Review Article

Advances in the Clinical Application of High-throughput Proteomics

Miao Cui[1,2#], Fei Deng[3], Mary L. Disis[4], Chao Cheng[5,6] and Lanjing Zhang[3,7,8*]

Chao Cheng; [1]Department of Pathology, Icahn School of Medicine at Mount Sinai, New York, NY, USA; [2]Department of Pathology, Mount Sinai West, New York, NY, USA; [3]Department of Chemical Biology, Ernest Mario School of Pharmacy, Rutgers University, Piscataway, NJ, USA; [4]UW Medicine Cancer Vaccine Institute, University of Washington, Seattle, WA, USA; [5]Department of Medicine, Baylor College of Medicine, Houston, TX, USA; [6]Dan L Duncan Comprehensive Cancer Center, Baylor College of Medicine, Houston, TX, USA; [7]Department of Pathology, Princeton Medical Center, Plainsboro, NJ, USA; [8]Rutgers Cancer Institute of New Jersey, New Brunswick, NJ, USA

Abstract

High-throughput proteomics has become an exciting field and a potential frontier of modern medicine since the early 2000s. While significant progress has been made in the technical aspects of the field, translating proteomics to clinical applications has been challenging. This review summarizes recent advances in clinical applications of high-throughput proteomics and discusses the associated challenges, advantages, and future directions. We focus on research progress and clinical applications of high-throughput proteomics in breast cancer, bladder cancer, laryngeal squamous cell carcinoma, gastric cancer, colorectal cancer, and coronavirus disease 2019. The future application of high-throughput proteomics will face challenges such as varying protein properties, limitations of statistical modeling, technical and logistical difficulties in data deposition, integration, and harmonization, as well as regulatory requirements for clinical validation and considerations. However, there are several noteworthy advantages of high-throughput proteomics, including the identification of novel global protein networks, the discovery of new proteins, and the synergistic incorporation with other omic data. We look forward to participating in and embracing future advances in high-throughput proteomics, such as proteomics-based single-cell biology and its clinical applications, individualized proteomics, pathology informatics, digital pathology, and deep learning models for high-throughput proteomics. Several new proteomic technologies are noteworthy, including data-independent acquisition mass spectrometry, nanopore-based proteomics, 4-D proteomics, and secondary ion mass spectrometry. In summary, we believe high-throughput proteomics will drastically shift the paradigm of translational research, clinical practice, and public health in the near future.

Introduction

During the post-genomic era, proteomics has become an exciting field and a potential frontier of modern medicine since the early 2000s.[1–3] High-throughput technologies enabled quantitative proteomics, revealing unprecedented new insights.[4–6] For example, GAPDH, traditionally considered a housekeeping protein consistently expressed in various tissues, was recently found linked to retinoblastoma, lung adenocarcinoma, and intrahepatic chol-
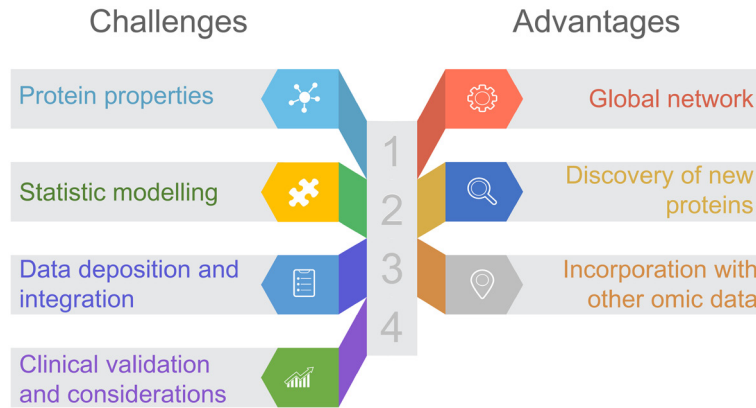
angiocarcinoma.[4,5,7] These findings led to increasing clinical applications of high-throughput proteomics, although these clinical applications remain relatively limited. Therefore, we here summarize recent advances in clinical applications of high-throughput proteomics and discuss the associated challenges, advantages, and future directions (Fig. 1).

The four most commonly used high-throughput proteomic techniques are mass spectrometry (MS), protein pathway array (PPA), next-generation tissue microarrays (ngTMA), and multiplex bead- or aptamer-based assays such as Luminex® and Simoa® (Fig. 2).[8] They each have their own methodological strengths and weaknesses and should be used accordingly.[8] Briefly, MS analyzes and quantifies proteins, their isoforms, and post-translational modifications through direct assessment of the fragments or specific proteolytic activities. Based on instrumental analysis methods, MS can be roughly classified into direct infusion MS, ion mobility system (IMS) MS, liquid chromatography-mass spectrometry (LC-MS), gas chromatography MS, and supercritical fluid chro-

Fig. 1. The challenges and advantages of high-throughput proteomics. The figure was generated using a template of Slidesgo (https://slidesgo.com/).

matography MS.[9,10] IMS MS is gaining popularity for its smaller chemical variations and higher speed than LC-MS.[9] The direct infusion shotgun proteome analysis combines shotgun and IMS MS methodologies and appears to be a highly efficient and accurate approach for high-throughput proteomics.[9,11] Isobaric tags for relative and absolute quantitation (iTRAQ) is another commonly used MS-based technique, covalently labeling the side-chain amines and/or N-terminus of targeted peptides.[12] It has been applied to discovering biomarkers of hepatocellular carcinoma and cervical cancer.[13,14] However, iTRAQ has its disadvantages, including isotopic use, contamination, and background noises, despite its very high accuracy (orders of magnitude).[15]

PPA uses a mixture of antibodies in a gel-based array to simultaneously detect corresponding antigens in a sample, making it high-throughput. ngTMA applies antibodies to a large number of tissue samples/cores simultaneously, usually formalin-fixed paraffin-embedded and arranged in an array on a single histologic slide. This allows large-scale antibody-based molecular analysis of multiple samples at the same time, improving time- and cost-efficiency and decreasing variations and the need for additional controls. Multiplex bead- or aptamer-based assays mix the samples with multiple beads or aptamers that simultaneously bind to various antigens in the samples through conjugated antibodies, aptamers, or probes. Most of these systems use fluorescent-based conjugation and detection systems. Finally, these technologies each have their unique strengths and weaknesses. For example, the acceptable sample

types vary by methodology. MS and PPA are mostly for processed proteins, while ngTMA and bead/aptamer-based assays require human tissue and blood samples, respectively. More methodological details can be found in other reviews.[8]

Advances in the clinical applications of proteomics

High-throughput proteomics methods have broad applications in translational research, clinical practice, and public health. They enable the exploration of molecular mechanisms and biological processes, the identification of novel diagnostic and prognostic biomarkers for precision medicine, and the discovery of therapeutic targets for personalized therapy.[16] Given the rapidly expanding high-throughput datasets,[17] high-throughput proteomics becomes increasingly important for translational and clinical research. It has been widely used in cancer research and other fields.[18–20] Several elegant reviews have focused on one or two disease areas and should be referred to for more details.[19,21–26] Here, we briefly summarize recent advances in the clinical application of high-throughput proteomics in selected diseases.

Breast cancer

Song *et al*.[27] performed both PPA and SmartChip, which is an mRNA microarray, and identified 1,243 cancer pathway-related genes in breast cancer. They revealed decreased protein and mRNA expression in *CDK6*, *Vimentin*, and *SLUG,* and different protein
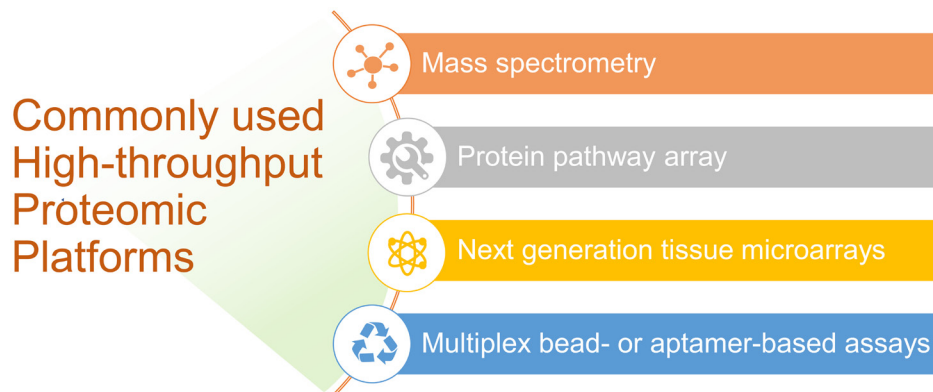
Fig. 2. Commonly used proteomics platforms. Bead-based array system includes commercially available Luminex and Meso-scale Discovery (MSD®) assays, while the mainstream aptamer-based proteomic system is SOMAscan® assay.

expression in *BCL6*, *CCNE1*, *PCNA*, *PDK1*, *SRC*, and *XIAP* between tumor and normal tissues, but no difference in mRNA expression in those genes. At the signaling network level, 15 altered pathways were identified in breast cancer. Among them, the p53, IL17, HGF, NGF, PTEN, and PI3K/AKT pathways, accounting for 6 pathways, were found to be shared between the mRNAs and proteins. Although many dysregulated pathways in breast cancer occur at both mRNA and protein levels, mRNA expression does not necessarily correlate with protein expression. It thus suggests different regulatory mechanisms for proteins and mRNAs in breast cancer pathogenesis.[27] Hadi *et al*.[28] used gas chromatography-mass spectrometry to identify potential protein markers for breast cancer. A partial least square discriminant analysis model was built to separate breast cancer patients, achieving a sensitivity of 96% and a specificity of 100% on the validation dataset. Models using the decision tree algorithm for grading, staging, and neoadjuvant status reached predictive accuracies of 71.5%, 71.3%, and 79.8%, respectively.[28] Interestingly, Aslebagh *et al*.[29] assessed protein expression patterns in human milk obtained from breastfeeding mothers who had breast cancer using 2D-polyacrylamide gel electrophoresis coupled with nano LC-MS/MS analysis. It showed that breast milk could be an essential and potentially informative biospecimen for breast cancer biomarker discovery.[29]

Colorectal cancer

Barberini *et al*.[30] applied gas chromatography-mass spectrometry to identify biomarkers in blood plasma for colorectal cancer and found the most significantly altered metabolic pathways in colorectal cancer involve monosaccharides, such as the catabolic pathway of fructose and D-mannose, and amino acids, such as methionine, valine, leucine, and isoleucine. Ang *et al*.[31] described a detailed protocol for revealing candidate protein markers in stool samples using 1D sodium dodecyl sulfate-polyacrylamide gel electrophoresis with LC-MS/MS. Their protein quantitation protocol and validation study will enhance the fecal proteome for the detection of potential fecal biomarkers. Additionally, using a high-density antibody microarray, Rho *et al*.[32] showed that plasma levels of several proteins/glycoproteins were associated with colon cancer diagnosis, including BAG4, IL6ST, and CD44. Adding CD44, EGFR, sialyl Lewis-A, and Lewis-X content further improved the panel's performance to an area under the curve (AUC) of 0.86 to 0.90.

Gastric cancer

In gastric cancer, Gao *et al*.[33] discovered that VCAM1, FLNA, VASP, CAV1, PICK1, and COL4A2 were differentially expressed using isobaric tags for relative and absolute quantitation (ITRAQ) labeling analysis with LC-MS. They identified VCAM1 as a potential biomarker for treatment, located at the center of the protein-protein interaction network by KEGG pathway analysis. Lian *et al*.[34] identified 20 proteins that were differentially expressed in Helicobacter pylori-associated gastric cancer using PPA. They found that both brassinosteroid-insensitive 1-associated kinase 1 and calpastatin were favorable prognostic factors in H. pylori-associated gastric cancers. The ERK/MAPK signaling pathway was the most significantly affected by H. pylori using PPA and ingenuity pathway analysis. He *et al*.[35] applied PPA to AFP-producing gastric adenocarcinoma, which is more aggressive and associated with liver metastasis, uncovering that *cyclin D1*, *RANKL*, *LSD1*, *Autotaxin*, *Calpain2*, *stat3*, *XIAP*, *IGF-Irβ*, and *Bcl-2* were up-regulated, and *ASC-R* and *BID* were down-regulated with significant differences. Furthermore, high levels of *XIAP* and *IGF-Irβ* were independent

prognostic factors. These factors can also be used to build a risk model with the pathological stage to separate AFP-positive gastric adenocarcinoma into two subgroups. The protein kinase A pathway was involved in the high-risk score group, while the PTEN pathway had significant enrichment in the low-risk score group by gene set enrichment analysis.[35] Tong *et al*.[36] uncovered nine serum markers using the Luminex system for the diagnosis of gastric cancer. Among them, pepsinogen I, pepsinogen II, ADAM8, VEGF, and Anti-H pylori IgG were identified as the panel of classifiers in the three algorithms, including logistic regression, random forest, and support vector machine, with accuracy in the validation set of 78.7%, 82.5%, and 86.1%, respectively.[36]

Bladder cancer

Proteomics has provided unique insights into the diagnostics, therapeutic targets, and pathogenesis of bladder cancer.[37] Chen *et al*.[38] applied differential 12C2-/13C2-dansylation labeling coupled with liquid chromatography/tandem MS to evaluate metabolite-based diagnostic biomarkers in urine for bladder cancer. They used ultra-performance liquid chromatography coupled with a high-resolution Fourier transform ion-cyclotron resonance MS system and an ion trap MS with multiple reactions for precise quantification. Among o-phosphoethanolamine, 3-amino-2-piperidone, uridine, and 5-hydroxyindoleacetic acid, o-phosphoethanolamine and uridine were differentially expressed in the urine of bladder cancer patients compared with controls. Furthermore, o-phosphoethanolamine was the most promising biomarker among the four, with an AUC of 0.709 for bladder cancer diagnosis. The AUC improved to 0.726 with the combination of o-phosphoethanolamine and uridine.[38] Hu *et al*.[39] demonstrated that 45 proteins were differentially expressed in bladder cancers compared with non-tumor samples. Among them, EGFR and cdc2p34 were associated with muscle invasion and higher histological grade. Moreover, ß-catenin, HSP70, autotaxin, Notch4, PSTPIP1, DPYD, ODC, cyclinB1, calretinin, and EPO can be employed as a classifier panel to classify muscle-invasive bladder urothelial carcinoma by prognosis. P2X7, cdc25B, and TFIIH p89 were identified as significant prognostic factors by Kaplan–Meier and log-rank analyses on overall survival.[39] Recent studies also identified 14 differentially expressed plasma proteins in cancer versus control groups, with apolipoprotein A1 being the most promising candidate (AUC = 0.906) and showed that Cadherin 12 is a predictor of neoadjuvant chemotherapy outcomes.[40,41]

Laryngeal squamous cell carcinoma

Sewell *et al*.[42] first identified stratifin, S100 calcium-binding protein A9, p21-ARC, stathmin, and enolase as proteomic markers for laryngeal squamous cell carcinoma. Chen *et al*.[43] discovered 16 proteins differentially expressed in laryngeal squamous cell carcinoma. Among them, TTF-1, CDK2, Eg5, PCNA, Bcl-xL, 14-3-3b, p27, SRC-1, and cytokeratin 18 were identified as markers for classification, and JAK2, keratin 10, and IL-3Ra were identified for prognosis. They also developed a risk model based on histological grade, T classification, N classification, JAK2, and IL-3Ra, which can predict the prognosis with 85.5% accuracy.[43] Pan *et al*.[44] developed a four-autoantibody-based early diagnostic panel, including TP53, HRAS, CTAG1A, and NSG1, for esophageal squamous cell carcinoma using the Luminex xMAP platform. The panel can discriminate early esophageal squamous cell carcinomas from controls with a sensitivity of 58.0% and specificity of 90.0% in an external validation dataset.[44] Using LC-MS, Zhao *et al*.[45] recently revealed that the fatty acid desaturase 1 expression was linked to poor prognosis and advanced clinical features in re-

current laryngeal squamous cell carcinoma patients treated with chemotherapy. They identified that fatty acid desaturase 1 is a potential promoter in laryngeal squamous cell carcinoma progression through the AKT/mTOR signaling pathway by protein-protein interactions (PPIs) and module analysis.

Coronavirus disease 2019 (COVID-19)

High-throughput proteomics is a technology that can be rapidly applied, as demonstrated by its use in studying COVID-19. Despite the progress made in combating COVID-19,[46,47] diagnostics and prognostication of COVID-19 and long COVID-19 remain challenging in the post-vaccination period.[48,49] Ray *et al*.[50] illustrated that proteomics techniques, including MS, antibody-based assays, and bioinformatics had tremendous potential to uncover the severe-acute-respiratory-syndrome-related coronavirus-2 (SARSr-CoV-2) pathobiology and inform therapeutics and vaccine development. LC-MS has helped identify the host cell pathways modulated by SARS-CoV-2 virus.[51] Hierarchical clustering analysis identified two main clusters of proteins: one consisting of proteins involved in cholesterol metabolism, which were reduced during infection, and another of proteins that were increased by infection. They showed that inhibition of these pathways can stop viral replication *in vitro* and thus can be targets for COVID-19 prevention and treatment.[51] Forster *et al*. successfully used phylogenetic networks to identify undocumented COVID-19 infection sources. Their team found three central variants marked by different amino acid profiles, named A, B, and C types. The A and C types are mostly found in Europeans and Americans, while the B type is more prevalent in East Asia.[52,53] Interestingly, the ancestral genome of type B seems to have mutated into derived B types before being transmitted beyond East Asia.[52,53]

Challenges

Protein property

Degradation has always been the biggest challenge in proteomics, compared to the considerable stability of DNA and cDNA. Protein stability and half-life are modulated by multiple post-translational modifications (PTMs), which regulate various signaling pathways and modify proteins with functional chemical groups, including phosphate, glycan, methyl, acetyl, ubiquitin, and others.[54] Although these modifications are significant for protein functions such as activity state, stability, localization, turnover, and interactions with other molecules, they are susceptible to degradation. This degradation can occur during protein extraction, sample collection, and temperature changes.[55] Missing PTM detection can lead to misinterpreted results and errors in data analysis. For example, the degradation of phosphorylation can mislead the activity status of proteins and dynamic protein-protein interactions. Loss of PTMs can also significantly change the original multi-dimensional structure of proteins. Low protein concentration and non-specific bindings are additional challenges for immunoassay-based techniques.

Statistical modeling

The missing or inappropriate normalization of data will lead to inaccurate or misleading statistical analysis, despite the use of proper statistical methods.[56–58] For example, although housekeeping proteins such as GAPDH and beta-actin are known for their consistent expression across biological sources, it is sometimes inaccurate if there are systematic errors or intrinsic linkages to a disease.[4,5,7]

These errors may affect protein detection in certain areas or types of protein. Using additional housekeeping proteins can help identify these errors and serve as internal controls.

Statistical modeling algorithms may also be affected by input data and selected features/factors (e.g., quality of samples and biomarker selection). For example, the clustering results can be greatly influenced by changes in samples and available features.[8] In such scenarios, unsupervised machine learning (ML) may be particularly useful since it does not rely on the labels/annotations provided by experts but is driven by the intrinsic relationships of the samples.[59,60] However, the performance of unsupervised ML may be worse than supervised ML and thus should be compared with that of supervised ML.[61] Sample selection, variables, and study goals need to be clarified beforehand to achieve a more meaningful result.

The same statistical method can be performed using various formulas, which focus on different principles or data types and generate different results. For example, clustering analysis can generate different heat maps using different distances as units, such as Euclidean distance, Manhattan distance, Pearson correlation, minimum distance, and maximum distance. Even for the same dataset, different ML models can achieve varying classification accuracy and performance.[8,62,63] Moreover, there is no best or standardized statistical algorithm to fit all data types, especially unknown data. Therefore, an optimized statistical model should not only be based on the data itself to seek the most reasonable formula but also on clinical significance and dataset characteristics. This is probably the biggest challenge during data analysis. Therefore, model optimization and comparison should also be performed to ensure that the best-fit model is identified and used while minimizing the risk of overfitting.[6,64–66]

Data deposition, integration and harmonization

Research and clinical communities are integrating and standardizing data from different studies and sources for a bigger picture of the signaling network and greater statistical power. However, several challenges remain in collecting and merging datasets currently.[67] (1) The two major omics data depositories, which include proteomic data, are the Gene Expression Omnibus hosted by the National Library of Medicine, National Institutes of Health, Bethesda, MD, USA, and the ArrayExpress by the European Molecular Biology Laboratory, European Bioinformatics Institute, Hinxton, Cambridgeshire, UK.[68] However, there are limited publicly available resources for acquiring proteomics and genomics data, which makes assessing signaling network changes among the DNA, mRNA, and protein levels difficult in a target disease due to limited overlapping molecules. Indeed, 33.8% of the datasets in the ProteomeXchange consortium were unreleased.[69] These two omics data depositories also focus on genomic and transcriptomic data. While the PRoteomics IDEntifications (PRIDE) database, part of the ProteomeXchange consortium, has more proteomic data,[70] how to incorporate the 3 omics data types remains challenging. (2) Particularly with clinical samples, protecting patient identity is becoming and should have become a priority for database repositories.[69,71,72] It is legally and ethically challenging to balance excessive administrative burdens with sufficient patient protection. (3) Sample preparations in different research or clinical settings will increase system errors and noises in multi-omics analysis. Therefore, it is essential to use a standardized protocol, common data standards, and annotation guidelines during the experiment and computational processing to simplify and acquire qualified data for further data sharing, merging, integration, and

mining. Efforts have been focused on standardized submission and data protocols.[67,68,73,74] (4) Nearly all proteomic data repositories are designed to accept MS-based data, while non-MS-based data such as that from ngTMA and multi-bead-based technology are currently incompatible.[69] This has become a future direction for the ProteomeXchange consortium.

Clinical validation and considerations

Approximately 3,000 genomic and proteomic biomarkers are currently used in clinical trials involving more than 2,000 diseases.[75] However, a significant bottleneck in developing useful and marketable proteomics-based assays is the step of moving into clinical validation, with or without a clinical trial.[22,76] Any qualified clinical validation study should have not only high sensitivity and specificity but also high precision and high accuracy.[77] These characteristics are affected by specimen collection, storage, and processing, which are often not assessed in the scientific research phase. Developing clinical tests, including multi-step procedures that must also be easy to perform with good reproducibility, can be difficult.[22] Besides, a complex clinical environment is usually not conducive to high-throughput assays. Cross-contamination is still common and difficult to completely remove/prevent, even for procedures performed in different areas such as the clean area (pre-test room), reaction area (test room), and contaminated area (post-test room).[78]

Furthermore, real-world clinical settings are more complex than research settings. Patients' existing treatments and comorbidities are major barriers between real-world and research settings. They may interfere with or confound the expected clinical usefulness of proteomic markers and thus decrease the generalizability of research studies.[78] MS might be highly specific, but clinical laboratories may need higher sensitivity from an (immune) assay platform.[79] Indeed, it is relatively straightforward to incorporate a particular protein variant into a clinical setting as a screening marker that is sensitive but less specific for a disease or diagnosis. Moreover, other reasons may also significantly delay the clinical application of proteomics, including researchers' lack of understanding of clinical validation requirements, long development turnaround times, the lack of ready-to-use quality control and calibrators, and the lack of necessary paperwork and regular maintenance. A sufficient number of both positive and negative samples for clinical validation is a unique but not uncommon challenge for small laboratories.

How to meet these challenges

Technological advances, stringent data validation, model optimization, and rigorous validation processes are key to successfully meeting these challenges in clinical proteomics.[20,80] PTM, as part of the challenging protein property, can be effectively monitored using advanced MS technologies, such as iTRAQ, multiplexed proteome dynamics profiling, and data-independent acquisition (DIA) MS.[81–83]

Several considerations are noteworthy for improving the statistical modeling of high-throughput clinical proteomics.[20] First, rigorous and high-performance statistical modeling relies on robust data validation and quality control processes, which must be ensured through document control and implementation. Second, all ML modeling must be compared with conventional or existing modeling and undergo an optimization (tuning) process for the best performance. Third, an external dataset should be used as the independent test set so that the generalizability of the final (chosen) model can be reliably and independently tested.

Finally, although proteomics-based clinical trials lag behind other diagnostic modalities (e.g., genomics), plasma proteomic and metabolomic data may greatly help guide precision oncology.[19,20,84–86] When developing clinical proteomic tests, attention should be directed to each of the pre-analytics, method development, performance evaluation, and implementation steps.[85] Several groups called for standardization and stringent quality control processes for the clinical use of proteomic biomarkers.[19,20,85] Education and training of laboratory staff are also important for the robust validation and implementation of clinical proteomics.[85]
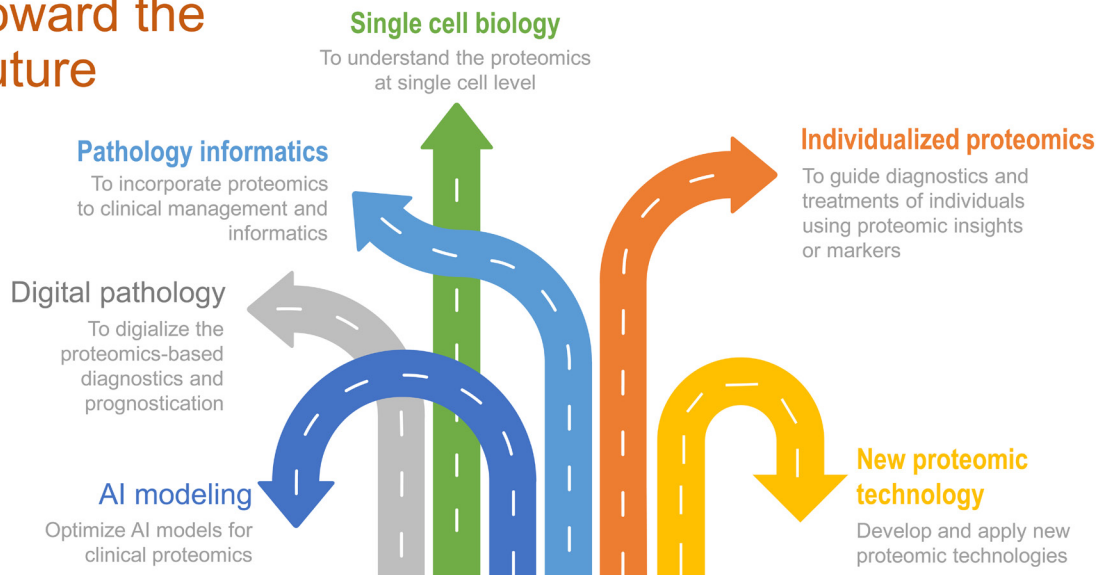
Advantages

Global networks

Understanding cell signaling networks involved in disease and carcinogenesis has significantly advanced our knowledge of disease mechanisms and cancer initiation. These networks provide a global picture of protein-protein interactions, pathway-pathway interactions, and the significant functions of each pathway and sub-network group.[87] Protein signaling network alterations, as part of a multi-step model of carcinogenesis, result from genetic, epigenetic, transcriptomics, etc. For example, PPA allows digitalized protein expression to be combined with genomic data to create a more comprehensive multi-dimensional signaling network of diseases.[8] This network can be further integrated with existing knowledge, such as epidemiological data and digital pathology. A proteomic network, including large-scale PPI discoveries, will thus bridge the gap between genomics and biological functions, refining or reshaping our understanding of diseases.[88,89] Furthermore, the entire network has great potential to investigate the functions and relationships of proteins and metabolites that reflect the disease's hallmarks, and to understand the strength of each group of PPIs, enabling the discovery of driver proteins or driver pathways.[90,91] The single protein expression with the most statistical differences is not necessarily the protein that affects biological functions the most in the network, nor are they the driver proteins that affect the entire network changes or the independent factors that affect disease progress. Therefore, biostatistical models and artificial intelligence can recombine all of the biomarkers and optimize them into a panel,[89] providing higher specificity than single-protein assays to meet different clinical needs, such as early diagnosis, prognosis prediction, and targeted treatment. This enables truly personalized medicine because each biomarker contributes differently to each clinical setting.

Discovery of new proteins

Unlike immunoassays, MS-based methods do not require the development of high-affinity antibodies specific to each protein epitope. They allow the simultaneous sequencing of hundreds of proteins in a wide variety of biological matrices, including fresh cells, frozen tissues, and formalin-fixed paraffin-embedded tissues.[92,93] MS-based methods can discover unknown proteins by powerful searching against a protein sequence database and quantifying them in a complex compound with high sensitivity, which is the cornerstone of identifying biomarkers and exploring the proteomics network.[18,19] Moreover, various types of chemical groups in the peptide structure, such as phosphate groups involved in PTMs, can be captured and mapped back to protein sequences to infer the expressed proteins.[94,95] Increasingly popular and improving MS technology makes protein discovery easier, resulting in more comprehensive proteome coverage for various organisms

Fig. 3. The roadmap toward the future includes the six major directions. The figure was generated using a modified template of Slidesgo (https://slidesgo.com/).

and generating a wealth of information stored in databases and bioinformatics repositories.

Multi-omics

The complex signaling pathway mechanisms that trigger oncogenesis or disease are not dependent on any single factor or only on the proteomic level but instead on the comprehensive effects from genomic and transcriptomic to proteomic alterations. Any individual protein is regulated by PPIs, mRNA, and DNA, working cooperatively in intricate signaling networks.[87,89,96] Moreover, protein levels cannot be directly predicted by mRNA abundance, and protein dynamics are also dependent on other factors such as epigenetic and transcriptional regulation, which work together to alter protein levels, abnormal structural conformation, and impair function. Therefore, a multi-omics approach is required to characterize the complex pathophysiology of oncogenesis and explore and reshape the mechanisms of pathogenesis at the molecular level.[97–99] Integration of data across -omics areas is promising because it provides a comprehensive view of genomic mutations and transcriptional abnormalities, promoting basic research on cells and animals. Thus, an exciting term and field, namely proteogenomics, has been developed.[100,101] For example, Cao *et al*.[102] conducted a comprehensive proteogenomic study on 50,000 MS runs of more than 900 projects. Among the 170,529 identified novel peptides, only about 1/30 (6,048) passed their strictest standard, which included being identified in more than two MS runs, more than one PRIDE project, and other criteria.

Currently, the diagnosis, evaluation, and treatment of most cancers are based on limited protein biomarkers or mutations combined with clinical presentation or characteristics. The integration of "omics" data can serve as the cornerstone of modern medicine to identify a new panel of biomarkers at the molecular level.[103] These profiles, generated by the statistical models of multi-omics data, can ultimately serve as risk factors for disease, diagnostic as-

says for early detection, therapeutic markers for personalized treatment, and many other applications in medicine. A single candidate, whether protein, gene, or clinicopathological characteristics, is often insufficient or ineffective in providing a comprehensive evaluation due to the complexity of human tumors and carcinogenesis.[104,105] Furthermore, as accumulating patient specimens undergo multi-omics analysis, a larger sample size with a more diverse population will allow the identification of additional low-frequency driver markers and mutations, especially in rare diseases.

Future directions

The clinical applications of high-throughput proteomics are still limited. Therefore, it is crucial to develop a roadmap for the future. We here recommend a roadmap focusing on single-cell biology, individualized proteomics, digital pathology, pathology informatics, deep learning modeling, and new proteomic technologies (Fig. 3).

Proteomics based single cell biology and its clinical applications

Single-cell mass cytometry is increasingly applied in various biomedical fields as the vast heterogeneity between cells of the same tissue is gradually recognized in medicine.[106–109] This technology will likely help create a new division of modern biology, termed single-cell proteomics, providing unique biological insights at the single-cell level.[110] For example, protein extraction-based proteomics techniques, such as Western blot or PPA, are challenging to identify whether the target protein is highly expressed in a small portion of cells or weakly expressed in most cells. A sufficiently large population of single cells can be studied as a time series of "snapshots" to recreate a timeline of dynamic biological processes of disease. Next-generation sequencing, as the ultra-high-throughput transcriptome analysis, has tremendously improved sensitivity and increased capacity in genomics, allowing the identification of numerous new genetic variables in rare cell populations.[25]

Single-cell proteomics can similarly provide maximal information to uncover the uniqueness of each cell in proteomics and has been recently applied to multiple myeloma, chimeric antigen receptor T cell therapy, and ovarian cancers.[111–113]

Li *et al*.[114] described a nanoliter-scale oil-air-droplet chip for multistep complex sample pretreatment and injection for single-cell proteomic analysis in the shotgun mode, identifying 355 proteins at the single-cell level (mouse oocyte). Zhu *et al*.[115] developed the nanoPOTS (nanodroplet processing in one pot for trace samples) platform, which can identify over 3,000 proteins from as few as 10 cells using the Match Between Runs algorithm of MaxQuant. They demonstrated the quantification of 2,400 proteins from a single human pancreatic islet within sections using this system. Ctortecka *et al*.[116] further developed the proteoCHIP for preparing single-cell proteomics samples and can detect 2,000 proteins per TMT10-plex in single cells that were 170 multiplexed across various human cell types. Krieg *et al*.[117] used high-dimensional single-cell mass cytometry for an in-depth characterization of immune cell subsets in the peripheral blood of patients with stage IV melanoma to predict responses to anti-PD-1 immunotherapy. Various new mass-based cytometry technologies significantly promote high-dimensional proteomics of single-cells,[118] opening an exciting field to understand the complicated relationship between tumor cells and the environment and bringing higher clinical value for precision medicine.

Individualized proteomics

Polymorphisms, as individual modifiers of distinct genetic traits, widely influence the genome-based global arrangement of proteomics, resulting in a unique proteomic signature for each individual. Moreover, the structure and expression of the proteomic network vary in functional efficiency, known as "network polymorphisms".[119,120] Furthermore, individual variations lead to different biochemical and physiological baselines. Various modifiable factors, such as smoking, stress, obesity, and nutrition, accumulate in each individual with aging, shaping their unique genome, epigenome, and proteome together to contribute to disease development.[121] Therefore, assessing and monitoring an individual's proteotype of oncogenic proteomic profiles will contribute to the evolution and expansion of individualized precision medicine, ensuring that the right intervention, including diagnosis or treatment, is provided to the right person at the right time.[26,119]

Pathology informatics and digital pathology

Pathology informatics and digital pathology have emerged in recent years with the rapid development of imaging and computational technology. They enable us to perform more clinical tasks in a shorter time and generate a large amount of medical data. Novel sources of data from electronic medical records, pathology images, and bio-information,[122] recorded by wearable personal trackers (e.g., heart rate, activity, sleep, weight), along with multi-omics data, are incorporated into a comprehensive dataset, providing a broader view of disease. No single analytical domain holds the keys to all aspects of disease development, diagnosis, and treatments.[37,92,123] These detailed multi-dimensional explorations enable proteomics to play a significant role in the near future. The new multi-dimensional data resources provide a new foundation to generate more comprehensive proteomics biomarkers or panels for modern precision medicine. In turn, proteomics in this integrated multidisciplinary context can provide robust information to rethink and uncover the pathogenesis or specific patterns during the course of disease.

Deep learning

Deep learning, a subdiscipline within artificial intelligence and ML, focuses on algorithms that enable computers to learn to solve problems from existing data (training data).[124] With its powerful computing and learning capabilities, deep learning can handle complex situations far beyond what the human brain alone can accomplish.[125] It is particularly well-suited for processing massive (big) data with strong internal correlations from high-throughput multi-omics, assisting in diagnosis.[126] Deep learning can accelerate the statistical analysis and data visualization of existing proteomics methods. For example, MS data are analyzed by peaks, that represent ions with a specific mass-to-charge ratio and can be biomarkers due to their similarity in the massive sequence database, without determining which peptides or proteins are actually present.[127] During this process, deep learning can be a powerful tool to identify biomarkers with higher accuracy.[89,128,129] It allows us to explore large, comprehensive datasets combining data from multi-omics.[130]

Although multiple ML applications have been used to assist with proteomic data analysis, there remains substantial room for improvement.[131] Traditional data analysis is based on digital matrices converted from raw image results through multiple steps. For example, the size and density of each band from the antibody-antigen reaction, generated by a protein pathway array, need to be manually converted to numeric data. This multi-step manual processing can cause various systematic errors and make data difficult to merge. Convolutional neural networks and recurrent neural networks, types of deep learning/neural network-based multilayer artificial neural networks, are particularly useful for image analysis. They allow algorithms and statistical models to be built directly on the original image results rather than manually converted digital data. This approach may significantly improve the accuracy and efficiency of proteomics data analysis and increase the availability of high-quality public data resources.[74,132,133]

New proteomic technology

New proteomic technologies are rapidly developing and currently include DIA MS, nanopore-based proteomics, Python-based high-efficiency data processing packages, 4-D proteomics, and secondary ion MS. For example, the SWATH-MS system, a type of DIA MS, systematically fragments and measures all ionized peptides within a predefined mass range, resulting in fewer biases and better consistency than using the window of precursor isolations.[134] In SWATH-MS measurements, peptide-centric scoring is often used and requires a thorough understanding of the peptide's chromatographic and mass spectrometric characteristics.[134] Based on a conceptually novel MS machine,[135] an ultra-fast label-free DIA MS (named narrow-window DIA MS) was recently proposed, combining high-resolution MS1 scans and parallel tandem MS/MS scans of ~200 Hz, delivering high sensitivity, specificity, and speed.[136] Nanopore, known for its low cost and high sensitivity in DNA/RNA sequencing, may also be applied to label-free proteomics.[137] A Python-based package (AlphaPept) was developed to efficiently process large high-resolution MS datasets using both central processing unit and graphics processing unit.[138] The introduction of 4-D proteomics, with the fourth dimension of ion mobility (system), expands the depth of proteomics and has been coupled with DIA MS.[139–142] Secondary ion MS involves the detection and mass-to-charge ratio analysis of secondary ions generated when sample surfaces are bombarded with energetic ions,[143] offering very granular surface chemical data and sub-monolayer sensitivity.

It is noteworthy that these new proteomic technologies can be

integrated to generate synergistic outcomes. For example, 4D-proteomics combined with DIA MS reportedly improves detection sensitivity.[142] DIA MS was also used to explore single-cell proteomics.[144]

We must balance innovation and compliance when applying new proteomic technologies to clinical problems. Innovations in technology will certainly shift the paradigm in clinical proteomics. However, without rigorous validation in multiple datasets and high-level clinical evidence, new technologies, including proteomics, must not be directly used for clinical care, even as a laboratory-developed test (LDT).[145,146] Indeed, the College of American Pathologists has a policy requiring rigorous validation of LDTs, and the U.S. Food and Drug Administration is also likely to regulate LDTs.[147]

Conclusions

High-throughput proteomics is increasingly being applied to translational research, clinical practice, and public health. While others have elegantly reviewed advances in pancreatic cancer, soft tissue sarcomas, or medicine as a whole, we briefly summarize recent advances in the clinical applications of high-throughput proteomics in breast cancer, colorectal cancer, gastric cancer, bladder cancer, laryngeal squamous cell carcinoma, and COVID-19. Future applications of high-throughput proteomics will face challenges related to various protein properties, limitations of statistical modeling, and technical and logistical difficulties in data deposition, integration, and harmonization, as well as regulatory requirements for clinical validation and considerations. However, we are encouraged by the advantages of high-throughput proteomics, including novel global protein networks, the discovery of new proteins, and synergistic incorporation with other omic data. We look forward to future advances in high-throughput proteomics, such as single-cell proteomics and its clinical applications, individualized proteomics, pathology informatics, digital pathology, and deep learning models for high-throughput proteomics. In our view, recent and future advances in high-throughput proteomics will in our view drastically shift the paradigms of translational research, clinical practice, and public health.

Conflict of interest

Lanijng Zhang is a co-editor-in-chief of the journal *Exploratory Research and Hypothesis in Medicine*. The authors declare no other conflict of interests.

Author contributions

Study conceptualization and design, ensuring the data access, ac-

curacy and integrity (LZ), and manuscript writing (MC and LZ). All authors, including MC, FD, MLD, CC and LZ contributed to the writing or revision of the review article and approved the final publication version.

References

[1] Ping P. Getting to the heart of proteomics. N Engl J Med 2009; 360(5):532–534. doi:10.1056/NEJMcibr0808487, PMID:19179323.

[2] te Pas MF, Claes F. Functional genomics and proteomics for infectious diseases in the post-genomics era. Lancet 2004;363(9418):1337. doi:10.1016/S0140-6736(04)16082-0, PMID:15110486.

[3] Banks R, Selby P. Clinical proteomics—insights into pathologies and benefits for patients. Lancet 2003;362(9382):415–6. doi:10.1016/S0140-6736(03)14096-2, PMID:12927423.

[4] Guo Y, Li Q, Ren W, Wu H, Wang C, Li X, *et al*. Quantitative Proteomics Reveals Down-Regulated Glycolysis/Gluconeogenesis in the Large-Duct Type Intrahepatic Cholangiocarcinoma. J Proteome Res 2022;21(10):2504–2514. doi:10.1021/acs.jproteome.2c00472, PMID:36066509.

[5] Chen H, Lai X, Zhu Y, Huang H, Zeng L, Zhang L. Quantitative proteomics identified circulating biomarkers in lung adenocarcinoma diagnosis. Clin Proteomics 2022;19(1):44. doi:10.1186/s12014-022-09381-x, PMID:36404333.

[6] Becker CH, Bern M. Recent developments in quantitative proteomics. Mutat Res 2011;722(2):171–182. doi:10.1016/j.mrgentox.2010.06.016, PMID:20620221.

[7] Galardi A, Stathopoulos C, Colletti M, Lavarello C, Russo I, Cozza R, *et al*. Proteomics of Aqueous Humor as a Source of Disease Biomarkers in Retinoblastoma. Int J Mol Sci 2022;23(21):13458. doi:10.3390/ijms232113458, PMID:36362243.

[8] Cui M, Cheng C, Zhang L. High-throughput proteomics: a methodological mini-review. Lab Invest 2022;102(11):1170–1181. doi:10.1038/s41374-022-00830-7, PMID:35922478.

[9] Jiang Y, DeBord D, Vitrac H, Stewart J, Haghani A, Van Eyk JE, *et al*. The Future of Proteomics is Up in the Air: Can Ion Mobility Replace Liquid Chromatography for High Throughput Proteomics? J Proteome Res 2024;23(6):1871–1882. doi:10.1021/acs.jproteome.4c00248, PMID:38713528.

[10] Gerhardtova I, Jankech T, Majerova P, Piestansky J, Olesova D, Kovac A, *et al*. Recent Analytical Methodologies in Lipid Analysis. Int J Mol Sci 2024;25(4):2249. doi:10.3390/ijms25042249, PMID:38396926.

[11] Meyer JG, Niemi NM, Pagliarini DJ, Coon JJ. Quantitative shotgun proteome analysis by direct infusion. Nat Methods 2020;17(12):1222–1228. doi:10.1038/s41592-020-00999-z, PMID:33230323.

[12] Wiese S, Reidegeld KA, Meyer HE, Warscheid B. Protein labeling by iTRAQ: a new tool for quantitative mass spectrometry in proteome research. Proteomics 2007;7(3):340–350. doi:10.1002/pmic.200600422, PMID:17177251.

[13] Omar MA, Omran MM, Farid K, Tabll AA, Shahein YE, Emran TM, *et al*. Biomarkers for Hepatocellular Carcinoma: From Origin to Clinical Diagnosis. Biomedicines 2023;11(7):1852. doi:10.3390/biomedicines11071852, PMID:37509493.

[14] Mukherjee A, Pednekar CB, Kolke SS, Kattimani M, Duraisamy S, Burli AR, *et al*. Insights on Proteomics-Driven Body Fluid-Based Biomarkers of Cervical Cancer. Proteomes 2022;10(2):13. doi:10.3390/proteomes10020013, PMID:35645371.

[15] Ow SY, Salim M, Noirel J, Evans C, Rehman I, Wright PC. iTRAQ underestimation in simple and complex mixtures: "the good, the bad and the ugly". J Proteome Res 2009;8(11):5347–5355. doi:10.1021/pr900634c, PMID:19754192.

[16] Targeted proteomics: Bench to bedside. J Proteomics 2018;189:iii–iv. doi:10.1016/j.jprot.2018.08.007, PMID:30103027.

[17] Liu DD, Zhang L. Trends in the characteristics of human functional genomic data on the gene expression omnibus, 2001-2017. Lab Invest 2019; 99(1):118–127. doi:10.1038/s41374-018-0125-5, PMID:30206311.

[18] Cho WC. Mass spectrometry-based proteomics in cancer research. Expert Rev Proteomics 2017;14(9):725–727. doi:10.1080/14789450.2017.1365604, PMID:28783987.

[19] Macklin A, Khan S, Kislinger T. Recent advances in mass spectrom-

etry based clinical proteomics: applications to cancer research. Clin Proteomics 2020;17:17. doi:10.1186/s12014-020-09283-w, PMID: 32489335.

[20] Boys EL, Liu J, Robinson PJ, Reddel RR. Clinical applications of mass spectrometry-based proteomics in cancer: Where are we? Proteomics 2023;23(7-8):e2200238. doi:10.1002/pmic.202200238, PMID: 35968695.

[21] Mehta S, Bernt M, Chambers M, Fahrner M, Föll MC, Gruening B, *et al*. A Galaxy of informatics resources for MS-based proteomics. Expert Rev Proteomics 2023;20(11):251–266. doi:10.1080/1478945 0.2023.2265062, PMID:37787106.

[22] Letunica N, McCafferty C, Swaney E, Cai T, Monagle P, Ignjatovic V, *et al*. Proteomic Applications and Considerations: From Research to Patient Care. Methods Mol Biol 2023;2628:181–192. doi:10.1007/978-1-0716-2978-9_12, PMID:36781786.

[23] Huang P, Gao W, Fu C, Tian R. Functional and Clinical Proteomic Exploration of Pancreatic Cancer. Mol Cell Proteomics 2023;22(7):100575. doi:10.1016/j.mcpro.2023.100575, PMID:37209817.

[24] Chadha M, Huang PH. Proteomic and Metabolomic Profiling in Soft Tissue Sarcomas. Curr Treat Options Oncol 2022;23(1):78–88. doi:10.1007/s11864-022-00947-3, PMID:35171456.

[25] Song Y, Xu X, Wang W, Tian T, Zhu Z, Yang C. Single cell transcriptomics: moving towards multi-omics. Analyst 2019;144(10):3172–3189. doi:10.1039/c8an01852a, PMID:30849139.

[26] Van Eyk JE, Snyder MP. Precision Medicine: Role of Proteomics in Changing Clinical Management and Care. J Proteome Res 2019;18(1):1–6. doi:10.1021/acs.jproteome.8b00504, PMID:30296097.

[27] Song D, Cui M, Zhao G, Fan Z, Nolan K, Yang Y, *et al*. Pathway-based analysis of breast cancer. Am J Transl Res 2014;6(3):302–311. PMID:24936222.

[28] Hadi NI, Jamal Q, Iqbal A, Shaikh F, Somroo S, Musharraf SG. Serum Metabolomic Profiles for Breast Cancer Diagnosis, Grading and Staging by Gas Chromatography-Mass Spectrometry. Sci Rep 2017;7(1):1715. doi:10.1038/s41598-017-01924-9, PMID:28496143.

[29] Aslebagh R, Channaveerappa D, Pentecost BT, Arcaro KF, Darie CC. Combinatorial Electrophoresis and Mass Spectrometry-Based Proteomics in Breast Milk for Breast Cancer Biomarker Discovery. Adv Exp Med Biol 2019;1140:451–467. doi:10.1007/978-3-030-15950-4_26, PMID:31347064.

[30] Barberini L, Restivo A, Noto A, Deidda S, Fattuoni C, Fanos V, *et al*. A gas chromatography-mass spectrometry (GC-MS) metabolomic approach in human colorectal cancer (CRC): the emerging role of monosaccharides and amino acids. Ann Transl Med 2019;7(23):727. doi:10.21037/atm.2019.12.34, PMID:32042743.

[31] Ang CS, Baker MS, Nice EC. Mass Spectrometry-Based Analysis for the Discovery and Validation of Potential Colorectal Cancer Stool Biomarkers. Methods Enzymol 2017;586:247–274. doi:10.1016/bs.mie.2016.10.019, PMID:28137566.

[32] Rho JH, Ladd JJ, Li CI, Potter JD, Zhang Y, Shelley D, *et al*. Protein and glycomic plasma markers for early detection of adenoma and colon cancer. Gut 2018;67(3):473–484. doi:10.1136/gutjnl-2016-312794, PMID:27821646.

[33] Gao Z, Wang J, Bai Y, Bao J, Dai E. Identification and Verification of the Main Differentially Expressed Proteins in Gastric Cancer via iTRAQ Combined with Liquid Chromatography-Mass Spectrometry. Anal Cell Pathol (Amst) 2019;2019:5310684. doi:10.1155/2019/5310684, PMID:31886120.

[34] Lian G, Wei C, Wang D, Cui M, Wang Z, Liu X, *et al*. Protein profiling of Helicobacter pylori-associated gastric cancer. Am J Pathol 2014;184(5):1343–1354. doi:10.1016/j.ajpath.2014.01.027, PMID: 24589339.

[35] He L, Ye F, Qu L, Wang D, Cui M, Wei C, *et al*. Protein profiling of alpha-fetoprotein producing gastric adenocarcinoma. Oncotarget 2016;7(19):28448–28459. doi:10.18632/oncotarget.8571, PMID:270 57629.

[36] Tong W, Ye F, He L, Cui L, Cui M, Hu Y, *et al*. Serum biomarker panels for diagnosis of gastric cancer. Onco Targets Ther 2016;9:2455–2463. doi:10.2147/OTT.S86139, PMID:27217769.

[37] López-Cortés R, Vázquez-Estévez S, Fernández JÁ, Núñez C. Proteomics as a Complementary Technique to Characterize Bladder Cancer. Cancers (Basel) 2021;13(21):5537. doi:10.3390/cancers13215537,

PMID:34771699.

[38] Chen YT, Huang HC, Hsieh YJ, Fu SH, Li L, Chen CL, *et al*. Targeting amine- and phenol-containing metabolites in urine by dansylation isotope labeling and liquid chromatography mass spectrometry for evaluation of bladder cancer biomarkers. J Food Drug Anal 2019;27(2):460–474. doi:10.1016/j.jfda.2018.11.008, PMID:30987717.

[39] Hu J, Ye F, Cui M, Lee P, Wei C, Hao Y, *et al*. Protein Profiling of Bladder Urothelial Cell Carcinoma. PLoS One 2016;11(9):e0161922. doi:10.1371/journal.pone.0161922, PMID:27626805.

[40] Nedjadi T, Albarakati N, Benabdelkamel H, Masood A, Alfadda AA, Al-Maghrabi J. Proteomic Profiling of Plasma-Derived Biomarkers in Patients with Bladder Cancer: A Step towards Clinical Translation. Life (Basel) 2021;11(12):1294. doi:10.3390/life11121294, PMID:34947825.

[41] Gouin KH 3rd, Ing N, Plummer JT, Rosser CJ, Ben Cheikh B, Oh C, *et al*. An N-Cadherin 2 expressing epithelial cell subpopulation predicts response to surgery, chemotherapy and immunotherapy in bladder cancer. Nat Commun 2021;12(1):4906. doi:10.1038/s41467-021-25103-7, PMID:34385456.

[42] Sewell DA, Yuan CX, Robertson E. Proteomic signatures in laryngeal squamous cell carcinoma. ORL J Otorhinolaryngol Relat Spec 2007;69(2):77–84. doi:10.1159/000097406, PMID:17127822.

[43] Chen W, Ye F, Cui M, Sikora AG, Wang X, Wang P, *et al*. Protein marker profiling in different T classification in laryngeal squamous cell carcinoma. Head Neck 2015;37(3):357–365. doi:10.1002/hed.23607, PMID:24753215.

[44] Pan J, Zheng QZ, Li Y, Yu LL, Wu QW, Zheng JY, *et al*. Discovery and Validation of a Serologic Autoantibody Panel for Early Diagnosis of Esophageal Squamous Cell Carcinoma. Cancer Epidemiol Biomarkers Prev 2019;28(9):1454–1460. doi:10.1158/1055-9965.EPI-18-1269, PMID:31239266.

[45] Zhao R, Tian L, Zhao B, Sun Y, Cao J, Chen K, *et al*. FADS1 promotes the progression of laryngeal squamous cell carcinoma through activating AKT/mTOR signaling. Cell Death Dis 2020;11(4):272. doi:10.1038/s41419-020-2457-5, PMID:32323698.

[46] Haendel MA, Chute CG, Bennett TD, Eichmann DA, Guinney J, Kibbe WA, *et al*. The National COVID Cohort Collaborative (N3C): Rationale, design, infrastructure, and deployment. J Am Med Inform Assoc 2021;28(3):427–443. doi:10.1093/jamia/ocaa196, PMID:32805036.

[47] Yuan X, Xu J, Hussain S, Wang H, Gao N, Zhang L. Trends and Prediction in Daily New Cases and Deaths of COVID-19 in the United States: An Internet Search-Interest Based Model. Explor Res Hypothesis Med 2020;5(2):1–6. doi:10.14218/ERHM.2020.00023, PMID:32348380.

[48] Feng G, Zhang L, Wang K, Chen B, Xia HH-X. Research, Development and Application of COVID-19 Vaccines: Progress, Challenges, and Prospects. J Explor Res Pharmacol 2021;6(2):31–43. doi:10.14218/JERP.2021.00004.

[49] Chen Z, Zhang L. Meet the Challenges of Mass Vaccination against COVID-19. Exploratory Research and Hypothesis in Medicine 2021;6(2):77–79. doi:10.14218/ERHM.2020.00075.

[50] Ray S, Srivastava S. COVID-19 Pandemic: Hopes from Proteomics and Multiomics Research. OMICS 2020;24(8):457–459. doi:10.1089/omi.2020.0073, PMID:32427517.

[51] Bojkova D, Klann K, Koch B, Widera M, Krause D, Ciesek S, *et al*. Proteomics of SARS-CoV-2-infected host cells reveals therapy targets. Nature 2020;583(7816):469–472. doi:10.1038/s41586-020-2332-7, PMID:32408336.

[52] Sánchez-Pacheco SJ, Kong S, Pulido-Santacruz P, Murphy RW, Kubatko L. Median-joining network analysis of SARS-CoV-2 genomes is neither phylogenetic nor evolutionary. Proc Natl Acad Sci USA 2020;117(23):12518–12519. doi:10.1073/pnas.2007062117, PMID: 32381733.

[53] Forster P, Forster L, Renfrew C, Forster M. Phylogenetic network analysis of SARS-CoV-2 genomes. Proc Natl Acad Sci USA 2020;117(17):9241–9243. doi:10.1073/pnas.2004999117, PMID:32269081.

[54] Ramazi S, Zahiri J. Posttranslational modifications in proteins: resources, tools and prediction methods. Database (Oxford) 2021;2021:baab012. doi:10.1093/database/baab012, PMID:33826699.

[55] Mirzaei H, Carrasco M. Modern Proteomics-Sample Preparation, Analysis and Practical Applications. Berlin/Heidelburg: Springer; 2016.

[56] Dressler FF, Brägelmann J, Reischl M, Perner S. Normics: Proteomic Normalization by Variance and Data-Inherent Correlation Structure. Mol Cell Proteomics 2022;21(9):100269. doi:10.1016/j.mcpro.2022.100269, PMID:35853575.

[57] Gokce E, Shuford CM, Franck WL, Dean RA, Muddiman DC. Evaluation of normalization methods on GeLC-MS/MS label-free spectral counting data to correct for variation during proteomic workflows. Journal of the American Society for Mass Spectrometry 2011;22(12):2199–208. doi:10.1007/s13361-011-0237-2, PMID:21952779.

[58] Karpievitch YV, Taverner T, Adkins JN, Callister SJ, Anderson GA, Smith RD, *et al*. Normalization of peak intensities in bottom-up MS-based proteomics using singular value decomposition. Bioinformatics 2009;25(19):2573–2580. doi:10.1093/bioinformatics/btp426, PMID:19602524.

[59] Li Y, Kuhn M, Zukowska-Kasprzyk J, Hennrich ML, Kastritis PL, O'Reilly FJ, *et al*. Coupling proteomics and metabolomics for the unsupervised identification of protein-metabolite interactions in Chaetomium thermophilum. PLoS One 2021;16(7):e0254429. doi:10.1371/journal.pone.0254429, PMID:34242379.

[60] Bittremieux W, Meysman P, Martens L, Valkenborg D, Laukens K. Unsupervised Quality Assessment of Mass Spectrometry Proteomics Experiments by Multivariate Quality Control Metrics. Journal of proteome research 2016;15(4):1300–1307. doi:10.1021/acs.jproteome.6b00028, PMID:26974716.

[61] Andreev VP, Gillespie BW, Helfand BT, Merion RM. Misclassification Errors in Unsupervised Classification Methods. Comparison Based on the Simulation of Targeted Proteomics Data. J Proteomics Bioinform 2016;S14:005. doi:10.4172/jpb.S14-005, PMID:27524871.

[62] Feng CH, Disis ML, Cheng C, Zhang L. Multimetric feature selection for analyzing multicategory outcomes of colorectal cancer: random forest and multinomial logistic regression models. Lab Invest 2022;102(3):236–244. doi:10.1038/s41374-021-00662-x, PMID:26974716.

[63] Deng F, Huang J, Yuan X, Cheng C, Zhang L. Performance and efficiency of machine learning algorithms for analyzing rectangular biomedical data. Lab Invest 2021;101(4):430–431. doi:10.1038/s41374-020-00525-x, PMID:33574440.

[64] Zhang SR, Shan YC, Jiang H, Liu JH, Zhou Y, Zhang LH, *et al*. The Null-Test for peptide identification algorithm in Shotgun proteomics. J Proteomics 2017;163:118–125. doi:10.1016/j.jprot.2017.05.010, PMID:28506863.

[65] Goeminne LJ, Gevaert K, Clement L. Peptide-level Robust Ridge Regression Improves Estimation, Sensitivity, and Specificity in Data-dependent Quantitative Label-free Shotgun Proteomics. Mol Cell Proteomics 2016;15(2):657–668. doi:10.1074/mcp.M115.055897, PMID:26566788.

[66] Granholm V, Noble WS, Käll L. A cross-validation scheme for machine learning algorithms in shotgun proteomics. BMC Bioinformatics 2012;13(Suppl 16):S3. doi:10.1186/1471-2105-13-S16-S3, PMID:23176259.

[67] Becnel LB, McKenna NJ. Minireview: progress and challenges in proteomics data management, sharing, and integration. Mol Endocrinol 2012;26(10):1660–1674. doi:10.1210/me.2012-1180, PMID:22902541.

[68] Kolesnikov N, Hastings E, Keays M, Melnichuk O, Tang YA, Williams E, *et al*. ArrayExpress update—simplifying data submissions. Nucleic Acids Res 2015;43(Database issue):D1113–D1116. doi:10.1093/nar/gku1057, PMID:25361974.

[69] Deutsch EW, Bandeira N, Perez-Riverol Y, Sharma V, Carver JJ, Mendoza L, *et al*. The ProteomeXchange consortium at 10 years: 2023 update. Nucleic Acids Res 2023;51(D1):D1539–D1548. doi:10.1093/nar/gkac1040, PMID:36370099.

[70] Perez-Riverol Y, Bai J, Bandla C, García-Seisdedos D, Hewapathirana S, Kamatchinathan S, *et al*. The PRIDE database resources in 2022: a hub for mass spectrometry-based proteomics evidences. Nucleic Acids Res 2022;50(D1):D543–D552. doi:10.1093/nar/gkab1038, PMID:34723319.

[71] Watanabe Y, Yoshizawa AC, Ishihama Y, Okuda S. The jPOST Repository as a Public Data Repository for Shotgun Proteomics. Methods in molecular biology (Clifton, NJ) 2021;2259:309–22. doi:10.1007/978-1-0716-1178-4_20, PMID:33687724.

[72] Ma J, Chen T, Wu S, Yang C, Bai M, Shu K, *et al*. iProX: an integrated

[73] Perez-Riverol Y, Csordas A, Bai J, Bernal-Llinares M, Hewapathirana S, Kundu DJ, *et al*. The PRIDE database and related tools and resources in 2019: improving support for quantification data. Nucleic Acids Res 2019;47(D1):D442–D450. doi:10.1093/nar/gky1106, PMID:30395289.

[74] Jarnuczak AF, Vizcaino JA. Using the PRIDE Database and ProteomeXchange for Submitting and Accessing Public Proteomics Datasets. Curr Protoc Bioinformatics 2017;59:1–12. doi:10.1002/cpbi.30, PMID:28902400.

[75] Piñero J, Rodriguez Fraga PS, Valls-Margarit J, Ronzano F, Accuosto P, Lambea Jane R, *et al*. Genomic and proteomic biomarker landscape in clinical trials. Comput Struct Biotechnol J 2023;21:2110–2118. doi:10.1016/j.csbj.2023.03.014, PMID:36968019.

[76] Christians U, Klawitter J, Klawitter J. Biomarkers in Transplantation—Proteomics and Metabolomics. Ther Drug Monit 2016;38(Suppl 1):S70–S74. doi:10.1097/FTD.0000000000000243, PMID:26418702.

[77] Dogan A. Advances in clinical applications of tissue proteomics: opportunities and challenges. Expert Rev Proteomics 2014;11(5):531–533. doi:10.1586/14789450.2014.953062, PMID:25187078.

[78] Wright I, Van Eyk JE. A Roadmap to Successful Clinical Proteomics. Clin Chem 2017;63(1):245–247. doi:10.1373/clinchem.2016.254664, PMID:27864386.

[79] Solier C, Langen H. Antibody-based proteomics and biomarker research-current status and limitations. Proteomics 2014;14(6):774–83. doi:10.1002/pmic.201300334, PMID:24520068.

[80] Sauerbrei W, Taube SE, McShane LM, Cavenagh MM, Altman DG. Reporting Recommendations for Tumor Marker Prognostic Studies (REMARK): An Abridged Explanation and Elaboration. J Natl Cancer Inst 2018;110(8):803–811. doi:10.1093/jnci/djy088, PMID:29873743.

[81] Yang Y, Qiao L. Data-independent acquisition proteomics methods for analyzing post-translational modifications. Proteomics 2023;23(7-8):e2200046. doi:10.1002/pmic.202200046, PMID:36036492.

[82] Lee JM, Hammárén HM, Savitski MM, Baek SH. Control of protein stability by post-translational modifications. Nat Commun 2023;14(1):201. doi:10.1038/s41467-023-35795-8, PMID:36639369.

[83] Savitski MM, Zinn N, Faelth-Savitski M, Poeckel D, Gade S, Becher I, *et al*. Multiplexed Proteome Dynamics Profiling Reveals Mechanisms Controlling Protein Homeostasis. Cell 2018;173(1):260–274.e25. doi:10.1016/j.cell.2018.02.030, PMID:29551266.

[84] Edsjo A, Russnes HG, Lehtio J, Tamborero D, Hovig E, Stenzinger A, *et al*. High-throughput molecular assays for inclusion in personalised oncology trials - State-of-the-art and beyond. J Int Med 2024;295(6):785–803. doi:10.1111/joim.13785, PMID:38698538.

[85] Smit NPM, Ruhaak LR, Romijn FPHTM, Pieterse MM, van der Burgt YEM, Cobbaert CM. The Time Has Come for Quantitative Protein Mass Spectrometry Tests That Target Unmet Clinical Needs. J Am Soc Mass Spectrom 2021;32(3):636–647. doi:10.1021/jasms.0c00379, PMID:33522792.

[86] Gastman B, Agarwal PK, Berger A, Boland G, Broderick S, Butterfield LH, *et al*. Defining best practices for tissue procurement in immuno-oncology clinical trials: consensus statement from the Society for Immunotherapy of Cancer Surgery Committee. J Immunother Cancer 2020;8(2):e001583. doi:10.1136/jitc-2020-001583, PMID:33199512.

[87] Keskin O, Tuncbag N, Gursoy A. Predicting Protein-Protein Interactions from the Molecular to the Proteome Level. Chem Rev 2016;116(8):4884–909. doi:10.1021/acs.chemrev.5b00683, PMID:27074302.

[88] Xu L, Lu Z, Yu S, Li G, Chen Y. Quantitative global proteome and phosphorylome analyses reveal potential biomarkers in kidney cancer. Oncol Rep 2021;46(5):237. doi:10.3892/or.2021.8188, PMID:34528699.

[89] Vora DS, Kalakoti Y, Sundar D. Computational Methods and Deep Learning for Elucidating Protein Interaction Networks. Methods in molecular biology (Clifton, NJ) 2023;2553:285–323. doi:10.1007/978-1-0716-2617-7_15, PMID:36227550.

[90] Kustatscher G, Grabowski P, Schrader TA, Passmore JB, Schrader M, Rappsilber J. Co-regulation map of the human proteome enables identification of protein functions. Nat Biotechnol 2019;37(11):1361–1371. doi:10.1038/s41587-019-0298-5, PMID:31690884.

[91] Öztürk M, Freiwald A, Cartano J, Schmitt R, Dejung M, Luck K, *et

al. Proteome effects of genome-wide single gene perturbations. Nat Commun 2022;13(1):6153. doi:10.1038/s41467-022-33814-8, PMID:36257942.

[92] Katona B, Lindskog C. The Human Protein Atlas and Antibody-Based Tissue Profiling in Clinical Proteomics. Methods Mol biol (Clifton, NJ) 2022;2420:191–206. doi:10.1007/978-1-0716-1936-0_15, PMID:34905175.

[93] Wingren C. Antibody-Based Proteomics. Adv Exp Med Biol 2016;926:163–179. doi:10.1007/978-3-319-42316-6_11, PMID:27686812.

[94] Hu A, Zhang J, Shen H. Progress in Targeted Mass Spectrometry (Parallel Accumulation-Serial Fragmentation) and Its Application in Plasma/Serum Proteomics. Methods Mol biol (Clifton, NJ) 2023;2628:339–352. doi:10.1007/978-1-0716-2978-9_22, PMID:36781796.

[95] Li X, Wang W, Chen J. Recent progress in mass spectrometry proteomics for biomedical research. Sci Chin Life Sci 2017;60(10):1093–1113. doi:10.1007/s11427-017-9175-2, PMID:29039124.

[96] Gallien S, Domon B. Advances in high-resolution quantitative proteomics: implications for clinical applications. Expert Rev Proteomics 2015;12(5):489–498. doi:10.1586/14789450.2015.1069188, PMID:26189960.

[97] Xiao Q, Zhang F, Xu L, Yue L, Kon OL, Zhu Y, *et al*. High-throughput proteomics and AI for cancer biomarker discovery. Adv Drug Delivery Rev 2021;176:113844. doi:10.1016/j.addr.2021.113844, PMID:34182017.

[98] Deng F, Zhou H, Lin Y, Heim JA, Shen L, Li Y, *et al*. Predict multicategory causes of death in lung cancer patients using clinicopathologic factors. Comp Biol Med 2021;129:104161. doi:10.1016/j.compbiomed.2020.104161.

[99] Hristova VA, Chan DW. Cancer biomarker discovery and translation: proteomics and beyond. Expert Rev Proteomics 2019;16(2):93–103. doi:10.1080/14789450.2019.1559062, PMID:30556752.

[100] Low TY, Mohtar MA, Ang MY, Jamal R. Connecting Proteomics to Next-Generation Sequencing: Proteogenomics and Its Current Applications in Biology. Proteomics 2019;19(10):e1800235. doi:10.1002/pmic.201800235, PMID:30431238.

[101] Nesvizhskii AI. Proteogenomics: concepts, applications and computational strategies. Nat Methods 2014;11(11):1114–1125. doi:10.1038/nmeth.3144, PMID:25357241.

[102] Cao X, Sun S, Xing J. A Massive Proteogenomic Screen Identifies Thousands of Novel Peptides From the Human "Dark" Proteome. Mol Cell Proteomics 2024;23(2):100719. doi:10.1016/j.mcpro.2024.100719, PMID:38242438.

[103] Mobadersany P, Yousefi S, Amgad M, Gutman DA, Barnholtz-Sloan JS, Velázquez Vega JE, *et al*. Predicting cancer outcomes from histology and genomics using convolutional networks. Proc Natl Acad Sci U S A 2018;115(13):E2970–E2979. doi:10.1073/pnas.1717139115, PMID:29531073.

[104] Gonzalez CG, Zhang L, Elias JE. From mystery to mechanism: can proteomics build systems-level understanding of our gut microbes? Expert Rev Proteomics 2017;14(6):473–476. doi:10.1080/14789450.2017.1311211, PMID:28335651.

[105] Deng F, Shen L, Wang H, Zhang L. Classify multicategory outcome in patients with lung adenocarcinoma using clinical, transcriptomic and clinico-transcriptomic data: machine learning versus multinomial models. Am J Cancer Res 2020;10(12):4624–4639. PMID:33415023.

[106] Spitzer MH, Nolan GP. Mass Cytometry: Single Cells, Many Features. Cell 2016;165(4):780–791. doi:10.1016/j.cell.2016.04.019, PMID:27153492.

[107] Verdier J, Fayet OM, Hemery E, Truffault F, Pinzón N, Demeret S, *et al*. Single-cell mass cytometry on peripheral cells in Myasthenia Gravis identifies dysregulation of innate immune cells. Front Immunol 2023;14:1083218. doi:10.3389/fimmu.2023.1083218, PMID:36793723.

[108] Deng J, Zeng X, He C, Zhong D, Wu Y, Liu N, *et al*. Exploring the Accumulation Behavior and Heterogeneity of Perfluorooctanesulfonic Acid in Zebrafish Primary Organ Cells by Single-Cell Mass Cytometry. Anal Chem 2023;95(37):13750–13755. doi:10.1021/acs.analchem.3c02398, PMID:37669419.

[109] Ha MK, Kwon SJ, Choi JS, Nguyen NT, Song J, Lee Y, *et al*. Mass Cytometry and Single-Cell RNA-seq Profiling of the Heterogeneity in Human Peripheral Blood Mononuclear Cells Interacting with Silver Nanopar-

ticles. Small 2020;16(21):e1907674. doi:10.1002/smll.201907674, PMID:32163679.

[110] Perkel JM. Single-cell proteomics takes centre stage. Nature 2021;597(7877):580–582. doi:10.1038/d41586-021-02530-6, PMID:34545225.

[111] Ghaffari S, Saleh M, Akbari B, Ramezani F, Mirzaei HR. Applications of single-cell omics for chimeric antigen receptor T cell therapy. Immunology 2024;171(3):339–364. doi:10.1111/imm.13720, PMID:38009707.

[112] Funingana IG, Bedia JS, Huang YW, Delgado Gonzalez A, Donoso K, Gonzalez VD, *et al*. Multiparameter single-cell proteomic technologies give new insights into the biology of ovarian tumors. Semin Immunopathol 2023;45(1):43–59. doi:10.1007/s00281-022-00979-9, PMID:36635516.

[113] Setayesh SM, Ndacayisaba LJ, Rappard KE, Hennes V, Rueda LYM, Tang G, *et al*. Targeted single-cell proteomic analysis identifies new liquid biopsy biomarkers associated with multiple myeloma. NPJ Precis Oncol 2023;7(1):95. doi:10.1038/s41698-023-00446-0, PMID:37723227.

[114] Li ZY, Huang M, Wang XK, Zhu Y, Li JS, Wong CCL, *et al*. Nanoliter-Scale Oil-Air-Droplet Chip-Based Single Cell Proteomic Analysis. Anal Chem 2018;90(8):5430–5438. doi:10.1021/acs.analchem.8b00661, PMID:29551058.

[115] Zhu Y, Piehowski PD, Zhao R, Chen J, Shen Y, Moore RJ, *et al*. Nanodroplet processing platform for deep and quantitative proteome profiling of 10-100 mammalian cells. Nat Commun 2018;9(1):882. doi:10.1038/s41467-018-03367-w, PMID:29491378.

[116] Ctortecka C, Hartlmayr D, Seth A, Mendjan S, Tourniaire G, Udeshi ND, *et al*. An Automated Nanowell-Array Workflow for Quantitative Multiplexed Single-Cell Proteomics Sample Preparation at High Sensitivity. Mol Cell Proteomics 2023;22(12):100665. doi:10.1016/j.mcpro.2023.100665, PMID:37839701.

[117] Krieg C, Nowicka M, Guglietta S, Schindler S, Hartmann FJ, Weber LM, *et al*. High-dimensional single-cell analysis predicts response to anti-PD-1 immunotherapy. Nat Med 2018;24(2):144–153. doi:10.1038/nm.4466, PMID:29309059.

[118] Bensaddek D, Nicolas A, Lamond AI. Signal enhanced proteomics: a biological perspective on dissecting the functional organisation of cell proteomes. Curr Opin Chem Biol 2019;48:114–22. doi:10.1016/j.cbpa.2018.10.011, PMID:30551035.

[119] Huang W, Zhan D, Li Y, Zheng N, Wei X, Bai B, *et al*. Proteomics provides individualized options of precision medicine for patients with gastric cancer. Sci China Life Sci 2021;64(8):1199–1211. doi:10.1007/s11427-021-1966-4, PMID:34258712.

[120] Forler S, Klein O, Klose J. Individualized proteomics. J Proteomics 2014;107:56–61. doi:10.1016/j.jprot.2014.04.003, PMID:24732725.

[121] Sengupta S, Sun SQ, Huang KL, Oh C, Bailey MH, Varghese R, *et al*. Integrative omics analyses broaden treatment targets in human cancer. Genome Med 2018;10(1):60. doi:10.1186/s13073-018-0564-z, PMID:30053901.

[122] Cui M, Zhang DY. Artificial intelligence and computational pathology. Lab Invest 2021;101(4):412–422. doi:10.1038/s41374-020-00514-0, PMID:33454724.

[123] Ramón Y, Cajal S, Hümmer S, Peg V, Guiu XM, De Torres I, Castellvi J, *et al*. Integrating clinical, molecular, proteomic and histopathological data within the tissue context: tissunomics. Histopathology 2019;75(1):4–19. doi:10.1111/his.13828, PMID:30667539.

[124] Grapov D, Fahrmann J, Wanichthanarak K, Khoomrung S. Rise of Deep Learning for Genomic, Proteomic, and Metabolomic Data Integration in Precision Medicine. OMICS 2018;22(10):630–636. doi:10.1089/omi.2018.0097, PMID:30124358.

[125] Granter SR, Beck AH, Papke DJ Jr. AlphaGo, Deep Learning, and the Future of the Human Microscopist. Arch Pathol Lab Med 2017;141(5):619–621. doi:10.5858/arpa.2016-0471-ED, PMID:28470900.

[126] Iqbal MJ, Javed Z, Sadia H, Qureshi IA, Irshad A, Ahmed R, *et al*. Clinical applications of artificial intelligence and machine learning in cancer diagnosis: looking into the future. Cancer Cell Int 2021;21(1):270. doi:10.1186/s12935-021-01981-1, PMID:34020642.

[127] Oveland E, Muth T, Rapp E, Martens L, Berven FS, Barsnes H. Viewing the proteome: how to visualize proteomics data? Proteom-

ics 2015;15(8):1341–1355. doi:10.1002/pmic.201400412, PMID: 25504833.

[128] Swan AL, Mobasheri A, Allaway D, Liddell S, Bacardit J. Application of machine learning to proteomics data: classification and biomarker identification in postgenomics biology. OMICS 2013;17(12):595–610. doi:10.1089/omi.2013.0017, PMID:24116388.

[129] Rashidi HH, Tran NK, Betts EV, Howell LP, Green R. Artificial Intelligence and Machine Learning in Pathology: The Present Landscape of Supervised Methods. Acad Pathol 2019;6:2374289519873088. doi:10.1177/2374289519873088, PMID:31523704.

[130] La Porta CAM, Zapperi S. Explaining the dynamics of tumor aggressiveness: At the crossroads between biology, artificial intelligence and complex systems. Semin Cancer Biol 2018;53:42–47. doi:10.1016/j.semcancer.2018.07.003, PMID:30017637.

[131] Kelchtermans P, Bittremieux W, De Grave K, Degroeve S, Ramon J, Laukens K, *et al*. Machine learning applications in proteomics research: how the past can boost the future. Proteomics 2014;14(4-5):353–366. doi:10.1002/pmic.201300289, PMID:24323524.

[132] Rafay A, Aziz M, Zia A, Asif AR. Automated Retrieval of Heterogeneous Proteomic Data for Machine Learning. J Pers Med 2023;13(5):790. doi:10.3390/jpm13050790, PMID:37240960.

[133] Mathivanan S. Integrated Bioinformatics Analysis of the Publicly Available Protein Data Shows Evidence for 96% of the Human Proteome. J Proteomics Bioinform 2014;7(2):41–49. doi:10.4172/0974-276X.1000301.

[134] Ludwig C, Gillet L, Rosenberger G, Amon S, Collins BC, Aebersold R. Data-independent acquisition-based SWATH-MS for quantitative proteomics: a tutorial. Mol Syst Biol 2018;14(8):e8126. doi:10.15252/msb.20178126, PMID:30104418.

[135] Kuster B, Tushaus J, Bayer FP. A new mass analyzer shakes up the proteomics field. Nat Biotechnol 2024. doi:10.1038/s41587-024-02129-y, PMID:38302752.

[136] Guzman UH, Martinez-Val A, Ye Z, Damoc E, Arrey TN, Pashkova A, *et al*. Ultra-fast label-free quantification and comprehensive proteome coverage with narrow-window data-independent acquisition. Nat Biotechnol 2024. doi:10.1038/s41587-023-02099-7.

[137] Dorey A, Howorka S. Nanopore DNA sequencing technologies and their applications towards single-molecule proteomics. Nat Chem 2024;16(3):314–334. doi:10.1038/s41557-023-01322-x, PMID:38448507.

[138] Strauss MT, Bludau I, Zeng WF, Voytik E, Ammar C, Schessner JP, *et al*. AlphaPept: a modern and open framework for MS-based proteomics. Nat Commun 2024;15(1):2168. doi:10.1038/s41467-024-46485-4, PMID:38461149.

[139] Delafield DG, Lu G, Kaminsky CJ, Li L. High-end ion mobility mass spectrometry: A current review of analytical capacity in omics applications and structural investigations. TrAC Trends in Analytical Chemistry 2022;157:116761. doi:10.1016/j.trac.2022.116761.

[140] Vasilopoulou CG, Sulek K, Brunner AD, Meitei NS, Schweiger-Hufnagel U, Meyer SW, *et al*. Trapped ion mobility spectrometry and PASEF enable in-depth lipidomics from minimal sample amounts. Nat Commun 2020;11(1):331. doi:10.1038/s41467-019-14044-x, PMID:31949144.

[141] Mun DG, Budhraja R, Bhat FA, Zenka RM, Johnson KL, Moghekar A, *et al*. Four-dimensional proteomics analysis of human cerebrospinal fluid with trapped ion mobility spectrometry using PASEF. Proteomics 2023;23(10):e2200507. doi:10.1002/pmic.202200507, PMID:36752121.

[142] Chen M, Zhu P, Wan Q, Ruan X, Wu P, Hao Y, *et al*. High-Coverage Four-Dimensional Data-Independent Acquisition Proteomics and Phosphoproteomics Enabled by Deep Learning-Driven Multidimensional Predictions. Anal Chem 2023;95(19):7495–7502. doi:10.1021/acs.analchem.2c05414, PMID:37126374.

[143] Lockyer NP, Aoyagi S, Fletcher JS, Gilmore IS, van der Heide PA, Moore KL, *et al*. Secondary ion mass spectrometry. Na Rev Methods Primers 2024;4(1):32. doi:10.1038/s43586-024-00311-9.

[144] Petrosius V, Aragon-Fernandez P, Üresin N, Kovacs G, Phlairaharn T, Furtwängler B, *et al*. Exploration of cell state heterogeneity using single-cell proteomics through sensitivity-tailored data-independent acquisition. Nat Commun 2023;14(1):5910. doi:10.1038/s41467-023-41602-1, PMID:37737208.

[145] Stone JA, van der Gugten JG. Quantitative tandem mass spectrometry in the clinical laboratory: Regulation and opportunity for validation of laboratory developed tests. J Mass Spectrom Adv Clin Lab 2023;28:82–90. doi:10.1016/j.jmsacl.2023.03.001, PMID:36937811.

[146] Caldera JR, Gray HK, Garner OB, Yang S. FDA trial regulation of laboratory developed tests (LDTs): An academic medical center's experience with Mpox in-house testing. J Clin Virol 2023;169:105611. doi:10.1016/j.jcv.2023.105611, PMID:37866092.

[147] Miller MB, Watts ML, Samuel L. FDA's proposed rule for the regulation of laboratory-developed tests. J Clin Microbiol 2024;62(2):e0148823. doi:10.1128/jcm.01488-23, PMID:38206042.