled peptide.

11. The method according to claim 10, wherein the heavy isotope label is ^{2}H, ^{13}C, or ^{15}N.

12. The method according to claim 10, wherein the specific labeled peptide comprises an amino acid sequence of any of SEQ ID NO:1 to SEQ ID NO:30.

13. The method according to claim 10, wherein the specific labeled peptide corresponds to a fragment of a human antibody heavy chain of IgG, IgA, IgM, IgD, or IgE.

14. The method according to claim 10, wherein the specific labeled peptide corresponds to a fragment of a human antibody heavy chain of IgG and comprises the amino acid sequence ALPAPIEK (SEQ ID NO:4).

15. The method according to claim 10, wherein the specific labeled peptide corresponds to a fragment of a human antibody kappa light chain or lambda light chain.

16. The method according to claim 10, wherein the specific labeled peptide corresponds to a fragment of a human antibody kappa light chain and comprises the amino acid sequence DSTYSLSSTLTLSK (SEQ ID NO:28).

17. The method according to claim 10, wherein the specific labeled peptide corresponds to a fragment of a human serum albumin or human beta-2-microglobulin.

18. The method according to any of claims 4 to 8, wherein the target protein is denatured prior to fragmentation.

19. The method according to claim 18, wherein the target protein is denatured by treatment with urea, disulfide reduction, and/or cysteine alkylation.

20. The method according to any of claims 4 to 8, wherein the target protein is fragmented by proteolytic enzyme digestion.

21. The method according to claim 20, wherein the proteolytic enzyme is trypsin.

22. The method according to any of claims 4 to 8, wherein said treating step comprises one or more of size exclusion chromatography, gel electrophoresis, and/or affinity chromatography to isolate the target protein.

23. The method according to any of claims 4 to 8, wherein the mass spectrometry comprises liquid chromatography coupled to multiple reaction monitoring (LC-MRM).

24. The method according to claim 23, wherein the mass spectrometry is conducted on a triple quadrupole mass spectrometer.

25. The method according to claim 10, wherein the amino acid substitution comprises substitution of alanine for glycine.

26. The method according to claim 4, wherein the method is used to monitor for progression of monoclonal gammopathy of undetermined significance in the person or animal to multiple myeloma.

27. The method according to claim 4, wherein the method is used to monitor the efficacy of a treatment regimen on a person or animal with multiple myeloma or MGUS.

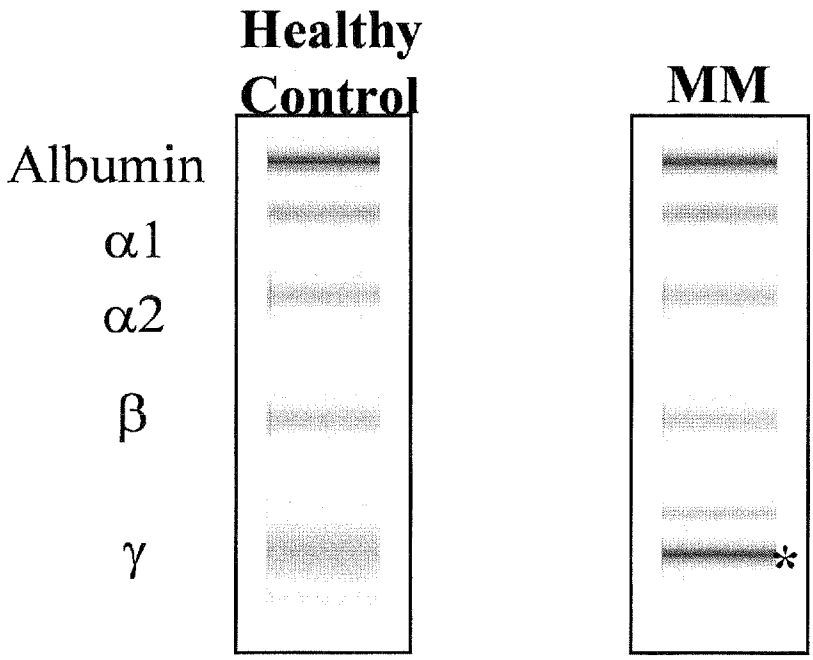

FIG. 1

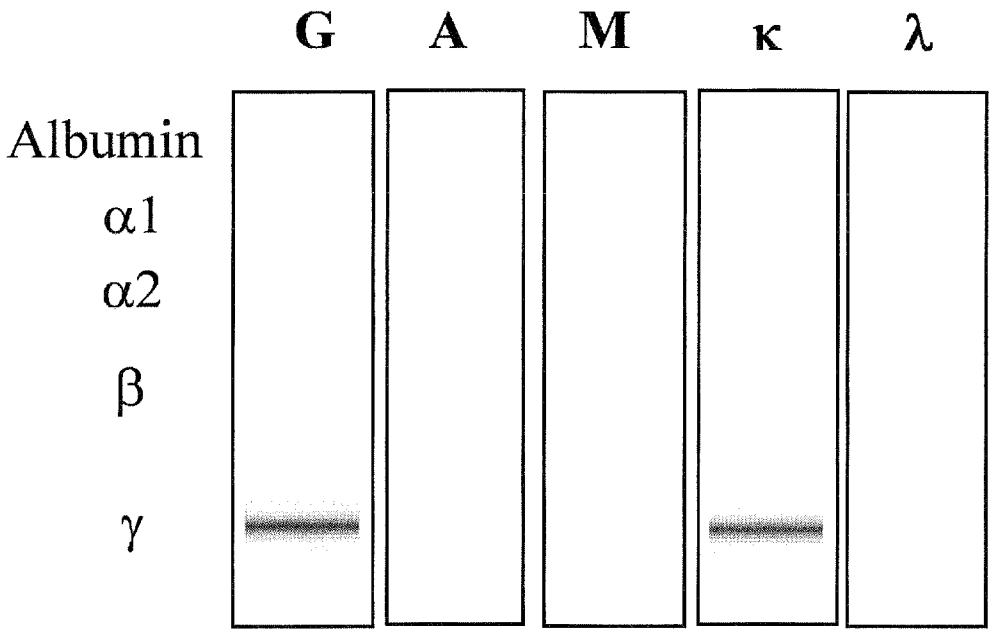

FIG. 2

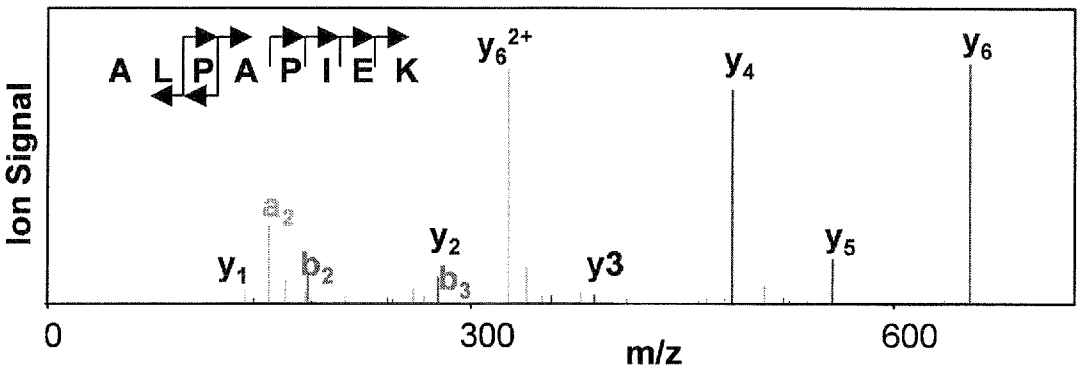
A L P A P I E K
y_6^{2+}
y_4
y_6
Ion Signal
a_2
y_2
y_1
b_2
b_3
y3
y_5
0
300
600
m/z

FIG. 3

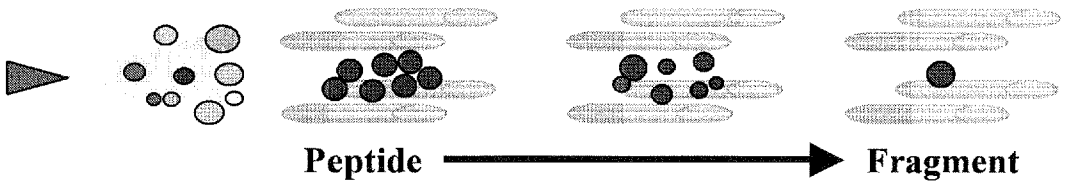
Peptide
Fragment

FIG. 4

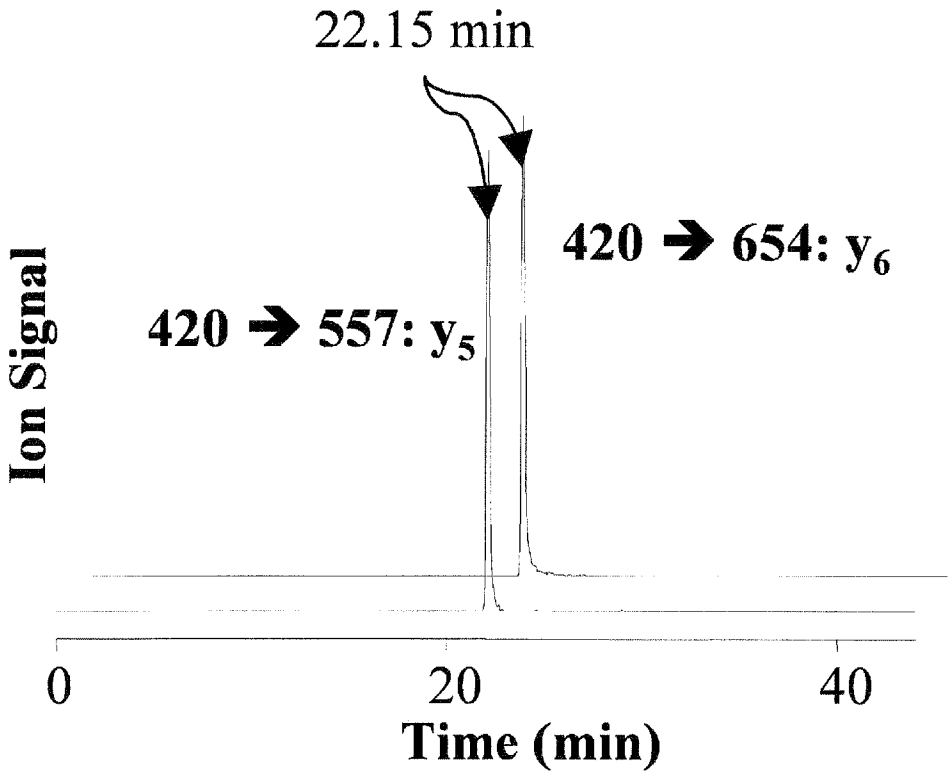
22.15 min
420 → 654: y6
420 → 557: y5
Ion Signal
0
20
40
Time (min)

FIG. 5

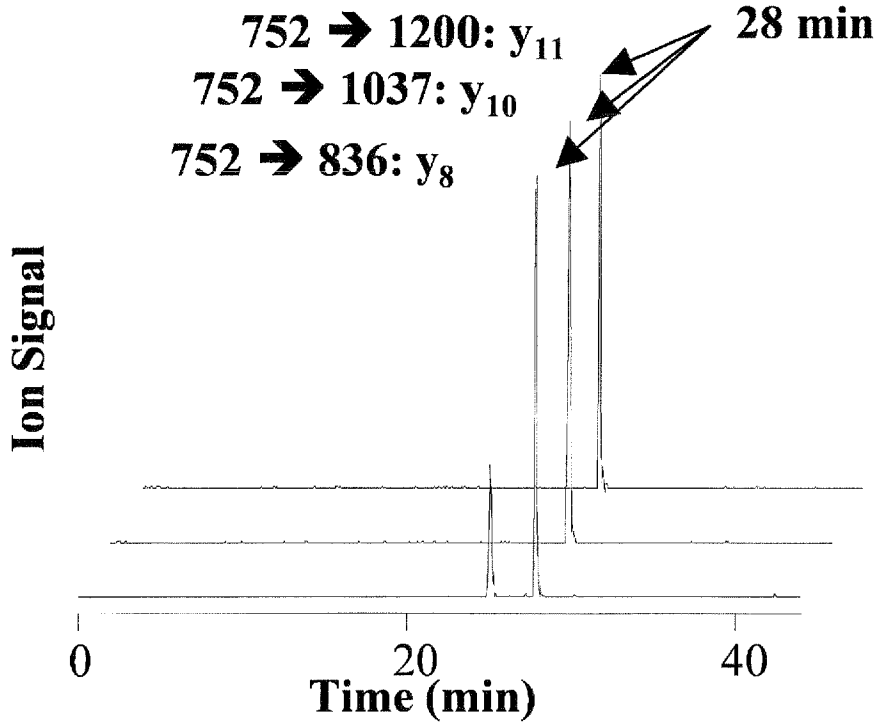
752 → 1200: y11
752 → 1037: y10
752 → 836: y8
28 min
Ion Signal
0
20
40
Time (min)

FIG. 6