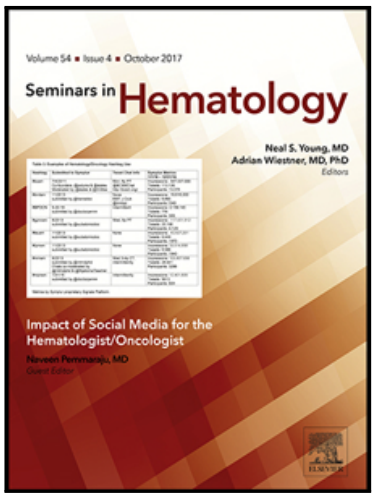

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Mass spectrometry and proteomics in hematology

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Abstract

Mass spectrometry-based techniques now enable the unbiased identification of proteins in complex mixtures including proteins isolated from cells and tissues. These powerful tools permit near-complete annotation of proteins expressed in cells, tissues or organs. Further, these techniques permit the interrogation of the numerous post translational modifications that govern cell-specific responses to signaling cues and underlie the functional heterogeneity of cellular composition and contribute to biological complexity. Parallel developments in technologies such as mass cytometry and multicolor ion-beam imaging which permit multi-parameter detection of numerous proteins at the single cell and in-situ level respectively are poised to radically impact our understanding of the functional and translational importance of proteins in hematologic conditions. Importantly, the field of proteomics is poised to realize the immensely powerful opportunities in integration with genomic information that is being discovered at an unprecedented pace for many hematologic conditions.

Introduction

Over the last 20 years, the relationship between cellular genotype and phenotype has been interrogated based on the assumption that there is a direct link between gene and protein function. Technologies to interrogate the genome at global scale such as whole genome sequencing (WGS), whole exome sequencing (WES) or RNA-sequencing (RNA-Seq) are in wide use. However, with the exception of few single gene defects, most disease phenotypes are more complex and remain largely unknown. Although proteins are the effectors of the cell's genetic code many studies have demonstrated a poor correlation between mRNA and protein

expression level [1, 2]. Thus, while recent advances in WGS, WES and RNA-Seq have made unparalleled discoveries in the field of hematology and hematologic malignancies, there remains a critical need for methods to analyze proteins and their functions in a high throughput and large-scale manner. Mass spectrometry (MS) and in particular tandem mass spectrometry (MS/MS) allows simultaneous identification and quantification of thousands of proteins in complex biological samples. Accordingly MS-based proteomic approaches permit the identification of disease biomarkers, protein-protein interactions involved in signaling and post-translational modifications such as phosphorylation and N-glycosylation which are critical events in a variety of hematologic disorders. In this review, we will discuss basic principles of MS-based proteomics and their applications in a variety of hematologic disease. Figure 1 summarizes the specimens, components and selected approaches encompassed by proteomics.

Mass spectrometry-based proteomics

Principles and instrumentation

Mass spectrometry measurements are carried out in the gas phase on ionized analytes. By definition, a mass spectrometer consists of an ion source, a mass analyzer and a detector. Since the first description of a MS experiment in the early 1900s by J.J. Thomson [3], the MS-based interrogation of the proteome has significantly accelerated within the last two decades with the description in the late 1980s of two different soft ionization methods named MALDI (Matrix-Assisted Laser Desorption/Ionization) [4, 5] and ESI (ElectroSpray Ionization) [6] that allow the ionization of biomolecules which are subsequently amenable to interrogation by mass analyzer. After ionization, the sample reaches the mass analyzer which separates ions by their mass-to–

charge ratio (*m/z*). Ion motion in a mass analyzer can be manipulated by electric or magnetic fields to direct the ions to the detector that registers the number of ions at each *m/z* value. In high-resolution tandem mass spectrometry (MS/MS), two stages of mass analysis are used in a single experiment. The MS1 scan refers to the *m/z* values of the precursor ions, whereas the MS2 scan refers to the *m/z* values recorded for their fragmented ionic products. Different types of mass analyzers are currently used: time-of-flight (TOF), ion trap and quadrupoles. All differ considerably in sensitivity, resolution, mass accuracy and the capacity to generate and analyze fragment peptide ions which results in mass spectra with high content of information (MS/MS spectra). The choice of the combination of the ion source with the mass analyzer and the detector depends mainly on the application.

MS-based proteomic analyses can be divided into top-down or bottom-up. In top-down proteomics, intact proteins or polypeptides are directly analyzed by mass spectrometry. On the other hand, bottom-up proteomics entails digestion of complex protein mixtures into peptides using a proteolytic enzyme (typically trypsin). Resulting peptides are separated by liquid chromatography (LC) and analyzed by tandem mass spectrometry (MS/MS). Proteins are identified by matching experimental spectra with those from theoretical spectra of translated genomic databases generated by "*in silico*" cleavage using specific enzymes.

Quantification methods

Global quantification of proteins can be achieved by stable isotope labeling of proteins/peptides and the use of stable isotopically-labeled synthetic peptides as standards or label-free quantitation. Isotope labels can be introduced metabolically (i.e. *in vivo*), chemically or enzymatically (i.e. *in vitro*). Since metabolic labeling (stable isotope labeling with amino acids in

cell culture, SILAC) requires maintaining cells in culture for several passages in presence of amino acids that incorporate stable isotope, its direct application to clinically relevant scenarios is not feasible. *In vitro* methods for labeling include isotope-coded affinity tagging (ICAT), isobaric tags for relative and absolute quantitation (iTRAQ) and tandem mass tag (TMT). These methods may be adaptable to utilization in clinical scenarios since cell viability is not required for chemical labeling. The AQUA (absolute quantification) quantitative approach uses isotope-labeled synthetic peptides as standards spiked at a known amount into a sample preparation [7]. Label-free quantitation methods are increasingly being employed as an alternative to label-based approaches. These techniques do not use isotopic labeling, but instead directly compare signal intensities across different mass spectrometry runs using the signal intensity of peptide precursors.

Identification of post-translational modification of proteins

The proteome is a rather complex bioanalyte with diverse parameters for measurement beyond the levels of protein expression. Indeed, the functional diversity of proteins is largely increased by post-translational modifications (PTMs) which modulate protein stabilities, protein subcellular localizations, enzyme activities, protein interactions, as well as mediate signal transduction in response to extracellular and intracellular stimuli. As many as 300 post-translational modifications of proteins are known to occur physiologically [8]. The most frequently interrogated PTMs are listed in **Table 1** and include phosphorylation, N- and O-glycosylations, ubiquitination, acetylation, methylation and palmitoylation. Modern triple mass accuracy MS instruments are capable of both high resolution and high mass measurement accuracies for both MS1 and MS2 scans simultaneously. Having high mass accuracy has

increased the reliability and efficiency of PTM identifications and precise localizations of the modified amino acid within peptide sequences.

Targeted proteomics by selected or multiple reaction monitoring

Selected reaction monitoring (SRM) is a targeted approach to detect and quantify peptides of special interest. In contrast to unbiased and global mass spectrometry-based proteomic experiments aiming to identify and quantify all proteins present in a biological sample, SRM involves the interrogation of a specific *m/z* window for a precursor ion as well as selected fragment ions following MS/MS analysis. It utilizes two rounds of mass selection by quadrupole mass analyzers to discriminate specific peptide ion within a complex sample based on *m/z* ratios[9]. Typically, precursor peptide ions with predetermined *m/z* values are selected in the first quadrupole (Q1) and transmitted to the second quadrupole (Q2) where fragmentation occurs. The produced fragment ions are transmitted to the third quadrupole (Q3) where the detection and identification of the targeted analyte is done. By ignoring all other ions that flow into the mass spectrometer the experiments gain considerably in sensitivity while maintaining exquisite accuracy. The application of SRM to multiple ions is referred as multiple reaction monitoring (MRM). The establishment of a SRM/MRM assay could be time consuming as a thorough characterization of the behavior of the selected peptides in the mass spectrometer is essential. Indeed, the selected ions should identify uniquely the targeted protein (or one isoform thereof), be most likely observed in the experiment and provide the strongest specific signal [10]. However, once the SRM/MRM experiment is appropriately established, a large number of samples can be analyzed in a timeline compatible with clinical practice.

Recently, Mohammed *et al.* developed a MRM assay to evaluate the concentrations of 31 coagulation/fibrinolysis-related plasmatic proteins in a cohort of 25 healthy controls, 25 patients with venous thrombosis (VT) and 25 patients with VT who were also diagnosed with cancer [11]. When compared to traditional antibody/activity-based methods of measurement, the MRM assay demonstrated a good correlation (Pearson correlation: 0.77). In addition, MRM strategy offered higher sensitivity (mean regression slope: 0.81) and higher multiplicity in a single run. The combination of coagulation factor concentrations highlighted signatures on VT and cancer samples that were not obvious from a traditional single measurement demonstrating the power of implementing multiplexed targeted proteomics strategies.

In a proof-of-principle paper, Conlon *et al*. [12] utilized this sensitive approach to identify and quantify fusion peptides derived from chimeric proteins encoded by fusion genes from chromosomal translocations recurrent in specific form of hematologic malignancies including NPM-ALK+ anaplastic large cell lymphoma (ALCL). Peptides containing amino acid substitutions resulting from point mutations can also be detected/quantified by SRM/MRM and be used as biomarkers as it was demonstrated in a non-hematologic malignancy harboring a KRAS missense mutation [13].

Mass cytometry

Mass cytometry (or CyTOF for cytometry by time-of-flight) is a hybrid technology combining principles of flow cytometry with inductively coupled plasma (ICP) mass spectrometry. It pioneered a new era of high-dimensional single-cell analysis since its introduction and application to the study of immune responses [14]. The innovative concept was

to use stable rare isotopes of elements not normally found in cells (mainly lanthanides) to tag antibodies (or other target-specific probes) for labeling single-cell suspensions. After running through a nebulizer the labeled single cells are flowing through the ICP torch within an argon stream at about 7,200°C resulting in atomization and ionization of the cells. The resulting single-cell derived cloud of ions is further analyzed by a mass spectrometer (time-of-flight analyzer). By tagging each antibody with a unique lanthanide isotope the readout from each isotope can be correlated to levels of antigen associated within an individual cell.

Mass cytometry has been used in the hematology arena to identify new cellular subsets and their regulation during hematopoiesis. Bendall *et al.* examined healthy human bone marrow and their signaling responses to cytokines and kinase inhibitors [14]. The assembly of related cell types defined by surface antigen expression and the mapping of cell signaling responses to stimulation/inhibition revealed previously unappreciated features of signaling responses from subset cell populations. In 2012 Bodenmiller *et al*. described drug responses to 27 small-molecule inhibitors across human peripheral blood mononuclear cells [15]. This high-dimensional analysis revealed how different cell types are uniquely affected by these inhibitors as well as their off-target effects. Using 44 B-cell centric parameter single-cell mass cytometry data collected from human bone marrow Bendall *et al*. provided a more comprehensive analysis of B-cell differentiation cascade [16]. More recently Bengsch *et al*. investigated the cytotoxic programs of T/NK-cells at different stages of differentiation and identified relationship of cytotoxic programs and CD8+ T-cell differentiation [17]. Similarly, the exploration of bone marrow aspirates from 41 acute myeloid leukemia (AML) patients with two 39-antibody staining panels enabled characterization of the *in vivo* function properties of leukemia stem cells [18].

Imaging cytometry by Time of Flight (CyTOF) and multiplexed ion-beam imaging (MIBI)

The ability to perform multiplexed imaging on single cells at the *in-situ* level on tissue sections is also desirable and offers significant advantages for better understanding of cellular diversity and heterogeneity as well as micro-anatomical topography and elucidation of functionally relevant adjacencies. In this regard, new technologies employing mass spectrometry have been developed. These include imaging CyTOF [19] and multiplexed ion-beam imaging [20]. In CyTOF imaging mass cytometry, tissues are stained with multiple antibodies which are labelled with metals. A high-resolution laser ablation system is then applied to transfer the tissue spot-by-spot with a resolution of 1,000nm generating particles that are directed into the CyTOF mass analyzer through a gas stream. The CyTOF analyzer identifies the metal isotope by m/z and quantifies epitope expression levels by signal abundance. By comparison, in multiplexed ion beam imaging a primary (oxygen) ion beam is generated using a duoplasmatron ion source which is rasterized over the tissue of interest at a resolution of ~200nm. The primary ions dislodge antibody-body metal isotopes as secondary ions which are analyzed in a magnetic sector mass analyzer [20]. Imaging CyTOF and MIBI are complementary and while currently early in their adoption cycles for clinical applications in hematopoietic conditions are likely to play an important role in the investigation of these diseases.

Proteomic studies of myeloid diseases

Myeloproliferative neoplasms (MPN) are often associated with a dysregulation of protein tyrosine kinase such as constitutive activation of JAK2 via V617F mutation and aberrant expression of the chimeric protein tyrosine kinase, BCR-ABL. Such aberrant proteins offer great potential for treatment and small molecule inhibitors targeting these tyrosine kinases

demonstrated significant benefits in term of symptom reduction, durable cytogenetic and molecular responses. However, these inhibitors are rarely curative. MS-based proteomic explorations of these oncogenic tyrosine kinases offer an unbiased approach to expand our understanding of the effects of these oncogenes in hematopoietic cells with the aim of improving treatment strategies. To gain an understanding into the JAK2 V617F-driven transformation process Pearson *et al*. performed a global proteomic screen on cells expressing wild-type JAK2 and mutated JAK2 V617F[21]. Out of the 5,021 identified proteins, 140 proteins exhibited a greater than 2-fold change between wild-type and mutated JAK2 expressing cells including transcription factors. A proteome and phosphoproteome analysis of the nuclear proteins revealed that 38 of the 2,090 quantified phosphopetides significantly changed as a consequence of both JAK2 V617F and JAK2 K539L expression. Pathway analysis indicated a disruption of both p53 and MYC signaling pathways. These observations were confirmed in primary CD34+ cell isolated from patients with JAK2 V617F mutation and healthy donors. Simultaneous inhibition of MYC and upregulation of p53 led to the depletion of JAK2 V617F-positive CD34+ cells, demonstrating a potential therapeutic benefit from combinatorial therapeutic approaches. By comparison, Reckel *et al* investigated the differential signaling networks of the 2 major isoforms of the oncogenic BCR-ABL tyrosine kinase (p210 and p190) resulting from the Philadelphia chromosome translocation [22]. The p210 isoform usually associated with chronic myelogenous leukemia (CML) whereas p190 occurs mainly in B-cell acute lymphoblastic leukemia (ALL) [23]. Many differences in both the interactome and phosphoproteome between the 2 isoforms were identified including a preferential interaction with the phosphatase STS1 as well as a stronger activation of the transcription factor STAT5 and the kinases ERK1/2 for the p210 isoform. Imatinib mesylate was the first molecularly targeted therapy designed to specifically

inhibit BCR-ABL tyrosine kinase activity. Despite the effectiveness of imatinib, drug resistance in sanctuary site like bone marrow occurs [24, 25]. Since it has been demonstrated that the bone marrow stroma is abnormal in CML and plays an important role in promoting survival and maintenance of resistance [26], Pizzatti *et al*. used a high-resolution label-free MS proteomic approach to identify differential protein expression in the bone marrow plasma of patients responsive and resistant to imatinib [27]. Their studies implicated two altered mechanisms in imatinib-resistant patients that could act independently in CML bone marrow and contribute to the resistance phenotype namely lipid metabolism and Wnt pathway activation.

Lenalidomide is a highly effective treatment of myelodysplatic syndrome (MDS) with deletion of the chromosome 5q (del(5q)) with cytogenetic remission in more than 50% of patients. This immunomodulatory agent has been shown to promote the ubiquitination and degradation of specific substrates by the $CRL4^{CRBN}$ E3 ubiquitin ligase. Using a K-ε-GG enrichment strategy to study the ubiquitome, Kronke *et al.* demonstrated that lenalidomide induces the ubiquitination and therefore the degradation of casein kinase 1A1 (CK1α) which is encoded by a gene within the common deletion region for del(5q) MDS.

Acute promyelocytic leukemia (APL) comprises 5-8% of AML and responds remarkably to the differentiation-induction by all-trans-retinoic acid (ATRA). To gain insights molecular networks underlying the synergy between ATRA and arsenic trioxide in APL Zheng *et al*. applied an approach integrating cDNA microarray and 2D gel electrophoresis with MS [28]. Their study revealed a coordinated regulation of transcription factors and cofactors, activation of the calcium signaling pathway, stimulation of the interferon pathway, and degradation of the chimeric oncoprotein PML-RARα. Similarly Hofmann *et al*. identified 500 membrane proteins including 137 CD annotated cell surface proteins in two APL-derive cell lines [29]. Consistent

with previous observations they observed a decrease of CD71 after ATRA treatment as well as several markers of granulocytic differentiation and other membrane proteins such the zinc transporter (S38AE), the amino acid transporter (SATT) and plexin D1.

Proteomic studies of lymphoid diseases

Mass spectrometry-based proteomic strategies have been leveraged to carry out comprehensive assessment of proteomics of both immune cells (B and T) as well as malignancies derived from B- and T-cells. To unravel the complex interactions between immune cells at the protein level, Rieckmann *et al*. used MS approaches to characterize 28 hematopoietic cell types sorted by flow cytometry from 3-4 human healthy donors [30]. The MS data was acquired from seven major lineages including granulocytes, monocytes, dendritic cells, natural killer, B-cells, CD4+ T-cells, CD8+ T-cells, erythrocytes and platelets. They analyzed the cellular proteomes in their steady state as well as in activated states. They identified and quantified more than 10,000 different proteins with an average of 7,500 proteins per measurement. Gene ontology analysis revealed >80% coverage of proteins with known immune-related functions and principal component analysis (PCA) yielded a clear distinction between lymphoid and myeloid immune cells. T and NK-cells formed a dense cluster confirming their close functional relationship and an independent PCA analysis of T and NK-cells resolved the effector classes from helper to cytotoxic as well as differentiation forms from naïve to effector memory. In 2011 Navarro *et al*. reported an unbiased analysis of the cytotoxic T-cells phosphoproteome [31]. They identified more than 2,000 phosphorylations in cytotoxic T-cells of which approximately 450 were regulated by T cell receptor (TCR) signaling, including transcription activators, corepressors and chromatin regulators. They found that class I histone

deacetylase (HDAC) HDAC1 and HDAC2 and class IIa HDAC HDAC7 were constitutively phosphorylated in cytotoxic T-cells. Furthermore, they functionally validated the importance of HDAC7 for the control of cytotoxic T-cell function.

Protein interaction networks of oncogenes important for the pathogenesis of lymphomas have led to important biologic insights for B-cell and T-cell lymphomas. For examples, the comprehensive interactome of BCL6 [32] revealed novel signaling targets and broadened the functional relevance of the key transcription factor in B-cell lymphomas. Similarly, the interacting proteins of the oncogenic tyrosine kinase NPM-ALK identified through tandem MS were found to mediate diverse cellular pathways critical for oncogenesis [33].

In order to identify differentially expressed proteins and network patterns in diffuse large B-cell lymphoma (DLBCL) Ruetschi *et al.* performed a SILAC-based shotgun quantitative proteomic analysis of fresh-frozen tumor tissues from 5 patients diagnosed with ABC subtype of DLBCL and 5 patients diagnosed with GCB subtype of DLBCL [34]. Out of the 3,500 quantified proteins 87 were differentially expressed between the 2 groups. The progression-free patient group was correlated with the overexpression of proteins involved in the regulation and organization of the actin cytoskeleton. Large scale proteomics has been performed from proteins extracted from FFPE samples [35]. Deeb *et al*. quantified almost 9,000 proteins from 20 FFPE samples of DLBCL and were able to segregate the samples according to the cell of origin. Interestingly a panel of 4 proteins (PALD1, MME, TNFAIP8 and TBC1 D4) demonstrated the highest segregation power [36].

McDonnell *et al*. used an integrated phosphoproteomic and metabolomics strategy to gain insights of the mechanisms underlying the pathogenesis of chimeric kinase NPM-ALK in anaplastic large cell lymphoma (ALCL) [37]. The phosphorylation of the tumor-specific isoform

of pyruvate kinase (PKM2) by NPM-ALK was shown to mediate the metabolic shift toward aerobic glycolysis and biomass production. Furthermore, the Wiskott-Aldrich syndrome protein (WASp), a novel phosphorylation substrate of NPM-ALK was found to contribute to oncogenesis in ALCLs [38]. More recently, Corso *et al*. investigated the regulation of cell survival in Burkitt lymphoma comparing tonic and activated B-cell receptor (BCR) signaling by phosphoproteomics to identify novel BCR effectors [39]. They found that out of the 16,000 quantified phosphosites 909 were regulated by tonic BCR whereas 984 were regulated upon BCR engagement. A comprehensive dataset of tonic and activated BCR signaling could be exploited to develop new therapeutic approaches. Similarly, a differential phosphoproteomic analysis of pre-germinal center derived lymphoma and germinal center derived lymphoma underlined the prominent role of the BCR signaling pathway in germinal center derived lymphoma [40]. As the classification of lymphomas are based in part on the utilization of the detection/semi-quantification of membrane proteins, mainly CD markers [41], a comprehensive evaluation of membrane proteins expressed by different subtypes of lymphoma represents a useful database for diagnostic purpose but also as potential therapeutic targets. Based on the premise that N-glycosylation of proteins facilitates protein trafficking to membranes, Rolland *et al* demonstrated that unsupervised clustering of 751 unique N-glycoproteins successfully classified lymphomas according to lineage, cell of origin and subtype [42]. Many of these N-glycosylated membrane proteins could be potential targets for precision therapies and linked to the pathogenesis of the various lymphomas.

Proteomic studies of hemostatic diseases

Upon vascular damage or injury, circulating platelets are rapidly activated to initiate the coagulation cascade and limit the bleeding. Since adenosine diphosphate (ADP) is a major physiological enhancer of platelet activation, Beck *et al.* used a temporal phosphoproteomic approach to study ADP-mediated signaling within healthy donor platelets [43]. They provided a temporal profile of 4,797 phosphopeptides out of which 608 were significantly regulated by ADP stimulation. They identified well known activating functions such as degranulation and cytoskeletal reorganization, but also less-understood pathways involving ubiquitin ligases. In 2011 Niessen *et al*. analyzed the coagulation reaction by studying the coagulation degradome [44]. They identified the majority of the expected proteolytic events as well as multiple potential new proteolytic events including cleavage of two transmembrane proteins that may be shed from the surface of blood cells.

Conclusions

Application of MS-based proteomic analysis has contributed broadly to the understanding of many hematologic disorders. The rapid development of sophisticated instrumentation, bioinformatics tools and improved sample interrogation methods and parallel advances in single-cell genomic analyses will provide exciting new opportunities for the enhanced utilization of MS-based proteomics in the investigation and clinical evaluation of hematologic conditions.

References

[1] Anderson L, Seilhamer J. A comparison of selected mRNA and protein abundances in human liver. Electrophoresis. 1997;18:533-7.

[2] Gygi SP, Rochon Y, Franza BR, Aebersold R. Correlation between protein and mRNA abundance in yeast. Mol Cell Biol. 1999;19:1720-30.

[3] Thomson JJ. Cathode rays. Philosophical Magazine. 1897;44.

[4] Karas M, Bachamann D, Bach U, Hillenkamp F. Matrix-assisted ultraviolet laser desorption of non-volatile compounds. Int J Mass Spectrom Ion Proc. 1989;78:53-68.

[5] Tanaka K, Waki H, Ido Y, Akita S, Yoshida Y, Yoshida T. Protein and polymer analysis up to m/z 100 000 by ionization time-flight mass spectrometry. Rapid Commun Mass Spectrom. 1988;2.

[6] Fenn JB, Mann M, Meng CK, Wong SF, Whitehouse CM. Electrospray ionization for mass spectrometry of large biomolecules. Science. 1989;246:64-71.

[7] Aebersold R, Mann M. Mass spectrometry-based proteomics. Nature. 2003;422:198-207.

[8] Walsh CT. Posttranslational Modification of Proteins: Expanding Nature's Inventory. 1st edition ed. Greenwood Village, CO: Roberts and Company, Publishers; 2005.

[9] Lange V, Picotti P, Domon B, Aebersold R. Selected reaction monitoring for quantitative proteomics: a tutorial. Mol Syst Biol. 2008;4:222.

[10] Mallick P, Schirle M, Chen SS, Flory MR, Lee H, Martin D, et al. Computational prediction of proteotypic peptides for quantitative proteomics. Nat Biotechnol. 2007;25:125-31.

[11] Mohammed Y, van Vlijmen BJ, Yang J, Percy AJ, Palmblad M, Borchers CH, et al. Multiplexed targeted proteomic assay to assess coagulation factor concentrations and thrombosis-associated cancer. Blood Adv. 2017;1:1080-7.

[12] Conlon KP, Basrur V, Rolland D, Wolfe T, Nesvizhskii AI, MacCoss MJ, et al. Fusion peptides from oncogenic chimeric proteins as putative specific biomarkers of cancer. Mol Cell Proteomics. 2013;12:2714-23.

[13] Wang Q, Chaerkady R, Wu J, Hwang HJ, Papadopoulos N, Kopelovich L, et al. Mutant proteins as cancer-specific biomarkers. Proc Natl Acad Sci U S A. 2011;108:2444-9.

[14] Bendall SC, Simonds EF, Qiu P, Amir el AD, Krutzik PO, Finck R, et al. Single-cell mass cytometry of differential immune and drug responses across a human hematopoietic continuum. Science. 2011;332:687-96.

[15] Bodenmiller B, Zunder ER, Finck R, Chen TJ, Savig ES, Bruggner RV, et al. Multiplexed mass cytometry profiling of cellular states perturbed by small-molecule regulators. Nat Biotechnol. 2012;30:858-67.

[16] Bendall SC, Davis KL, Amir el AD, Tadmor MD, Simonds EF, Chen TJ, et al. Single-cell trajectory detection uncovers progression and regulatory coordination in human B cell development. Cell. 2014;157:714-25.

[17] Bengsch B, Ohtani T, Herati RS, Bovenschen N, Chang KM, Wherry EJ. Deep immune profiling by mass cytometry links human T and NK cell differentiation and cytotoxic molecule expression patterns. J Immunol Methods. 2018;453:3-10.

[18] Behbehani GK, Samusik N, Bjornson ZB, Fantl WJ, Medeiros BC, Nolan GP. Mass Cytometric Functional Profiling of Acute Myeloid Leukemia Defines Cell-Cycle and Immunophenotypic Properties That Correlate with Known Responses to Therapy. Cancer Discov. 2015;5:988-1003.

[19] Giesen C, Wang HA, Schapiro D, Zivanovic N, Jacobs A, Hattendorf B, et al. Highly multiplexed imaging of tumor tissues with subcellular resolution by mass cytometry. Nat Methods. 2014;11:417-22.

[20] Angelo M, Bendall SC, Finck R, Hale MB, Hitzman C, Borowsky AD, et al. Multiplexed ion beam imaging of human breast tumors. Nat Med. 2014;20:436-42.

[21] Pearson S, Williamson AJK, Blance R, Somervaille TCP, Taylor S, Azadbakht N, et al. Proteomic analysis of JAK2V617F-induced changes identifies potential new combinatorial therapeutic approaches. Leukemia. 2017;31:2717-25.

[22] Reckel S, Hamelin R, Georgeon S, Armand F, Jolliet Q, Chiappe D, et al. Differential signaling networks of Bcr-Abl p210 and p190 kinases in leukemia cells defined by functional proteomics. Leukemia. 2017;31:1502-12.

[23] Hantschel O. Structure, regulation, signaling, and targeting of abl kinases in cancer. Genes Cancer. 2012;3:436-46.

[24] Apperley JF. Part I: mechanisms of resistance to imatinib in chronic myeloid leukaemia. Lancet Oncol. 2007;8:1018-29.

[25] Nair RR, Tolentino J, Hazlehurst LA. The bone marrow microenvironment as a sanctuary for minimal residual disease in CML. Biochem Pharmacol. 2010;80:602-12.

[26] Bhatia R, McGlave PB, Dewald GW, Blazar BR, Verfaillie CM. Abnormal function of the bone marrow microenvironment in chronic myelogenous leukemia: role of malignant stromal macrophages. Blood. 1995;85:3636-45.

[27] Pizzatti L, Panis C, Lemos G, Rocha M, Cecchini R, Souza GH, et al. Label-free MSE proteomic analysis of chronic myeloid leukemia bone marrow plasma: disclosing new insights from therapy resistance. Proteomics. 2012;12:2618-31.

[28] Zheng PZ, Wang KK, Zhang QY, Huang QH, Du YZ, Zhang QH, et al. Systems analysis of transcriptome and proteome in retinoic acid/arsenic trioxide-induced cell differentiation/apoptosis of promyelocytic leukemia. Proc Natl Acad Sci U S A. 2005;102:7653-8.

[29] Hofmann A, Gerrits B, Schmidt A, Bock T, Bausch-Fluck D, Aebersold R, et al. Proteomic cell surface phenotyping of differentiating acute myeloid leukemia cells. Blood. 2010;116:e26-34.

[30] Rieckmann JC, Geiger R, Hornburg D, Wolf T, Kveler K, Jarrossay D, et al. Social network architecture of human immune cells unveiled by quantitative proteomics. Nat Immunol. 2017;18:583-93.

[31] Navarro MN, Goebel J, Feijoo-Carnero C, Morrice N, Cantrell DA. Phosphoproteomic analysis reveals an intrinsic pathway for the regulation of histone deacetylase 7 that controls the function of cytotoxic T lymphocytes. Nat Immunol. 2011;12:352-61.

[32] Miles RR, Crockett DK, Lim MS, Elenitoba-Johnson KS. Analysis of BCL6-interacting proteins by tandem mass spectrometry. Mol Cell Proteomics. 2005;4:1898-909.

[33] Crockett DK, Lin Z, Elenitoba-Johnson KS, Lim MS. Identification of NPM-ALK interacting proteins by tandem mass spectrometry. Oncogene. 2004;23:2617-29.

[34] Ruetschi U, Stenson M, Hasselblom S, Nilsson-Ehle H, Hansson U, Fagman H, et al. SILAC-Based Quantitative Proteomic Analysis of Diffuse Large B-Cell Lymphoma Patients. Int J Proteomics. 2015;2015:841769.

[35] Crockett DK, Lin Z, Vaughn CP, Lim MS, Elenitoba-Johnson KS. Identification of proteins from formalin-fixed paraffin-embedded cells by LC-MS/MS. Lab Invest. 2005;85:1405-15.

[36] Deeb SJ, Tyanova S, Hummel M, Schmidt-Supprian M, Cox J, Mann M. Machine Learning-based Classification of Diffuse Large B-cell Lymphoma Patients by Their Protein Expression Profiles. Mol Cell Proteomics. 2015;14:2947-60.

[37] McDonnell SR, Hwang SR, Rolland D, Murga-Zamalloa C, Basrur V, Conlon KP, et al. Integrated phosphoproteomic and metabolomic profiling reveals NPM-ALK-mediated phosphorylation of PKM2 and metabolic reprogramming in anaplastic large cell lymphoma. Blood. 2013;122:958-68.

[38] Murga-Zamalloa CA, Mendoza-Reinoso V, Sahasrabuddhe AA, Rolland D, Hwang SR, McDonnell SR, et al. NPM-ALK phosphorylates WASp Y102 and contributes to oncogenesis of anaplastic large cell lymphoma. Oncogene. 2017;36:2085-94.

[39] Corso J, Pan KT, Walter R, Doebele C, Mohr S, Bohnenberger H, et al. Elucidation of tonic and activated B-cell receptor signaling in Burkitt's lymphoma provides insights into regulation of cell survival. Proc Natl Acad Sci U S A. 2016;113:5688-93.

[40] Rolland D, Basrur V, Conlon K, Wolfe T, Fermin D, Nesvizhskii AI, et al. Global phosphoproteomic profiling reveals distinct signatures in B-cell non-Hodgkin lymphomas. Am J Pathol. 2014;184:1331-42.

[41] WHO classification of tumours of haematopoietic and lymphoid tissues. IARC: Lyon 2017;eds. Swerdlow S.H., Campo E., Harris N.L., Jaffe E.S., Pileri S.A., Stein H., Thiele J.

[42] Rolland DCM, Basrur V, Jeon YK, McNeil-Schwalm C, Fermin D, Conlon KP, et al. Functional proteogenomics reveals biomarkers and therapeutic targets in lymphomas. Proc Natl Acad Sci U S A. 2017;114:6581-6.

[43] Beck F, Geiger J, Gambaryan S, Solari FA, Dell'Aica M, Loroch S, et al. Temporal quantitative phosphoproteomics of ADP stimulation reveals novel central nodes in platelet activation and inhibition. Blood. 2017;129:e1-e12.

[44] Niessen S, Hoover H, Gale AJ. Proteomic analysis of the coagulation reaction in plasma and whole blood using PROTOMAP. Proteomics. 2011;11:2377-88.

Table 1: Major post-translational modifications of proteins and their mass values

Post-translational modifications	Mass differences (Dalton)
Phospho-serine	79.966
Phospho-threonine	79.966
Phospho-tyrosine	79.966
Acetyl-lysine	42.011
Methyl-lysine	14.016
Dimethyl-lysine	28.031
Trimethyl-lysine	42.047
Methyl-arginine	14.016
Dimethyl-arginine	28.031
Palmitoyl	238.230
Ubiquitin	114.043 (Gly-Gly tag remains after tryptic digestion)
Methionine oxidation	15.995
Tryptophan oxidation	3.995, 15.995 and 31.990

Figure legend

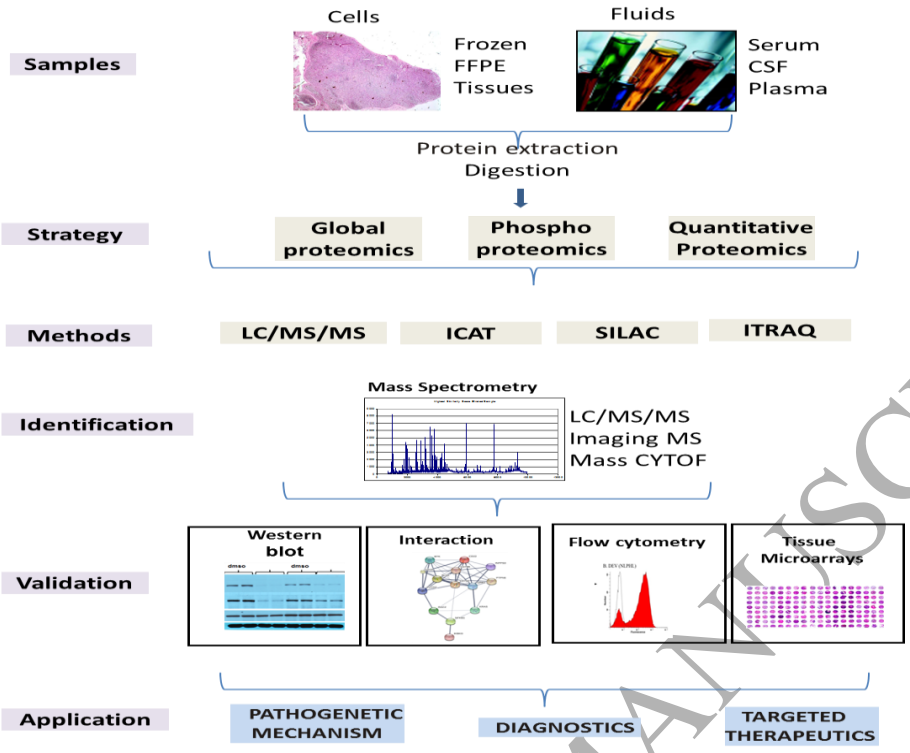

Figure 1. Overview of mass spectrometry-based proteomic analysis of complex biologic mixtures and its applications. Proteins are extracted from a variety of biologic source including frozen cell pellet, cells in culture, fluids or formalin-fixed paraffin-embedded tissues and digested with a protease, typically trypsin. Peptides are separated by using a combination of reverse-phase and cationic exchange liquid chromatography prior to electrospray ionization. Tissues can be used for imaging MS without peptide separation techniques. The peptides are identified by MS. The data are validated by western blot, flow cytometry, interactions with other proteins as well as expression in tissue microarrays. Proteomics is a multifaceted approach for the comprehensive analysis of proteins which can be applied for the identification of pathogenetic mechanisms, diagnostic biomarkers as well as therapeutic targets.