

REVIEW

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# Applying mass spectrometry based proteomic technology to advance the understanding of multiple myeloma

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## Abstract

Multiple myeloma (MM) is the second most common hematological malignancy in adults. It is characterized by clonal proliferation of terminally differentiated B lymphocytes and over-production of monoclonal immunoglobulins. Recurrent genomic aberrations have been identified to contribute to the aggressiveness of this cancer. Despite a wealth of knowledge describing the molecular biology of MM as well as significant advances in therapeutics, this disease remains fatal. The identification of biomarkers, especially through the use of mass spectrometry, however, holds great promise to increasing our understanding of this disease. In particular, novel biomarkers will help in the diagnosis, prognosis and therapeutic stratification of MM. To date, results from mass spectrometry studies of MM have provided valuable information with regards to MM diagnosis and response to therapy. In addition, mass spectrometry was employed to study relevant signaling pathways activated in MM. This review will focus on how mass spectrometry has been applied to increase our understanding of MM.

## Multiple Myeloma

Multiple myeloma (MM), the second most common blood cancer in adults, is a neoplasm of terminally differentiated B-cells characterized by clonal expansion of malignant plasma cells in the bone marrow. The most common symptoms associated with MM include lytic bone lesions, renal failure, calcium dysregulation, anemia and susceptibility to infections. The median age at diagnosis of MM is 62 years for men and 61 years for women, with less than 2% of those diagnosed at an age less than 40 years. The incidence of MM in the USA is more common among men (7.1 per 100,000) than women (4.6 per 100,000). In addition, MM is two times more frequent in the black population than in the white population [1]. Despite advances in clinical care, MM remains an almost universally fatal disease with a median survival of 3-4 years following conventional treatment and 5-7 years with high dose therapy followed by autologous stem cell transplantation [1].

The development of MM constitutes a series of progressive genetic events. A seminal event is the inappro-

priate translocation of oncogenes from partner chromosomes into the immunoglobulin heavy chain switch region (IgH) locus on chromosome 14q32. In the past several years, five recurring translocation partners have been defined and mapped to the earliest stages of the developing MM clone [2-4]. The translocations involve partner oncogenes cyclin D1 (11q13), cyclin D3 (6p21), fibroblast growth factor receptor 3 (FGFR3, 4p16), c-maf (6q23) and mafB (20q11). These recurrent translocations are identified with high frequency in primary patient samples and, between them, are found in approximately 50% of MM [5,6]. The remaining 50% of MM lack translocations and are characterized by chromosomal duplication (hyperdiploidy) with associated up-regulation of cyclins D1, D2 and D3 although the molecular pathogenesis is unclear [5]. An equally early event in the genesis of MM appears to be loss of part of chromosome 13 at 13q14.3, although the specific tumor suppressor gene(s) in this region have yet to be identified [7,8]. These events all occur early in disease onset and are often present during an asymptomatic and stable form of the disease called monoclonal gammopathy of unknown significance or MGUS. Active disease must therefore

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require subsequent genetic events such as mutation/deletion of *p53* or *Ras* mutations [9].

We have evaluated the prognostic significance of recurrent genomic aberrations including del(13q), t(11:14)/CyclinD1, t(4:14)/FGFR3, t(14:16)/c-Maf, del(17p)(p53), 1q21(CKS1B) amplification, 1p21/CDC14a deletion, and PTEN deletions, as well as CD56 expression in large cohorts of MM patients uniformly treated at our center [10-26]. In addition we have evaluated the impact of chromosomal aberrations on MM patients receiving novel therapies such as the proteasome inhibitor, bortezomib or the immunomodulatory drug lenalidomide. While high-risk genetic factors (t(4;14), del(17p)(p53) deletion, or 13q deletion) did not affect the response or survival of refractory/relapsed MM patients treated with bortezomib [27], del(17p)(p53) deletion had a negative influence on progression free and overall survival of MM patients receiving lenalidomide and dexamethasone [28].

In addition to the cytogenetic studies which have given us significant insight into MM diagnosis and prognosis, gene-expression profiling of MM has also significantly contributed to our understanding of this disease. Due to the highly heterogeneous nature and complexity of MM, gene expression profiling is well suited to study this cancer as it allows for the identification and differentiation of hundreds of genes between various disease states. Several groups have used gene expression arrays, for example, to evaluate drug response in MM patients. Mulligan *et al.* identified a pretreatment expression pattern and predictive markers that could differentiate between bortezomib and dexamethasone response [29]. Other groups have used expression arrays to identify genes involved in doxorubicin and dexamethasone resistance in MM [30,31]. Gene expression arrays have also been used to determine the genetic differences between plasma cells and MGUS and MM cells [32]. These studies have made significant contributions to our understanding of the molecular development as well as mechanisms of drug resistance of MM.

A complementary approach to the study of gene expression profiling is proteomic profiling. The advantages of this approach, which has been increasing in popularity over the past several years, is the ability to determine protein expression levels, post-translational modifications and protein-protein interactions, all of which may have a direct consequence to cell function; such information cannot be obtained through gene expression profiling. Furthermore, several studies found that there is not a significant overlap between gene and protein expression profiles [33-35]. Therefore direct approaches to studying the protein profile of MM are necessary.

Traditional methods to study proteins, such as western blot analysis or immunohistochemistry, have their short-

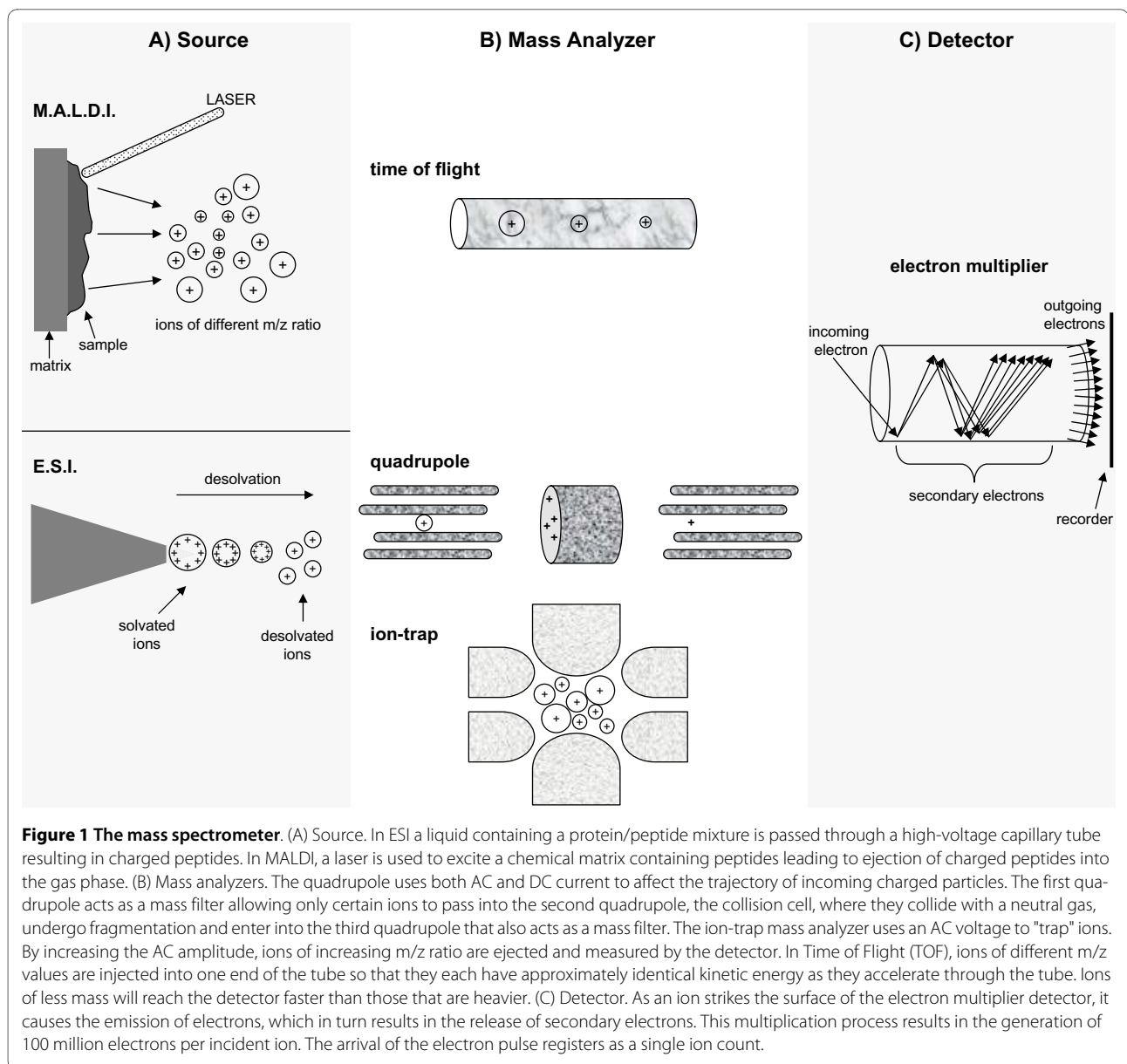
comings as high throughput solutions for protein profiling including the need for large amounts of tissue as well as the availability of well-characterized antibodies. Mass spectrometry techniques, on the other hand, offer a robust, high throughput method that overcomes many of these limitations [36]. Most important to cancer research, mass spectrometry can be employed to identify known and novel differentially expressed proteins between different tumor samples. This would allow for the identification of biomarkers that can be used in diagnosis, prognosis, and treatment assessment.

### Mass Spectrometry

A mass spectrometer determines the mass of a molecule by measuring its mass-to-charge ratio ( $m/z$ ). Each mass spectrometer consist of three components; i) the source, which generates ions from a sample either by matrix-assisted laser desorption ionization (MALDI) or electrospray ionization (ESI), ii) a mass analyzer, which resolves peptide ions according to their  $m/z$  ratio, and iii) a detector which determines ion abundance for each corresponding ion resolved by the mass analyzer according to their  $m/z$  value and generates a mass spectrum (Figure 1). Depending on the type of mass spectrometer used, peptide mass (MS) and/or peptide sequence (MS/MS) data can be obtained from the mass spectrum. This information is then used to search public databases for protein identification such as those maintained by the National Center for Biotechnology Information (NCBI) and the European Bioinformatics Institute (EBI).

### Mass Spectral Analysis

Biological samples including cell lines maintained in culture, biopsy specimens, and serum are very complex in nature as they contain not only an abundance of proteins, but also a large amount of lipids and nucleic acids [36]. Although the mass spectrometer is capable of resolving complex mixtures, protein identification can be greatly simplified if this complexity is reduced. Biological samples are typically lysed with detergents that solubilize proteins, separating them from lipids and nucleic acids. Subsequent procedures can then be employed to further simplify the protein mixture. One dimensional gel electrophoresis can be used to separate proteins according to their molecular weight. Alternatively, two-dimensional gel electrophoresis can be used to achieve greater protein separation by resolving proteins according to their isoelectric value (pI) and molecular weight. Following electrophoresis proteins are stained with dyes such as Coomassie blue, excised, digested "in-gel" into peptides and then analyzed by the mass spectrometer. Although gel electrophoresis is capable of reducing the complexity of a mixture it has its limitations [37]. Most notably, gel electrophoresis has a limited dynamic range of detection



as protein bands are excised only if they can be visualized following staining. The level of detection by MS, however, is below the level of detection of staining and hence many relevant proteins may be missed. A further disadvantage of 2D separation is that it is often difficult to reproduce and some proteins cannot be resolved according to their pI value [37].

An alternative method to reduce the complexity of a protein mixture is the use of liquid chromatography (LC) [36]. Typically, proteins are first digested into peptides and then resolved by LC. The separation of peptides is usually achieved according to charge and molecular weight. Often, the peptides that are resolved by LC are directly analyzed by the mass spectrometer. The main

advantage of LC is that this method avoids the low dynamic range limitation encountered by gel staining.

#### Quantitative Proteomics

In order to improve the diagnosis, prognosis and treatment stratification of those afflicted by cancer the identification of biomarkers indicative of these parameters are necessary. Quantitative protein analysis by mass spectrometry in which a tumor cell may be compared to a normal cell or a drug resistant tumor cell is compared to a drug sensitive tumor cell, provides an effective way to discover these biomarkers. There are two main methodologies to quantify proteins within a sample, stable isotope

labeling and label free methods. Both these techniques have been widely used for biomarker discovery [38-45].

One of the most commonly used stable isotopes is the isobaric tags for relative and absolute quantification (iTRAQ). The iTRAQ method allows the simultaneous comparison of up to 8 different samples. The iTRAQ reagent labels the N-terminus of tryptic peptides as well as the amino group side chain of lysine residues [36]. Proteins from different samples are first digested to yield peptides. Each peptide sample is then labeled with one of the iTRAQ reagents. Each reagent consists of i) a reporter group with a molecular weight of 113, 114, 115, 116, 117, 118, 119, or 121 Da; ii) a linker group that also varies in molecular weight to 'balance' the difference of the reporter group; and iii) a peptide reactive group that reacts with the N-terminus of peptides and lysine side chains. Labeled samples are then mixed together and analyzed by the mass spectrometer. Collision induced dissociation of iTRAQ-labeled peptides generates sequence information as well as relative quantification data between the samples.

Recent trends in discovery proteomics are now inclined towards using label-free relative quantification based on the linear relationship between sampling statistics observed using LC-MS/MS and relative protein abundance [46]. Sampling statistics evaluated as potential measures of relative protein abundance include 1) the mean peak area intensity of all peptides identified for an individual protein in a complex sample [47]; 2) the peptide count, or total number of peptides identified from a given protein in a LC-MS/MS experiment [46,48]; and 3) spectral counts, or the total number of tandem mass spectra generated on a given peptide in an LC-MS/MS experiment [47,49-52].

The use of label free techniques has several advantages [53]. First it is more cost effective and less time consuming compared to labeling methods since the labeling reagents do not have to be purchased and experiments to incorporate the stable isotopes into samples are bypassed. Label free methods therefore require less sample modification and avoid increasing sample complexity associated with mixtures of tagged peptides. A second advantage of label free methods is that theoretically there is no limit to the number of samples that can be compared whereas with isotope labeling such as iTRAQ a maximum of 8 samples can be compared at a time. Another advantage of the label free method is that it may provide a higher dynamic range in terms of quantification, although this comes at the expense of unclear linearity and relatively low accuracy [53]. Although there are several advantages to label free methods, it is essential that these methods are robust and reliable in order to control for any experimental variables and that sample processing does not affect the outcome of analyses [54].

### **Mass Spectrometry to study Multiple Myeloma Serum markers for MM diagnosis and prognosis**

One of the greatest challenges we face in the clinical setting is the development of tests that would allow for the early detection of cancer. It is well accepted that the earlier tumor cells are detected, the better the prognosis. Certain cancers, including breast, colon and prostate can be detected at an early stage through routine physical exams. For example, screening for prostate specific antigen (PSA) may be useful for the early detection of prostate cancer. Unfortunately, there are no reliable biomarkers that can be used for the early detection of MM and patients are often diagnosed after presenting with clinical manifestations.

A recent study has found that virtually all cases of MM arise from MGUS [55]. On the other hand, the majority of MGUS cases will not develop into MM. Although the status of M-protein may offer insight into the development of MM, it is not absolute, and thus there is a need to identify biomarkers that can predict progression to MM in patients diagnosed with MGUS.

Several groups have been using mass spectrometry based techniques in order to identify potential biomarkers that are early predictors of cancer development [56-61]. Elucidation of these early biomarkers for various cancers, including MM, would be most easily identified from plasma or serum. The advantage of screening blood is that it is easily obtained and contains a large amount of proteins which increase the likelihood of biomarker discovery [62]. One strategy for the early detection of cancer relies on the immune response, which is believed to make auto-antibodies against cancer cells and because the immune response involves an amplification process, these antibodies may be present in sufficient quantities for detection [63,64]. Regardless of the type of biomarker, it will be essential that they are both tumor specific and tissue specific so that the identification and location of the tumor can be determined. Because an overlap most likely exists in biomarker expression between different tumor types, a panel of biomarkers would have to be identified rather than relying on a single protein.

In addition to the identification of early biomarkers that can predict MM, it is also clinically relevant to identify markers that are used for the diagnosis and prognosis of MM. Currently these include calcium, creatinine, hemoglobin, albumin, beta2-microglobulin and monoclonal antibodies. In addition, disease relapse can be monitored by assessing the levels of monoclonal antibodies including heavy chains as well as  $\kappa$  and  $\lambda$  light chains. Koomen's group is currently developing mass spectral techniques that will allow for the quantitative detection of immunoglobulin associated peptides [65]. As MS analysis within the clinical setting becomes more accepted and affordable, successful development of these tests could offer

advantages over current clinical tests that are more qualitative in nature, slower, and of lower throughput [65].

Several groups are using mass spectrometry to identify additional biomarkers that may allow for a more specific and sensitive method to diagnose MM. Wang *et al.* employed MALDI-TOF-MS and identified a panel of three biomarkers that correctly identified 26 out of 30 (87%) MM patients and 34 out of 34 (100%) of all normal donors [66]. However, these markers were unable to differentiate between MM and other plasma cell dyscrasias including MGUS, Waldenstrom's macroglobulinemia, solitary plasmacytoma, as well as other tumors with osseous metastasis. Therefore, as the authors mention, it will be necessary to increase their samples size in order to identify additional markers that may unequivocally identify MM patients. Nevertheless this work demonstrates the usefulness of MALDI-TOF MS for the identification of novel biomarkers.

Another group also used mass spectrometry to identify serum biomarkers that might discriminate between patients with skeletal involvement [67]. This group screened serum samples from 48 patients either with evidence of skeletal involvement (24 patients) or without evidence of skeletal involvement (24 patients). Using a partial least squares discriminant analysis (PLS-DA), and a non-linear, random forest (RF) classification algorithm, they were able to predict skeletal involvement with an accuracy between 96-100% using the PLS-DA model and obtained a specificity and sensitivity of 87.5% each with the RF model based on four peaks. Although this study demonstrates the usefulness of proteomic profiling in the diagnosis and treatment of MM progression, further validation studies in additional patient samples are needed.

#### **Proteins that confer drug resistance in Multiple Myeloma**

As mentioned earlier, MM remains a largely incurable disease despite a plethora of chemotherapeutic drugs. This is mainly due to the acquisition of drug resistance by tumor cells. The molecular mechanisms responsible for drug resistance are not well understood. Moreover, it is likely these resistance pathways are unique for each drug. Two scenarios can be envisioned in the acquisition of drug resistance. First, tumor cells may express proteins prior to drug treatment that will render them resistant, and second, tumor cells may acquire resistance following drug administration. An understanding of the molecular signatures that confer drug resistance will be of significant benefit in treatment stratification and will enable the design of novel therapeutic strategies.

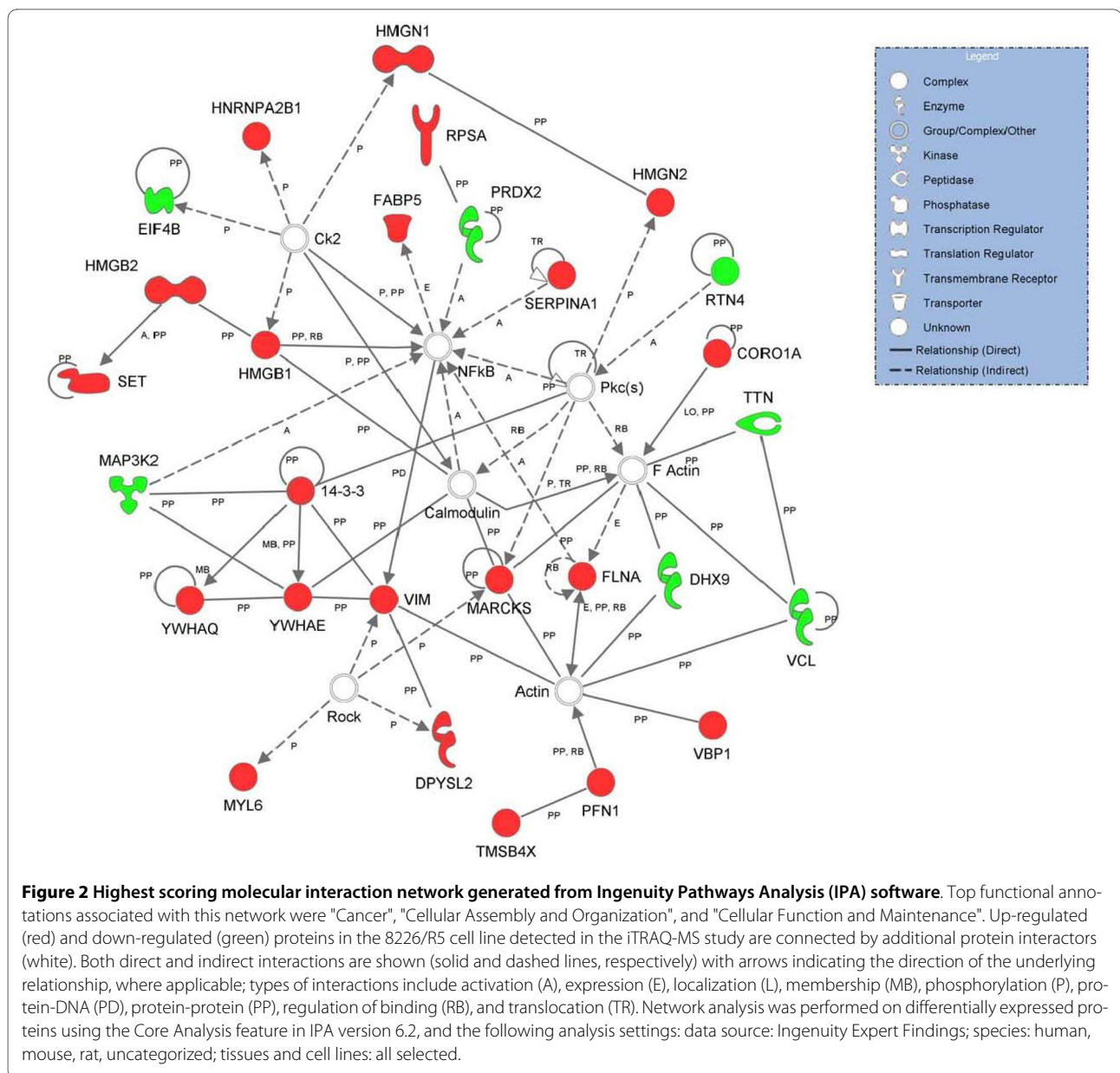
#### **Bortezomib**

Bortezomib, a proteasome inhibitor, has been approved for the treatment of MM patients who have received at least two prior therapies and progressed during the last treatment [68-70]. This drug has been shown to induce

apoptosis in various cancer cells, including MM and other lymphomas. It also affects nuclear factor- $\kappa$ B (NF- $\kappa$ B), the bone marrow microenvironment and various cytokine interactions, including, IL-6 [68-70]. Despite significant benefits with regards to time to progression, overall survival and a trend to a lower incidence of infections >grade 3, bortezomib induced an overall response rate of only 35% in refractory and relapsed MM patients (pivotal phase-II (SUMMIT) trial) [68]. In order to determine if recurrent molecular cytogenetic changes identified in MM contribute to the response of bortezomib therapy, we used fluorescence *in situ* hybridization combined with cytoplasmic immunoglobulin light chain stainings (cIg-FISH) and found that the response to bortezomib was independent of recurrent genomic aberrations in MM patients [27]. These observations were confirmed by two independent research groups [71,72].

In light of the above observations, our group is taking a proteomic based approach in order to identify biomarkers that may predict and contribute to bortezomib resistance. To undertake this study we have used iTRAQ analysis to identify differentially expressed proteins between the 8226/R5 bortezomib resistant multiple myeloma cell line and the 8226/S bortezomib sensitive multiple myeloma cell line. Using this approach we identified 30 proteins that were either significantly up or down regulated in the 8226/R5 cell line compared to the 8226/S cell line [73]. Biological systems analysis of these putative markers using Ingenuity Pathway Analysis software revealed that they were associated with cancer-relevant networks (Figure 2). Of particular interest is the MARCKS protein which we found to be over-expressed in the 8226/R5 cell line. MARCKS is a PKC substrate protein that has been found to be over-expressed in several cancers, including glioblastoma multiforme where it was shown to play a role in glioma cell invasion [74]. We have shown that MARCKS is over-expressed in 9 (50%) of 18 multiple myeloma cell lines. In addition, a preliminary screen of pre-bortezomib treatment MM patient samples by immunohistochemistry showed over-expression of MARCKS is associated with bortezomib resistance. We are currently evaluating whether MARCKS plays a role in drug resistance and/or contributes to other tumorigenic properties of MM.

Protein expression data obtained from our iTRAQ analysis comparing 8226/R5 versus 8226/S cell lines was also compared with gene expression array data from the literature that contrasted MM bortezomib resistant to bortezomib sensitive cells. As expected, there was minimal overlap between these datasets. However these lists of genes and proteins showed strong complementarity in terms of the functional and biological systems with which they are associated, suggesting the systems affected by them or those which they affect may be closely inter-



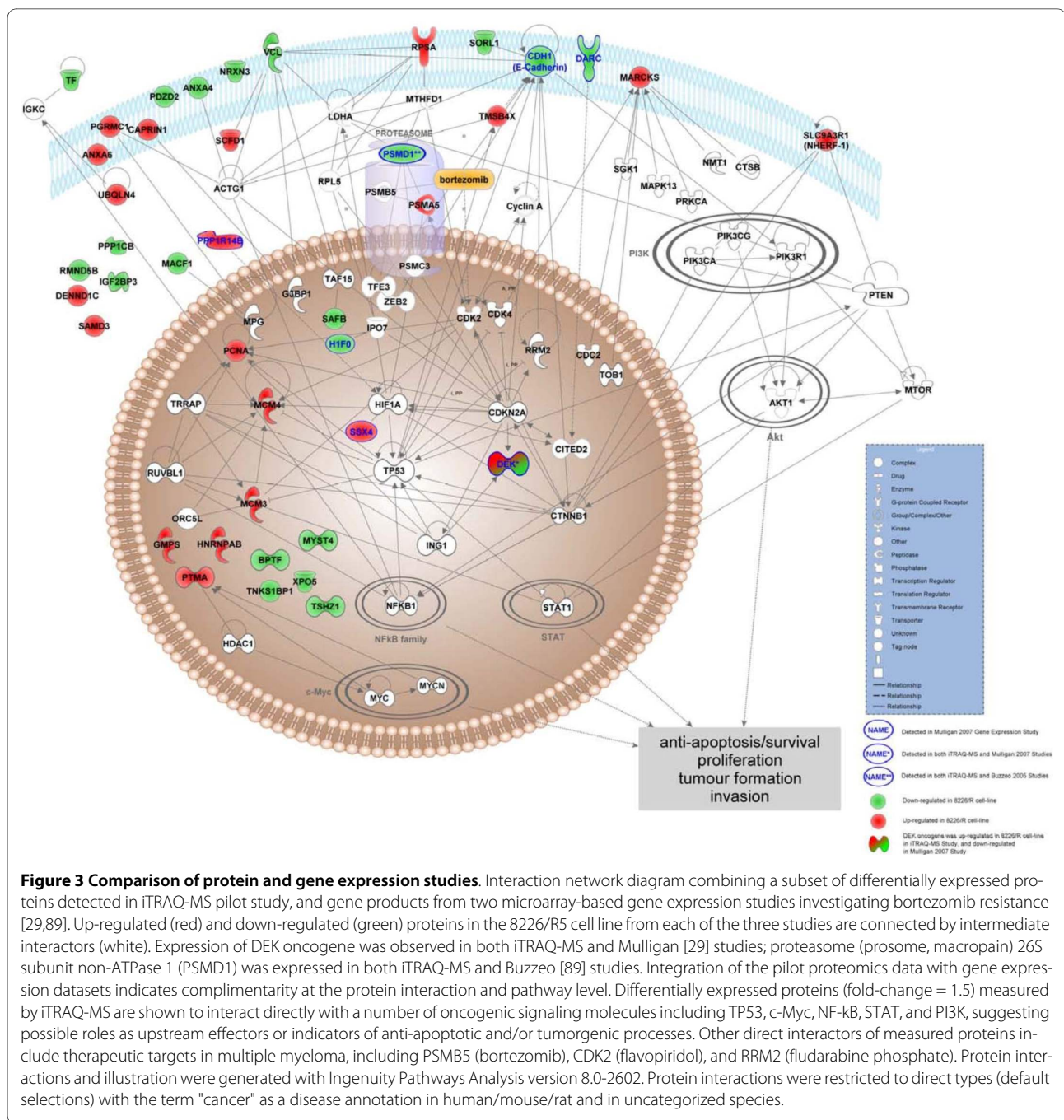
related (as illustrated by the network diagram in Figure 3). These data demonstrate the usefulness of proteomic profiling over conventional gene array approaches.

Recently, Hsieh *et al.* used mass spectrometry to identify early biomarkers of bortezomib resistance from the serum of MM patients [75]. They found both apolipoprotein C-I and apolipoprotein C-I' to be significantly more abundant in the non-responsive patients compared to the responsive patients 24-hours post drug administration. The results suggest that apolipoprotein C-I and apolipoprotein C-I' may be used as early biomarkers for bortezomib drug resistance. However, it will be necessary to carry out a time course experiment in a larger sample size in order to validate these findings. Additional experi-

ments are required to determine the functional relationship between these proteins and bortezomib response.

#### Dexamethasone

Dexamethasone (dex) is a synthetic steroid hormone that is also used in the treatment of MM. Clinical trials have shown response rates of up to 70% in MM patients [76]. Additional clinical trials observed a synergistic response when dex was used in combination with other drugs such as bortezomib and thalidomide [68,77]. The mechanisms of action of these drugs, however, are not well understood. In an attempt to improve clinical response Ressel-Unwin *et al.* used mass spectrometry to identify proteins that may play a role in dex induced apoptosis [76]. They found a panel of proteins that were differentially



expressed following dex treatment in the sensitive MM1S multiple myeloma cell line compared to the resistant MM1R cell line. Most notably, they identified FK binding protein 5 (FKBP5), which is involved in protein folding and trafficking to be over-expressed in the MM1S but not the MM1R cell line following dex treatment. These data are important as they shed light onto the signaling pathways that may induce dex-mediated apoptosis and thus may help direct rational drug design. However, before this is realized it will be necessary to gain further insight

into the signaling pathways in which these proteins are acting.

#### **Arsenic trioxide**

Arsenic trioxide (ATO) has been shown to induce growth inhibition and apoptosis in MM cells and has shown clinical activity in both Phase I and II clinical trials in patients with relapsed or refractory MM [78]. In order to determine the mechanisms of ATO activity, *Ge et al.* used 2D gel electrophoresis coupled with MALDI TOF/TOF analysis to evaluate proteins altered following ATO activity in

the U266 multiple myeloma cell line [78]. The most significant changes were observed in the up-regulation of HSP proteins and down regulation of 14-3-3 $\zeta$  protein and the members of the ubiquitin-proteasome system following ATO treatment. This group further demonstrated that the use of 14-3-3 $\zeta$  siRNA potentiated the effects of ATO induced apoptosis whereas over-expression 14-3-3 $\zeta$  reduced ATO-sensitivity in U266 cells. Furthermore, they showed that inhibition of HSP90, which is over-expressed following ATO treatment, sensitized cells to ATO treatment as well as potentiated the effect of 14-3-3 $\zeta$  knock-down. These results demonstrate the usefulness of identifying additional therapeutic targets that may be exploited to over-come drug resistance.

#### Post translation modifications of the MM proteome

Post-translation modifications (PTMs) play an important role in the maturation and regulation of proteins. One of the most common PTMs is phosphorylation. Phosphorylation of proteins is carried out by specific protein kinases and occurs at three specific residues: serine, threonine, and tyrosine. Protein dephosphorylation, on the other hand is carried out by phosphatases. Protein activity is controlled by cycles of phosphorylation and dephosphorylation. Because protein phosphorylation is crucial to protein activity and thus regulation of cellular behavior, knowledge of protein phosphorylation status within the cell would give significant insight into signaling mechanisms. Furthermore, this may help in the design of kinase or phosphatase inhibitors in an attempt to control cellular events.

In MM, several proteins are regulated through phosphorylation events, including fibroblast growth factor receptor-3 (FGFR3). Activation of FGFR3, through tyrosine phosphorylation, induces cell growth, survival and migration through activation of various signaling pathways including MAPK and PI3K [79,80]. Aberrant activation of FGFR3 has been observed in 15-20% of MM due to a t(4;14)(p16.3;q32) translocation and has been shown to contribute to the tumorigenesis of MM, including chemoresistance [81-83]. For these reasons several drugs have been designed to target this receptor [84].

The signaling networks activated downstream of FGFR3 are not fully known. Recently, St-Germain *et al.* studied the phosphotyrosine proteomic profile associated with FGFR3 expression, ligand activation, and drug inhibition in the KMS11 MM cell line by mass spectrometry [85]. They identified and quantified several phosphotyrosine sites as a result of FGFR3 activation and drug inhibition. Their results have substantially increased our understanding of FGFR3 function and provided a framework for studying appropriate signaling networks activated by this receptor in MM. Importantly their mass

spectrometry approach demonstrated the potential for pharmacodynamic monitoring.

#### The future of mass spectrometry in biomarker discovery

The use of mass spectrometry for biomarker discovery holds great promise. In order for this to be fully realized in the clinical setting however, various limitations must be addressed [62]. First, there exists a limited dynamic range for even the most sensitive mass spectrometers. Highly abundant proteins, such as albumin can mask less abundant proteins which may be important biomarkers. This is especially true during the early stages of tumor development when tumor biomarkers may be low and so care must be taken to simplify samples. Through sample purification, however, low-abundant proteins maybe lost through interactions with high abundant proteins such as albumin. Thus, all purifications steps should be analyzed.

It will also be important that biomarkers are validated in large, independent studies before entering the clinic. To this end, it will be necessary to standardize these experiments in terms of sample collection, storage and processing as well as bio-informatics and statistical analysis between various centers. Furthermore, careful consideration will need to be given to the normal group to which the cancer group is compared. Differences in age, sex, and ethnicity, as well as menopausal and nutritional status may all be confounding variables in biomarker discovery [86,87].

Although both cell lines and clinical specimens are valuable samples for biomarker discovery, they each have their limitations. Most notably, cell lines do not represent an *in vivo* model. Therefore, the influence of the microenvironment on the tumor biomarker signature cannot be evaluated resulting in potential misrepresentation of the true biomarkers. In terms of clinical specimens, as discussed above, the many confounding variable associated when comparing tumor to normal tissue also represents a hurdle impeding biomarker discovery. An alternative to these approaches is the use of genetically engineered mouse models. These mouse models offer the opportunity to standardize experiments through homogenized breeding and environmental control and by defining stages of tumor development [64]. Importantly it has been observed that the tumor antigen repertoire of tumor-bearing transgenic mice can predict human tumor antigens [88].

#### Conclusion

The use of mass spectral analysis will prove to be a valuable tool for the diagnosis, prognosis and response to drug treatment in cancer. Studies that have been carried out in MM have increased our understanding of this can-



cer; they have identified new serum biomarkers that may distinguish between MM and normal patients as well as serum markers that may identify patients with skeletal involvement. In addition, mass spectrometry has been used to identify biomarkers that indicate resistance to several chemotherapeutic drugs used to treat MM. Equally important mass spectrometry was used to investigate the phosphotyrosine signaling pathways downstream of FGFR3. These types of studies, that investigate signaling networks, are essential as they will help guide future investigations into the pathogenesis of MM.

Perhaps one of the greatest promises of mass spectrometry will be its use in helping direct therapy. Since current "one size fits all" therapy is complicated by serious toxicities and may be unnecessary in some good prognosis patients, it is critical to introduce risk-adapted therapy. The development of risk-adapted therapy requires better prognostic markers as the current prognostic models remain inadequate to predict disease outcome for individual patients. Through protein expression profiling by mass spectrometry we will be able to identify biomarkers that can be used to improve the diagnosis and prognosis of MM as well as increase our understanding of the mechanisms of drug resistance, which will help direct therapeutic strategies.

#### Competing interests

The authors declare that they have no competing interests.

#### Authors' contributions

JM and HC drafted manuscript; JM, MD, JC, SA, KV, LQ and HC participated in the design and analysis of the MM proteomic data described in the manuscript; all contributed to the critical revision of the manuscript. HC supervised the study, provided funding and approved the final manuscript. All authors read and approved the final manuscript.

#### Acknowledgements

The study is supported in part by grants from Canadian Institute of Health Research (CIHR), Leukemia and Lymphoma Society of Canada (LLSC) and Ontario Association of Medical Laboratories.

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Received: 25 January 2010 Accepted: 7 April 2010

Published: 7 April 2010

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doi: 10.1186/1756-8722-3-13

**Cite this article as:** Micallef et al., Applying mass spectrometry based proteomic technology to advance the understanding of multiple myeloma *Journal of Hematology & Oncology* 2010, **3**:13

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