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Integrative proteomics and metabolomics advance early cancer detection and targeted therapy with emerging technologies and clinical applications

Shayesteh Shakarami[1], Nisreen M. Saleh[2], Farshad Zare[3], Raed Fanoukh-Aboqader-Al-Aouadi[4], Nemat Allaberdiyev[5], Tulkin Buzrukov[6], Natrayan Lakshmaiya[7], Ibadulla Abdullaev[8*] and Mehrdad Nourizadeh[9]

*Correspondence:
Ibadulla Abdullaev
abdullayev_ibodulla@mamunedu.uz

Full list of author information is available at the end of the article

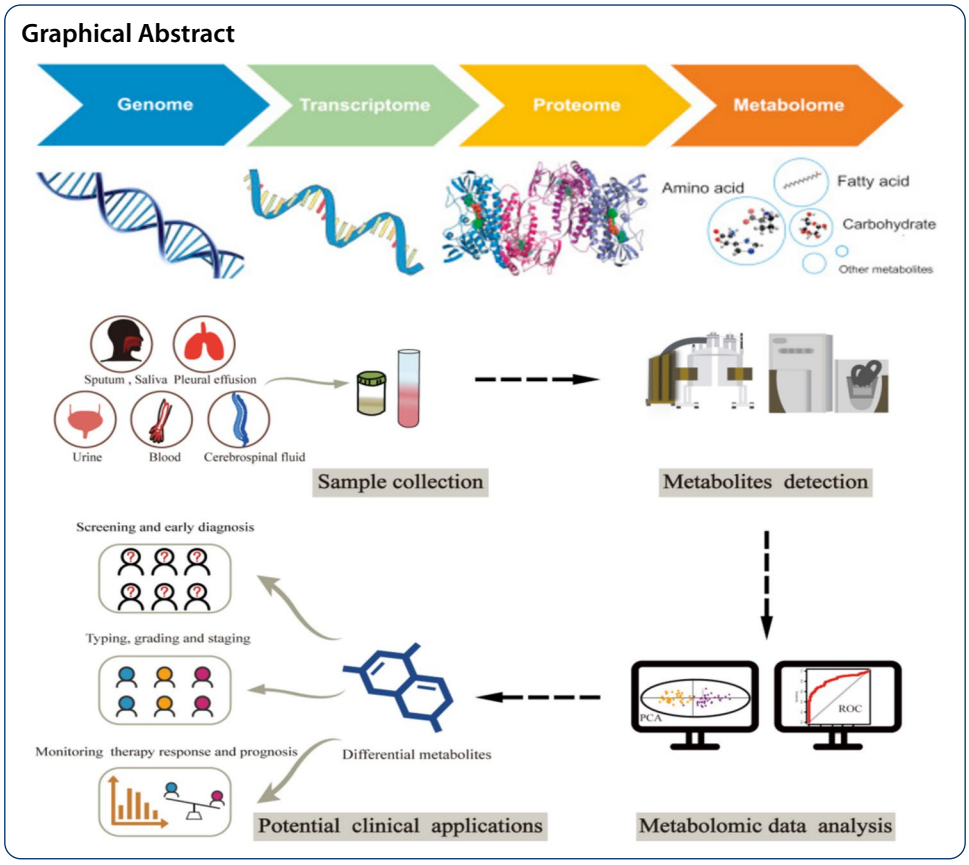
Abstract

Early detection and personalized therapy remain central challenges in modern oncology. Recent advancements in proteomics and metabolomics have unlocked powerful strategies for identifying molecular alterations associated with cancer onset, progression, and therapeutic response. Proteomics, employing high-resolution mass spectrometry and integrative bioinformatics, facilitates the discovery of differentially expressed proteins in blood and tissue samples, enabling early diagnostic and prognostic assessments. Metabolomics, on the other hand, profiles low-molecular-weight metabolites to capture dynamic biochemical changes. This review focuses primarily on high-performance liquid chromatography-mass spectrometry (HPLC–MS) as the primary method for metabolomic analysis, while also acknowledging the role of additional techniques such as nuclear magnetic resonance (NMR) and gas chromatography-mass spectrometry (GC–MS) in the broader landscape of metabolomics. By integrating these complementary approaches, we provide a comprehensive systems-level view of cancer biology that links protein function to metabolic networks. The altered proteomic and metabolomic signatures highlight robust biomarkers crucial for early screening, disease monitoring, and therapeutic stratification. This review discusses recent breakthroughs in these technologies, emphasizing their integrated role in enhancing cancer diagnostics and precision oncology.

Keywords Proteomics, Metabolomics, Cancer, Biomarker

1 Introduction

Cancer remains one of the most significant health challenges facing societies worldwide. In the United States, it accounts for one in five deaths each year, while globally, mortality rates range from 100 to 350 per 100,000 individuals affected by this disease [1, 2]. Cancer usually occurs due to a malfunction in the mechanisms that regulate cell growth and division. These malfunctions are mainly caused by genetic damage caused by chemicals,



hormones, and sometimes viruses. Therefore, cancer occurs when the mechanisms responsible for controlling and stabilizing cell growth are disrupted [3–5]. One of the most significant advancements in the field is the development of sophisticated analytical techniques such as mass spectrometry (MS) and high-performance liquid chromatography (HPLC), which enable the identification and quantification of metabolites and proteins with remarkable accuracy [6, 7]. These methods assist researchers in identifying specific metabolic and protein profiles within biological samples from cancer patients, thereby serving as potential biomarkers for early cancer diagnosis [8].

In light of a saturated field of multi-omics studies, this review specifically narrows its focus to metabolomics and proteomics due to several key factors that underscore their clinical maturity and applicability. Both disciplines are supported by advanced instrumentation, robust workflows, and interpretative frameworks that enhance their role in clinical settings. For instance, established proteomic platforms are widely accessible, enabling high-throughput analysis, while metabolomic assays demonstrate portability and rapid turnaround times that are crucial for clinical diagnostics. In addition, the use of bioinformatics technologies and machine learning algorithms in the analysis of metabolomics and proteomics data is rapidly expanding [9]. These tools help researchers to identify complex patterns and investigate the relationships between metabolites and proteins in different cancer contexts [10]. For example, the identification of new biomarkers through the analysis of metabolic and protein profiles can help predict response to treatment and determine the specific molecular profiles of each type of cancer [11].

Recent advances in proteomics and metabolomics have accelerated the development of targeted and personalized cancer therapies [14]. By profiling proteins and metabolites associated with tumor initiation and progression, researchers can design treatments that precisely target disease-specific molecular signatures. This strategy enhances therapeutic efficacy, minimizes off-target effects, and improves patient quality of life [15, 16]. Cancer often arises from mutations in three key gene classes: proto-oncogenes, tumor suppressor genes, and caretaker genes. Proto-oncogenes promote normal cell growth but can become oncogenes when mutated, driving uncontrolled proliferation [17, 18]. Tumor suppressor genes act as growth inhibitors, and their inactivation removes critical cell-cycle regulation. Caretaker genes, which maintain genomic integrity by repairing DNA damage, do not directly trigger tumorigenesis but their loss increases mutation rates across the genome. The combined dysregulation of these gene types contributes to cancer development, and their molecular signatures (when integrated with proteomic and metabolomic data) offer powerful targets for precision oncology [19, 20].

The emergence of genomics, proteomics, metabolomics, lipidomics, and transcriptomics underscores the need for a more integrated approach to investigate the pathogenesis of human diseases. Although each discipline is an independent field of study, recent research highlights how they complement and enhance one another. Proteomics emphasizes protein expression and function, whereas metabolomics focuses on the end products of cellular processes. Their synergy provides a comprehensive understanding of disease states by linking enzymatic functions to metabolic outcomes. This review seeks to articulate the selection criteria for focusing on proteomics and metabolomics while highlighting their maturity in the context of other omics disciplines (Fig. 1).

2 Advances in metabolomics

Metabolomics, as a valuable technique for studying in various fields including medicine, is considered one of the important achievements of the latest developments in science and technology [12]. In 1999, Professor Nicholson first proposed the concept of metabolomics. Metabolomics measures the end products of cellular metabolism (downstream products of the genome) and environmental changes in a biological system [10, 13]. Therefore, it is possible to identify metabolites derived from genes (endogenous) and changes caused by the environment (exogenous), which indicates the interaction

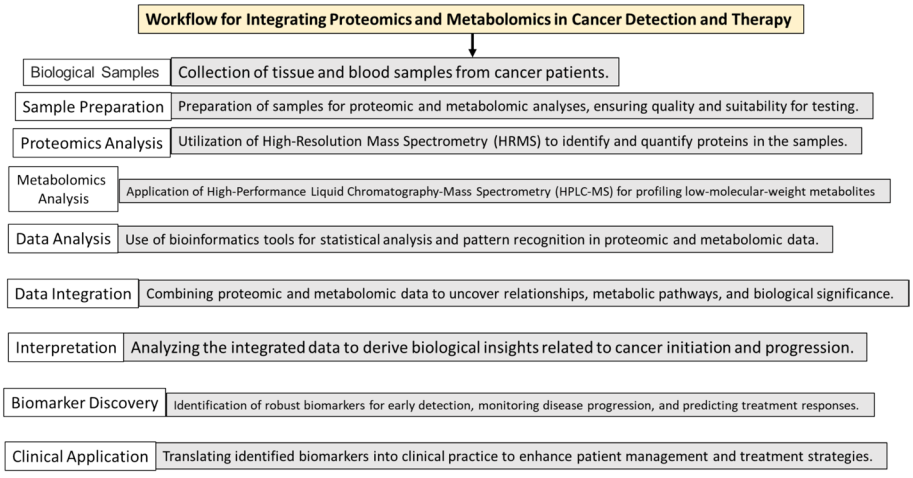


Fig. 1 Framework for multi-omics integration in cancer detection and therapy

between genes and the environment [14]. In other words, metabolomics analyzes metabolites with small molecular masses, typically less than 1.5 kDa, such as glucose, cholesterol, adenosine triphosphate, biogenic amine neurotransmitters, and lipid signaling molecules in body fluids and tissues [15, 16]. The study of metabolomics is enhanced when integrated with interdisciplinary techniques such as biochemistry, analytical chemistry, bioinformatics, and metabolic biology. Metabolomics is defined as the comprehensive measurement of small molecular metabolites resulting from the end products of gene expression in a biological organism [17].

Metabolomics employs both untargeted and targeted approaches, each with its unique strengths and limitations. The untargeted approach can be further divided into fingerprinting and profiling strategies. Metabolic fingerprinting allows for the assessment of the overall metabolic state of a cell, tissue, or organism without predetermined knowledge of specific metabolites. This approach is particularly useful for comparative analyses, such as identifying changes in metabolite levels between control and treatment groups. However, it is essential to address potential confounders, including batch effects, matrix effects, and normalization strategies, as these can significantly influence the reliability of results. The sensitivity and specificity required for clinical applications necessitate rigorous validation to ensure that observed metabolic differences are indeed indicative of disease states [18–20]. Conversely, metabolic profiling offers a more targeted analysis by quantifying specific metabolites and pathways within a biological sample, which can facilitate accurate disease diagnosis. Despite its advantages, this approach typically measures a smaller fraction of metabolites compared to fingerprinting, particularly when considering biological matrices like urine. While metabolic profiling can identify alterations associated with diseases, it faces similar challenges regarding sample handling and analytical variability, which can impede clinical validation [21, 22].

The targeted approach focuses on pre-defined metabolites or pathways associated with specific diseases. This method is effective for assessing the impact of drugs on disease processes or evaluating the effects of treatments or genetic modifications on particular enzymes [23, 24]. However, while it can provide precise insights into metabolic alterations, the limitation lies in its reliance on a predefined selection of metabolites, potentially overlooking other relevant metabolic changes that might provide additional insights into disease mechanisms [25, 26]. In the context of oncology, the application of metabolomic techniques must be critically evaluated to ensure clinical relevance. Understanding the strengths and limitations of each approach is crucial for their advancement and application in personalized medicine. For instance, while metabolic fingerprinting can facilitate disease classification, the clinical utility may be limited without rigorous validation and standardization protocols. On the other hand, targeted metabolomic studies may yield actionable insights into therapeutic responses but require comprehensive context regarding the underlying biological complexity [27]. By employing a critical analysis of these methodologies and their implications in the clinical setting, we can enhance the translational impact of metabolomics in oncology, ultimately paving the way for more effective diagnostic and therapeutic strategies. The process of metabolism is regulated by transcriptional mechanisms that ultimately lead to the production of metabolites [28].

The comprehensive study of metabolites, referred to as metabolomics, increases our understanding of cellular activities and changes in metabolic profiles. Metabolites

play important roles in biological systems and can be considered an attractive option for diagnosing disease phenotypes. Metabolomics, by providing a comprehensive profile of metabolites, serves as a valuable tool for disease diagnosis, the identification of new therapeutic targets, and the development of innovative treatment strategies [29]. Metabolites, including amino acids, sugars, nucleotides, lipids, and organic acids, perform various functions in a biological system [30]. These metabolites play a role in energy production, macromolecule synthesis, and cell signaling. It is currently controversial how many primary metabolites are present in biological systems. However, studies suggest that yeast contains a few hundred metabolites, humans contain thousands, and plants contain hundreds of thousands [31, 32]. The Human Metabolome Database records more than 114,000 metabolites, including peptides, lipids, amino acids, nucleic acids, carbohydrates, and organic acids, even at relatively low concentrations [33, 34]. Metabolites can be influenced by both internal and external factors, thus reflecting the true health status of an individual [35] (Fig. 2).

3 Advances in proteomics

Proteomics refers to the study and investigation of the proteome of an organism on a large scale. The proteome refers to the set of proteins expressed from the genome of an organism [17]. Proteins control the phenotype of an organism and form a vital part of biological systems that play a fundamental role in the metabolic and physiological pathways of cells [37]. The genome is largely consistent across all human cells and remains relatively stable throughout an organism's lifespan. In contrast, the proteins within a cell are far more abundant and vary significantly across different cell types and during the cell's lifetime [38–40]. While the genome comprises approximately 20,000 to 25,000

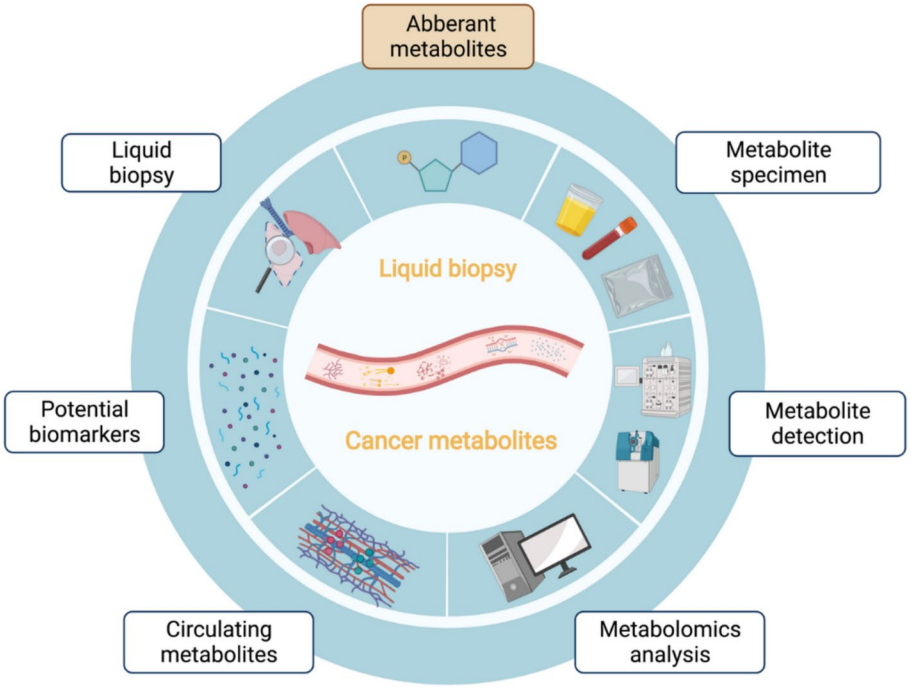


Fig. 2 Summary of overall content: the identification of abnormal metabolites in cancer, alongside advancements in high-throughput metabolomics, suggests that circulating metabolites may serve as promising biomarkers for the early detection and diagnosis of cancer in the context of liquid biopsy [36]

genes, the transcriptome includes around 100,000 transcripts, and the proteome contains over 1 million proteins [38–40]. For the example, Recent advancements in thermal proteome profiling and high-definition mass spectrometry have shed light on the modulation of cholesterol biosynthesis by next-generation galeterone analog VNPP433-3β in castration-resistant prostate cancer [41].

Defective proteins are known to be the main factors in the development of cancer and are therefore important markers for the diagnosis and treatment of this disease. In addition, proteins are the main targets for many drugs and form the basis for the design of various drugs [37, 42]. Additionally, findings demonstrate that SYVN1 mediates the ubiquitination and subsequent proteasomal degradation of MNK1/2, the only known kinases responsible for phosphorylating eIF4E. Phosphorylation of eIF4E is a rate-limiting step in the formation of the eIF4F translation initiation complex. The degradation of MNK1/2 by VNLG-152R and its analogs impedes dysregulated translation in triple-negative breast cancer (TNBC) cells, resulting in the inhibition of tumor growth [43]. Therefore, the study of proteomics is very useful for understanding their role in the development and control of cancer [44]. Proteomics can provide a deeper understanding of cells and the activities of living organisms. It focuses on studying the structure and function of the proteome, isoforms, conformational changes, post-transcriptional and translational modifications (such as phosphorylation and glycosylation) [45–47], and interactions with other proteins and drugs. It can also be used to compare mutant proteins with their normal counterparts. Proteomics is a more intricate and effective method than genomics due to the variability in proteome expression across different cells and at varying times [42, 48]. In proteomics, electrophoresis is usually used to separate proteins. This method allows proteins to be separated based on their charge and mass (isoelectric point and molecular weight) [49]. In the next steps, changes in expression and amino acid sequences that lead to the creation of new isoforms or post-transcriptional and translational modifications such as phosphorylation, glycosylation, conjugation and acetylation are examined [50].

One of the important applications of proteomics is in the design of new drugs by identifying and analyzing the cell proteome [51]. By identifying proteins associated with cancer, they can be targeted and drugs designed using computer software can be used [50, 52]. For instance, if a specific protein is implicated in cancer, understanding its three-dimensional structure can facilitate the design of an effective drug to inhibit its activity. Given that individuals possess unique genetic information, variations in protein expression will also occur among different people [53]. Therefore, one of the applications of proteomics is that by determining the proteome of each individual, a more specific drug can be designed to effectively treat each person [54, 55] (Fig. 3).

4 Advantages of metabolomics over other diagnostic methods

In recent years, the use of metabolomics in clinical studies has been expanding and is considered a complementary technique to genomics, transcriptomics, and proteomics [56]. Metabolomics analyzes the activity of small molecules less than 15 kDa resulting from cellular metabolism that cannot be identified by genomics and proteomics [57]. mRNA expression data and proteomic analyses alone do not fully capture the cellular dynamics; in contrast, metabolomics offers a comprehensive insight into the cell's physiological state. Additionally, metabolomics can reveal how the metabolic profile of

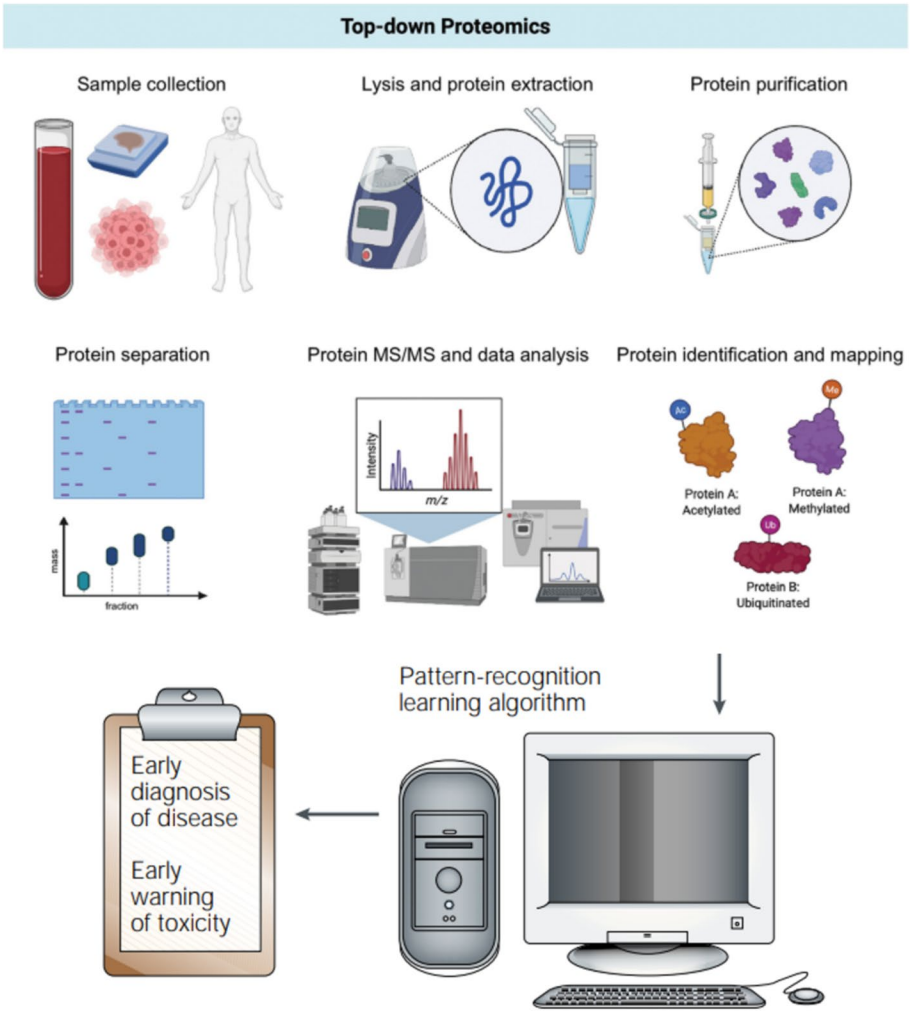


Fig. 3 Proteomics techniques can identify proteins based on their expression levels, as well as elucidate protein–protein interactions and post-translational modifications (PTMs), which are valuable for understanding the development of diseases

a complex biological system adapts in response to stressors, including harmful agents, environmental shifts, or physiological alterations [58]. This technique also requires small samples for diagnosis. Therefore, metabolomics can be used to identify the metabolome of cells in different conditions, such as for therapeutic purposes. In addition, this technology has several other advantages over other omics [59, 60]. Genomics/transcriptomics analyzes the first changes in protein levels caused by a specific disease. Because changes in gene expression and changes in protein levels are controlled by homeostatic systems, changes in gene levels cause changes in metabolite levels [61]. As a result, metabolite analysis provides more comprehensive information than other omics. Another advantage of metabolomics is the detection of changes in body fluids such as urine and blood and its non-invasiveness. In addition, because the number of metabolites is lower than that of genes and proteins [62, 63]. Significant changes in metabolites are caused by disease, which leads to their rapid identification [64].

5 Metabolomics-based analytical techniques

Advanced, accurate, and high-resolution spectroscopic techniques are essential for metabolomics studies. Today, nuclear magnetic resonance spectroscopy (NMR), spectroscopy coupled with liquid chromatography (LC) or gas chromatography (GC) are the three most common analytical methods in metabolomics [32]. In general, NMR has several advantages over MS, including high reproducibility, easy metabolite identification, and non-destructive analysis. However, its low resolution and sensitivity prevent the determination of metabolites at the millimolar level [35]. Many extensive epidemiological studies have shown the ability to quantify 50 to 70 metabolite peaks and more than 200 metabolic measurements, including ratios of these metabolite peaks, using NMR as part of routine practice [65].

NMR does not require careful sample preparation to accurately determine all compounds present in biological fluids, cell extracts, and tissues, and it allows the identification of compounds with the same mass, including compounds with different isotopomer distributions. Furthermore, NMR is highly reproducible, enabling large-scale metabolomics studies with higher throughput than LC–MS or GC–MS. NMR is also capable of detecting compounds such as sugars, organic acids, alcohols, lipoproteins, and inorganic ions [66–68]. NMR is also suitable for studying tissues, organs, and other solid or semi-solid samples. In contrast, LC–MS and GC–MS-based methods cannot be used for the analysis of living samples due to their inherently destructive nature. GC–MS is a versatile analytical technique used in metabolomics studies due to its high resolution, selectivity, sensitivity, and reproducibility, and the availability of mass spectral databases [69, 70]. Advantages of GC–MS include ease of use for analysis and low operating cost. However, the main prerequisite for GC–MS analysis is that the compound must be volatile and thermally stable [71]. Because most metabolites in physiological fluids are polar and nonvolatile, they cannot be directly analyzed by GC–MS without sample preparation. Therefore, metabolomic profiling by GC–MS usually requires modification of the polar functional group of the molecule to reduce polarity, increase thermal stability, and increase the volatility of the analytes [72, 73].

The challenges associated with LC–MS should be clarified, particularly in differentiating discovery and targeted modalities. While targeted LC–MS is indeed a quantitative method widely employed in clinical settings, it is essential to recognize that its effectiveness relies heavily on the implementation of appropriate controls. In contrast, discovery-based LC–MS techniques may not provide the same level of quantitation precision; they often focus on identifying a wide range of metabolites without standardized measurements. For targeted analyses, such as label-free and data-independent acquisition (DIA), semi-quantitative and quantitative results can successfully be achieved when proper controls are in place. However, in targeted LC–MS workflows, some limitations persist: absolute quantitation can be challenging due to the necessity of calibration curves and standards for each metabolite of interest. Conversely, relative quantitation might succeed in detecting changes in metabolite levels but may not reflect true concentrations unless carefully calibrated. Overall, while LC–MS offers diverse quantitation capabilities across different modalities, the specific approach dictates its strengths and limitations [74].

6 Application of metabolomics in disease diagnosis

Most diseases are one or more factors driven by a combination of genetic and environmental factors, resulting in a wide range of phenotypes [75]. Downstream of all genetic transcription and translation processes, metabolites are low molecular weight molecules (less than 1.5 kDa) that reflect the interaction between the genome, transcription, translation, and metabolism [76]. Metabolomics allows the simultaneous identification of thousands of biomarkers (including different metabolites) as indicators of biological processes, pathological processes, or drug responses to a therapeutic intervention [77]. Identification of disease-specific metabolic changes by metabolomics increases physicians' understanding of the pathophysiological mechanisms of the disease, which can help improve disease and prescribe treatment [78]. Diabetes is accompanied by significant changes in metabolic processes. Identification of prediabetic individuals with depleted β-cell mass is essential for the prevention of diabetes. However, it is difficult to detect ß-cell defects in vivo without early symptoms [79].

Metabolomics is a powerful tool that can detect the onset of disease before clinical manifestations [80]. Several studies have shown that a number of metabolites, such as choline acid (CA), chenodeoxycholic acid (CDCA), taurocholic acid (TCA), glycocholic acid (GCA), taurochenodeoxycholic acid (TCDCA), taurine, acylcarnitine, 2-exoglutarate, alanine, leucine, succinate, 3-hydroxybutyrate, betaine, allantoin, acetate, dimethylamine, creatine, creatinine, glucose, phenylacetylglycine, and hippurate, differ between healthy and diabetic subjects by analyzing serum and urine samples from diabetic patients and diabetic rats [81–83]. Cancer fundamentally alters cellular metabolism, making metabolomics a crucial tool for early diagnosis and therapeutic interventions. In clinical settings, various biomarkers are analyzed to predict disease prognosis. For instance, in breast cancer, increased levels of choline-containing compounds, primarily due to elevated phosphocholine, alongside decreased glycerophosphocholine and low glucose levels, have been associated with malignant tumors compared to benign tissues or healthy controls. Additionally, the overexpression of the glycolytic isoenzyme M2-type pyruvate kinase (M2-PK) has been documented in tumor tissues [84].

Thyroid cancer is the most common endocrine disorder. Compared with benign specimens, there are elevated levels of lactate, taurine, and lower levels of several lipids in the malignant group [85]. Another study showed that metabolites such as cholesterol, dihomo-gamma-linolenic acid, sphingosines (3-O-methylsphingosine, threosphingosine, 5-O-methylsphingosine and erythrosphingosine) are altered in men and women with thyroid cancer [86, 87]. Also, in hypothyroidism in mice, maximal intestinal citrate synthase activity and decreased glutarate dehydrogenase have been observed [88]. The liver is an important metabolic organ in the body that performs multiple functions, including the elimination of toxic substances, regulation of carbohydrate, protein, amino acid and lipid metabolism [89].

In recent studies, the analysis of urine and serum metabolic profiles using liquid chromatography-quadrupole time-of-flight mass spectrometry (LC-QTOF-MS) has revealed significant abnormalities in metabolites such as tryptophan, valine, leucine, isoleucine, and citrate cycle components, as well as sphingolipid and glycerophospholipid metabolites in liver fibrosis. These findings suggest potential early biomarkers for liver fibrosis diagnosis and indicate possible therapeutic targets [90]. Furthermore, research on mouse models of liver carcinoma has reported significant alterations in metabolic

profiles, including changes in glucose, lactate, choline, lipids, glycine, alanine, lysine, and aspartate within tumor tissues [91, 92]. Lung cancer remains one of the leading causes of mortality globally, with a dismal prognosis largely due to late-stage diagnoses [79, 93]. Increased levels of glucose, glutamine, glycerol, (iso)leucine, N-acetylated glycoproteins, threonine and valine and decreased levels of lactate and alanine have been reported in malignant cells [94]. In another study, metabolic changes observed in lung cancer included alterations in glycine, serine, threonine metabolism, oxidative stress pathways and aldarate metabolism, methionine and cysteine metabolism, glutamate metabolism, nucleotide metabolism (purine metabolism), and inflammation (leukotriene metabolism) [95] (Table 1).

7 Biomarkers

In recent years, much attention has been paid to the role of biomarkers in cancer diagnosis at different stages of clinical studies [96]. Recently, special attention was paid to proteomics and its place in the diagnosis and treatment of diseases, and a field called clinical proteomics was established. Biomarkers are important tools for the follow-up and study of cancer [97]. The use of proteomics for cancer diagnosis was first performed on ovarian cancer [98]. More than two-thirds of patients with this type of cancer become aware of their disease in advanced stages; because this cancer does not have specific symptoms until it reaches the malignant stage. However, if the diagnosis is made at an early stage (stage 1), the patient's chance of survival increases to more than 5 years [79, 98, 99]. Considering the recent advances in the field of proteomics, it has become possible to identify biomarkers in different types of cancers, as well as to examine the function and structure of proteins [100].

These advances in proteomics and genomics have led to the identification of a wide range of clinically valuable biomarkers. Understanding these biomarkers can help determine the stage of the disease and provide specific treatments [101]. Biomarkers are not only important in the timely diagnosis of cancer and monitoring the progression of the disease, but are also considered as evaluable factors in human populations. These biological molecules reflect the physiological state of an individual [55, 102]. Gene mutations and changes that occur in the transcription and translation processes of proteins can be defined as biomarkers of cancer. Changes in the serum proteome that occur following the progression of cancer can be considered as biomarkers of that cancer [103]. For cancer analysis, the identification of a biomarker alone does not provide sufficient information. It can also be valuable to pay attention to changes in the expression levels of different proteins [104]. Two-dimensional electrophoresis and spectroscopy are among the common methods used in the proteomic study of biomarkers [105, 106]. Mass spectrometry (MS) is recognized as a suitable method for analyzing biological data, especially in the field of cancer [107].

The high accuracy of this technique in identifying biomarkers is remarkable. Among the methods used in this field, MALDI-TOF–MS is a powerful tool for protein and peptide analysis and is capable of identifying changes even in very small proteins [91]. Given the importance of proteomics in these studies, advances in genomics, epigenetics, transcriptome, and metabolomics have also played an effective role in the identification of biomarkers, in addition to proteomics [83, 108]. Another important issue in the identification of biomarkers is the investigation of the best ones for the study and diagnosis of

Table 1 Previously reported biomarkers identified by proteomics and other methodologies

Disease	Differential metabolites	Biological pathways/mechanisms	Instrument/modality	Assay type	Study design
Hepatocellular carcinoma (HCC)	Elevated: bile acids (e.g. taurocholic acid), phosphatidylethanolamine, triglycerides; decreased: phosphatidylcholine, fatty acids	Alterations in lipid metabolism and bile acid synthesis	UHPLC-Q Exactive MS	Lipidomics	Integrated multiomics
Liver cancer (general)	Changes in amino acids (e.g. tryptophan, valine, leucine, isoleucine), citrate, sphingolipids, glycerophospholipids, lipids, glucose, lactate, choline	Disrupted glycolysis, TCA cycle, amino acid and lipid metabolism	LC–MS, GC–MS	Lipid profiling	Comprehensive metabolic profiling
Lung adenocarcinoma	Elevated: oxalate, glycolate, glycine, glyceric acid, aminomalonic acid, creatinine; decreased: phosphate, proline, serine, methionine	One-carbon metabolism (serine/glycine), glycolysis, TCA cycle, inflammation pathways	LC–MS	Untargeted metabolomics	Machine learning integration
Thyroid cancer	Altered levels of lactate, taurine, cholesterol, dihomo γ-linolenic acid, sphingosines (3-O-methylsphingosine, erythrosphingosine), citrate, glutarate	Fatty acid and sphingolipid metabolism, energy metabolism shifts	LC–MS	Targeted metabolomics	Metabolic disruption analysis
Breast cancer	Elevated: choline (phosphocholine), glycerophosphocholine, M2-PK enzyme; altered glucose metabolism	Altered membrane synthesis and glycolytic flux (Warburg effect)	NMR, LC–MS	Glycolyticenzyme assays	Tumor analysis using biomarkers

the disease, and the most reliable ones should be investigated [109, 110].Only 12 types of biomarkers for different cancers have been approved by the FDA, and therefore there is a need for additional studies using other techniques to distinguish the accuracy of biomarker detection from proteins of healthy, precancerous, and malignant cells. A list of some of the biomarkers identified in different cancers is given in Table 2 [80, 111, 112].

Table 2 Names of some biomarkers identified by proteomics and other methods

Biomarker	Cancer type(s)	Detection method	Source/ proteomics discovery	Clinical relevance
CA-125	Ovarian cancer	Serum immunoassay	Widely established cancer marker	Commonly used in diagnosis and monitoring of ovarian cancer
Carcinoembryonic antigen (CEA)	Ovarian, colorectal, lung, breast cancers	Serum immunoassay	Traditional clinical biomarker	Diagnostic and prognostic marker for multiple cancers
α-Fetoprotein (AFP)	Liver (HCC), testicular, pancreatic cancers	Serum immunoassay	Standard tumor marker	Used in screening and monitoring hepatocellular carcinoma
PSA (Prostate-specific antigen)	Prostate cancer (also hepatoma/testicular)	Serum immunoassay	Classical marker for prostate cancer	Widely used in prostate cancer screening and follow-up
Annexin A1	Prostate and lung cancer	Proteomics (tissue/urine profiling)	Differential expression in tumors	Potential diagnostic/ prognostic marker; associated with aggressive phenotypes
HSP90-β & HSP70	Lung, gastric, astrocytoma, breast, prostate	Proteomics, immunohistochemistry	Identified co-overexpression in cancer tissues	Linked to tumor survival, therapy resistance; potential therapeutic targets
Enolase-1 (α-enolase, ENO1)	Lung, hepatocellular, breast, prostate cancers	Proteomics expression profiling	Overexpressed in multiple tumors	Tumor invasion marker; target for therapy and immunologic detection
UQCRC1, RAC1, GAPDH, PFKP	Lung adenocarcinoma	Quantitative plasma proteomics	High diagnostic performance (AUC > 0.90)	Promising early-screening biomarkers with strong discrimination power
CPM, KRT8, ITIH2, RCN1	Prostate cancer	Urinary proteomics + network analysis	Newly proposed biomarkers via integrated approach	May improve risk stratification beyond PSA
Sarcosine dehydrogenase / Lamin B1	Hepatocellular carcinoma	Literature-reported proteomics	Historically reported markers	Associated with metabolic alteration and chromatin in HCC (legacy data)
PCNA (Proliferating cell nuclear antigen)	Multiple cancers (e.g. esophageal, gastric, prostate)	Proteomics, tissue assays	Marker of cell proliferation and cancer progression	Indicator of tumor growth and aggressiveness

These heat shock protein homologs are found in various cellular compartments, including the cytosol, nucleus, endoplasmic reticulum (ER), and mitochondria, where they carry out distinct functions that aid in tumor survival and proliferation [113, 114]. Key processes mentioned include epithelial-mesenchymal transition (EMT), the unfolded protein response (UPR), oxidative phosphorylation (OXPHOS), and chaperone-mediated autophagy (CMA) [115]. Additionally, they influence complement-dependent cytotoxicity (CDC) and interact with factors such as Bclaf1 (B-cell lymphoma 2-associated transcription factor 1), hypoxia-inducible factor 1 (HIF-1), and microtubule-associated serine/threonine kinase 1 (MAST1) [56, 116].

In conclusion, Clinical validation of biomarkers poses significant challenges, as it requires rigorous testing to ensure reliability and reproducibility across diverse patient populations and clinical settings. This process often demands extensive longitudinal studies, which can be resource-intensive and time-consuming. Regulatory hurdles can

complicate biomarker implementation, as approval from regulatory bodies like the FDA necessitates comprehensive evidence demonstrating that the biomarker is both effective and safe for clinical use. The pathway to regulatory approval is often fraught with uncertainty, and navigating this landscape requires expertise in regulatory compliance and the ability to generate substantial supporting data. Bioethical issues further complicate the biomarker discovery process, particularly regarding informed consent and patient privacy. As biomarkers can involve the analysis of genetic information, there are concerns about potential misuse of data and discrimination based on genetic predispositions. Ensuring that patients fully understand what their participation involves is crucial but can be challenging, especially in the context of complex scientific concepts. Additionally, the equitable access to newly developed biomarkers presents ethical dilemmas, as disparities in healthcare can hinder access among different populations. Addressing these clinical validation challenges, regulatory hurdles, and bioethical issues is essential to ensure that biomarker discovery and implementation align with ethical standards and ultimately improve patient outcomes. Collaboration between researchers, clinicians, regulatory agencies, and ethicists can foster a more comprehensive approach to overcoming these barriers in personalized medicine.

8 Proteomics methods and techniques

The use of proteomics techniques began in the 1970s; however, the term "proteomics" was not commonly used until 1997 [117]. The first use of two-dimensional electrophoresis was by O'Farrel and Klose in 1975, who were able to separate 1100 types of Escherichia coli proteins on separate bands in a gel [118]. Subsequently, the introduction of spectroscopy brought about a significant revolution in the field of proteomics. Numerous methods exist for extracting proteins from tissues and cells [54, 119]. In this regard, many factors are influential in achieving the desired results of proteomic analyses, including the presence of sufficient clinical information and appropriate sampling. If the sampling is performed incorrectly, even the most advanced technologies will not be able to analyze the information obtained, which unfortunately receives less attention [120]. Unlike nucleic acid, which can be prepared in sufficient quantities to examine a single cell using PCR, proteomic methods are limited by the inability to replicate the protein sample required for examination, and require the preparation of a sufficient sample for study [103]. Cancer proteomic research faces challenges because cancer tissues usually contain a mixture of cancer and healthy cells, requiring pure tumor proteins to study the tumor. Body fluids are a suitable source for proteomic analyses, which play an important role in tumor studies due to their easy access and ease of sampling, and also allow for the repetition of experiments [61, 121]. The use of patient body fluids has facilitated the ease of conducting repeated examinations to gain knowledge of the patient's condition [118] (Table 3).

However, due to the presence of proteins such as albumin, the identification of biomarkers in serum can be challenging. For this reason, scientists have developed techniques such as immunoassay extraction to facilitate the process of identifying relevant proteins [85, 119]. Plasma is also a valuable source for proteomic studies and serves as a suitable platform for a variety of body proteins. The Plasma Proteome Project was first initiated in 2002 by the HUPO organization in collaboration with several laboratories and this source is recognized as a suitable alternative to serum [122]. Urine is also

Table 3 Proteomics methods and techniques in cancer research

Method/technique	Primary application	Advantages	Limitations	Sample type
2D Electrophoresis	Protein separation and visualization	High-resolution profiling of complex samples	Requires large protein amounts; low reproducibility; misses hydrophobic proteins	Tissues, cell lysates
Spectroscopy (MS)	Protein identification and quantification	High sensitivity and specificity; sequence identification	Complex data analysis; cost-intensive	Tissues, plasma, serum, urine
Laser Capture Microdissection (LCM)	Isolation of pure cell populations	Minimizes contamination; precise targeting	Time-consuming; requires expertise	Tissue sections
DIGE (2D Difference Gel Electrophoresis)	Comparative protein expression analysis	Simultaneous analysis; high accuracy	Expensive dyes; limited dynamic range	Tissues, cultured cells
SELDI-TOF MS	Serum biomarker discovery	Minimal sample preparation; rapid screening	Low resolution; limited reproducibility	Serum, plasma
Immunoassay Extraction	Enrichment of low-abundance proteins	Improves detection sensitivity	Loss of protein complexity; potential bias	Serum, plasma
ELISA	Protein quantification and validation	High sensitivity and specificity; widely used	Limited to known proteins; single-target focus	Serum, urine
NMR Spectroscopy	Metabolomic and proteomic profiling	Non-destructive; quantitative	Low sensitivity; requires high sample volume	Fluids (e.g., urine, plasma)

considered an important diagnostic source for a variety of diseases, including cancer. By analyzing the proteins in urine, the presence or absence of biomarkers associated with urinary tract cancers and other types of cancer can be detected [123]. Techniques such as ELISA have been used for years to measure the expression of proteins. The first step in proteomic research involves taking a patient sample, and the quality of the sample preparation has a direct impact on the results. Limited human resources have led to the increased use of cell lines in this field [124]. Unlike tissue samples, cell lines are standard and suitable for biological studies, especially proteomics, although this method also faces limitations due to the lack of normal body conditions [125]. The protein composition of lysed cells or tissues should be identified on a two-dimensional electrophoresis gel. Ideally, these proteins should appear completely and unchanged on the gel, and contamination of the sample with unrelated proteins or peptides should be avoided [106]. If the amount of protein in a sample is high, careful preparation becomes even more important, as the concentration of contamination also increases [126].

The method of collecting the protein sample is very important. For example, it is relatively simple to isolate proteins from homogeneous protein solutions such as body fluids or lysed cells; however, when studying tissues, the type of cell to be analyzed must be carefully selected [127]. To avoid changes, the temperature of the sample must be kept very low and the preparation time must be minimized [128, 129]. Although techniques such as SELDI-TOF and spectroscopy are mainly used for serum analysis [106, 130], other methods such as isotopic labeling and reverse-phase protein arrays are also used intermittently [131]. However, the slight inconsistency between the results of these methods limits their use, and challenges such as small patient numbers and lack of standardized sampling methods add to the difficulties [132]. Despite these obstacles, proteomics is recognized as one of the best methods for analyzing and obtaining valuable information about the state of cancer [133]. Recently, there have been advances in

techniques related to two-dimensional electrophoresis that have increased its accuracy [94, 134]. The initial stage of separation is carried out on the basis of charge in an IEF gel and the second stage is carried out on the basis of size in an SDS gel, and finally the stained proteins are scanned on the gel [135]. One of the complementary techniques to electrophoresis is DIGE, which uses fluorescent labeling to easily identify expressed proteins [135, 136]. In the past, the Edman method was used to determine the sequence of proteins, in which the protein sequence was first attached to a side group at the amino terminus [137]. The terminal amino acid is then separated through a chemical reaction, and its identity is determined by comparing the retention time observed in HPLC with standard calibration plots. However, a significant limitation of this method is its speed and low sensitivity, which renders Edman's method less suitable for comprehensive proteomic analyses [104].

9 Integration and computational approaches

Metabolomics, a powerful approach in the realm of cancer research, has significantly advanced due to sophisticated analytical techniques such as nuclear magnetic resonance (NMR), gas chromatography-mass spectrometry (GC–MS), and the recent innovations in high-resolution mass spectrometry (HR-MS) and liquid chromatography (LC). These technologies enable researchers to accurately identify and quantify low-molecular-weight metabolites present in various biofluids and tissues. Particularly in cancer research, the ability to detect metabolic alterations associated with different cancer stages can unveil critical insights that propel biomarker discovery and inform personalized treatment strategies. Recent developments in HR-MS and LC have markedly enhanced the sensitivity, resolution, and throughput of metabolomic studies. For example, HR-MS can achieve parts-per-trillion sensitivity, allowing for the detection of metabolites that are present in exceedingly low concentrations, often characteristic of early cancer stages. This high sensitivity is crucial for identifying potential biomarkers that could lead to earlier diagnosis of cancers or provide insights into treatment efficacy. Integrating proteomics with metabolomics takes these capabilities to the next level [151, 154, 155].

The simultaneous analysis of proteins and metabolites yields a more comprehensive understanding of cancer biology. Consider the case of breast cancer, where researchers have successfully correlated specific metabolic profiles with proteomic signatures to elucidate the tumor microenvironment's metabolic dysregulation. Techniques such as data fusion strategies allow for combining datasets from different omics layers, enabling a holistic view of the underlying biological processes. A compelling example of the effectiveness of integrating proteomics and metabolomics in cancer research can be found in studies examining ovarian cancer. Research utilizing combined platforms revealed unique metabolic signatures associated with different tumor stages, coupled with proteomic data that identified related signaling pathways. For instance, through coordinated analysis, elevated levels of specific amino acids were found to correlate with the expression of certain oncogenic proteins, indicating a metabolic reprogramming essential for cancer progression. Moreover, the integration of these methodologies can address challenges in clinical applications, such as the variability typically seen in individual patient responses to therapy. Case studies have shown how multi-omics approaches can guide personalized treatment options by identifying patients who exhibit specific metabolomic

and proteomic profiles responsive to particular therapies. For example, patients with certain histone modification profiles, identified through proteomics, have been linked to altered metabolic pathways through metabolomic analysis, guiding clinicians in tailoring more effective, individualized treatment plans [151, 154, 155].

Integrating proteomic and metabolomic data allows researchers to correlate protein activity with metabolic pathways, providing a systems-level perspective on cancer progression. Multi-omics platforms, including genomics, transcriptomics, proteomics, and metabolomics, are now being utilized in tandem to identify novel therapeutic targets [156–158]. Although integrating such datasets poses computational and biological challenges, it enhances the precision of biomarker panels and improves our understanding of tumor heterogeneity [159, 160]. However, despite these advancements, the integration of proteomics and metabolomics is not without challenges. Data normalization, variability in sample preparation, and differences in analytical sensitivity can complicate the interpretation of combined datasets. Addressing these challenges requires the development of robust bioinformatics tools capable of managing and integrating large, multidimensional datasets. Successful applications in clinical practice will necessitate ongoing collaboration between analytical chemists, biologists, and clinicians to refine methodologies and translate findings into actionable strategies. The culmination of these efforts lies in the potential to develop comprehensive diagnostic and prognostic tools that leverage both proteomic and metabolomic insights, transforming the landscape of cancer treatment. As the field continues to evolve, it will be imperative to document and publish integrative case studies that not only illustrate the potential of combined omics approaches but also demonstrate their tangible impact on patient outcomes. This ongoing dialogue will ensure that the scientific value of integrated methodologies is continuously reaffirmed and expanded.

10 Application of proteomics in cancer studies

Investigating the applications of proteomics in cancer studies is very challenging due to the complexity of biological systems (each cell produces an average of 10 polypeptides) and requires extensive and comparative research that must be carried out by study groups [103, 132]. One of the applications of proteomics is the identification of biomarkers for early diagnosis of diseases [54]. By comparing the information obtained from the changes in isoforms and various modifications in protein molecules, the status of cancer can be understood. Identifying cancer biomarkers and distinguishing them from isoforms is of great importance [22, 138]. Before the emergence of new techniques such as spectroscopy and bioinformatics to study proteins, the Edman and Beadle and Taum methods were used [139]. Recently, the use of ICAT isotope labels in spectroscopy to measure the amount of proteins has attracted much attention. One of the applications of proteomics is patient monitoring, which can be used to study the process of cancer changes and therapeutic uses and drug design by examining patient serum proteins [22, 74, 140].

One of the goals of cancer therapy is to target glycoprotein proteins that play a key role in metastasis and immune responses [141]. The branching of the sugar chain of glycoproteins in cancer cells usually increases, and this increase in branching causes the connection of these chains with sialic acid, which is recognized and interacted with by lectin [142, 143]. These interactions between sialic acid and lectin provide the ability to

metastasize to cancer cells [144]. Another application of proteomics is in drug design, which uses various sciences such as genomics, proteomics, metabolomics, bioinformatics, X-ray crystallography, synthetic chemistry, pharmacology, microbiology, biotechnology, and molecular medicine. The steps in this process are that first the cancer-causing agent is identified, and this will be possible with the help of genomics, proteomics, and metabolomics techniques [145, 146]. The cancer agent can have significant effects on proteomics, so that this science plays an important role in the analysis of cancer-related proteins [147]. On the other hand, metabolomics can identify biochemical interactions between genes and protein dysfunctions [148]. Therefore, by identifying factors that are effective in the occurrence of cancer, we will be able to design drugs to treat people. Proteins themselves are known to be cancer-causing agents and therefore are suitable targets for drugs. Using bioinformatics, we can design the desired drugs that are first tested on animal models in biochemical and toxicity tests and then tested on humans [149–151].

11 Some limitations of proteomics in diagnosis

Despite the advantages of electrophoresis, this technique also has limitations. One of these limitations is the need for a large amount of protein to use this method, so that small amounts of protein cannot be a reliable source for electrophoresis [133, 152, 153]. In addition, healthy and tumor tissues contain a mixture of different cells, and for the identification of biomarkers and research in this field, we need pure samples containing only a specific cell type to achieve reliable results. In cancer studies, pure samples are essential, and this method cannot completely separate proteins from healthy and diseased cells [154]. Therefore, there is a need for complementary methods such as Laser Capture Microdissection (LCM), which is a precise method for separating pure cells from complex tissues without contact and contamination [155, 156]. Although LCM is time-consuming and difficult, this technique is widely used and allows the harvesting of isolated cells or selected sections of tissue [157]. Because only focused light is involved in the transfer process, this process is completely hands-free [125].

By purifying the cells under study, the problem of tissue purification is solved. In studies such as serum, the presence of abundant proteins such as albumin, which is 10 to 10 times more abundant than other proteins, can cause problems in identifying other proteins [158–160]. This problem is also observed in other tissues; for example, in tissues such as skin and cartilage, the high abundance of glycoproteins prevents access to other proteins [155]. In addition, the time-consuming and expensive nature of this method is another problem. Given that 2D electrophoresis is the main technique of proteomics, it is also important to examine the specific limitations of this method; among its disadvantages is the failure to identify some specific proteins, such as highly acidic and hydrophobic proteins [112, 156, 161]. Also, low reproducibility is another problem with these methods. Despite the disadvantages and limitations mentioned, proteomics studies are considered the best option using complementary techniques to identify proteins associated with diseases [63, 162, 163].

Despite the considerable promise of proteomics and metabolomics in advancing cancer research, several limitations and technical challenges persist that must be addressed. In proteomics, low abundance proteins often pose a significant challenge, as many clinically relevant proteins exist at low concentrations, making them difficult to

Table 4 Advantages, challenges, and complementary roles of proteomics versus metabolomics in advancing cancer research

Aspect	Proteomics	Metabolomics
Advantages	Provides insight into protein expression, function, and interactions Enables identification of disease-specific protein biomarkers High-throughput analysis techniques are available	Captures dynamic biochemical changes in response to environmental factors Profiles low-molecular-weight metabolites, reflecting cellular processes Rapid analysis and high sensitivity
Challenges	Complexity of protein structures and post-translational modifications Sample variability and degradation can affect results Data interpretation is often complex and requires advanced bioinformatics	Metabolite identification can be challenging due to their diverse nature Rapid turnover of metabolites can lead to temporal inconsistencies Limited capacity to provide mechanistic insights compared to proteomics
Complementary Roles	Sheds light on metabolic pathways influenced by protein activity Helps in linking proteomic changes to metabolomic profiles for a holistic understanding of cancer biology	Offers insights into metabolic dysregulations that occur as a result of cancer and influences protein alterations Provides a temporal snapshot of cellular states, complementing the static nature of proteomic data

detect amidst higher concentrations of more abundant proteins. This complexity is further exacerbated by the intricate nature of biological samples, such as blood or tissue, which contain a myriad of proteins, including various isoforms and post-translational modifications. Consequently, distinguishing these components can complicate analysis and interpretation. Additionally, data reproducibility is a notable concern, as variability in sample preparation, instrument performance, and analytical methods can lead to inconsistent results that complicate comparisons across studies. Metabolomics faces its own set of challenges, particularly in the dynamic range of metabolite concentrations, which can widely vary within biological samples. Ensuring accurate quantification across both high and low concentrations necessitates sophisticated techniques. Furthermore, matrix effects in biological samples can interfere with metabolite detection, leading to inaccurate results and emphasizing the need for robust sample preparation methods. As the integration of multi-omics data gains traction, issues related to data normalization and batch effects become increasingly critical. Implementing proper normalization is essential to eliminate systematic biases resulting from variations in sample handling and analytical conditions. Batch effects can introduce variations during the processing of different sample sets, skewing results and hindering consistent conclusions. Moreover, ensuring cross-platform reproducibility is vital, as different technologies may yield divergent results for the same analytes. Establishing standardized experimental protocols can facilitate comparability and enhance findings. Therefore, continuous advances in bioinformatics tools are essential for analyzing complex datasets from both proteomics and metabolomics, while the evolution of mass spectrometry platforms and sample preparation methods will increase robustness and reproducibility in these fields. Addressing these technical issues is crucial for realizing the full potential of proteomics and metabolomics in cancer detection and personalized therapy [63, 162, 163].

The Table 4, presents a comparison of the advantages, challenges, and complementary roles of proteomics and metabolomics in cancer research. Proteomics offers insights into protein expression and biomarker identification but faces complexities related to protein structures and data interpretation. In contrast, metabolomics captures dynamic biochemical changes and reflects cellular processes, though it struggles with metabolite

identification and temporal inconsistencies. Both disciplines contribute valuable information, with proteomics linking changes to metabolic pathways, while metabolomics highlights metabolic dysregulations. Together, they provide a comprehensive understanding of cancer biology and enhance research outcomes.

12 Clinical translation and future directions

As we look ahead, the integration of proteomics and metabolomics will be crucial in shaping the landscape of cancer diagnostics and treatment. This multifaceted approach promises to enhance our ability to develop predictive models that can accurately assess a patient's cancer risk, monitor treatment responses in real-time, and elucidate the underlying mechanisms of drug resistance. The incorporation of advanced analytical techniques such as MALDI-TOF–MS (Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry) [164], LC-QTOF-MS (Liquid Chromatography Quadrupole Time-of-Flight Mass Spectrometry) [165], and SWATH-MS (Sequential Windowed Acquisition of All Theoretical Mass Spectra) is driving innovation in biomarker discovery [166]. These methods not only provide enhanced sensitivity and specificity for detecting a wide array of biomolecules but also facilitate the analysis of complex biological samples with increased throughput [167, 168]. For instance, MALDI-TOF–MS is invaluable for its rapid analysis capabilities in clinical settings, while SWATH-MS allows for comprehensive profiling by capturing a broad spectrum of protein modifications [167, 168].

To translate these promising technologies into routine clinical practice, robust multicenter validation studies are essential. Such studies can establish the reproducibility and reliability of findings across diverse patient populations and clinical environments. Consistency in results will enhance the credibility of identified biomarkers and ensure that they can be effectively utilized in various healthcare settings. By validating findings across multiple institutions, we can establish standardized protocols that maintain high-quality control during analysis. Additionally, the development of standardized analytical pipelines is key to ensuring the successful implementation of proteomic and metabolomic technologies in clinical settings. These pipelines should include well-defined workflows and quality assurance measures that can be easily adopted by different laboratories. They will facilitate the reliable processing, analysis, and interpretation of complex data, ultimately leading to significant advancements in personalized medicine. Close collaboration among researchers, clinicians, and data scientists will also be vital in this endeavor. This interdisciplinary approach will foster knowledge exchange and the pooling of resources, ensuring that the latest research findings are effectively integrated into clinical practice. By working together, experts from various fields can identify unmet clinical needs, develop targeted interventions, and optimize patient care strategies based on individual biomarker profiles [167, 168].

The ultimate goal is to harness the full potential of systems biology, which offers a holistic view of cancer as a complex system rather than isolated components. By integrating insights from proteomics, metabolomics, genomics, and transcriptomics, we can create comprehensive models that accurately reflect tumor heterogeneity and behavioral dynamics. These models will enable more precise predictions regarding treatment efficacy, facilitate the identification of optimal therapeutic regimens, and enhance our understanding of individual responses to therapy. In summary, the future directions in

cancer research lie in the seamless integration of proteomics and metabolomics within clinical frameworks. By leveraging advanced technologies, validating findings across multiple centers, developing standardized methodologies, and fostering collaboration, we can push the boundaries of personalized cancer treatment. The successful application of these strategies promises to not only improve patient outcomes but also usher in a new era of cancer care that is responsive to the unique biological landscapes of individual patients [63, 166, 167].

13 Discussion and conclusion

Proteomics is widely regarded as an essential technique in oncology research, playing a crucial role in prevention, early diagnosis, and the development of targeted therapies through drug design [169]. In many cases, cancer is diagnosed at advanced stages and the patient realizes his disease when the malignancy has reached advanced stages. Therefore, proteomics increases the chances of treatment and the use of effective methods by enabling timely diagnosis of malignancy [98]. The information obtained from proteomics includes details about the intracellular processes that control cell division and differentiation or information about apoptosis in normal cells and factors that cause dysfunction of healthy cells and the initiation of cancer [73]. The methods used in proteomics need to be improved to be more efficient. One of the prospects of proteomics is to increase the volume and quality of extracted proteins from a sample and reduce the amount of proteins to be analysed [170]. Also, the use of automation (meaning the use of tools such as robots to replace humans), more efficient software, and complementary techniques with higher sensitivity and specificity can provide greater accuracy for diagnosing cancer malignancy [108, 171]. Moreover, metabolomics is a crucial technique in research, as it plays a pivotal role in early disease diagnosis, identifying disease mechanisms, and monitoring treatment outcomes [172]. In fact, metabolomics as a screening method can help diagnose the disease by identifying changes in metabolomic profiles related to each disease before the phenotype or occurrence of the disease is observed [49]. Metabolomics focuses on the function of metabolites and related metabolic enzymes in the first stage. Secondly, in-vivo and in-vitro experiments are performed to confirm the metabolomics findings, thus increasing the confidence in the metabolomics study [173]. In recent years, the progress of metabolomics in cancer and various metabolic diseases has been investigated, and the development of this science is also predicted in the future. However, the use of this method or its greater efficiency requires the development of advanced software and complementary techniques with greater sensitivity and specificity [174, 175]. Also, using this method will reduce the treatment costs of the individual and the healthcare organization, whereas if the disease progresses, the treatment becomes more difficult and the cost also increases. Therefore, early diagnosis of diseases will greatly help the individual and the medical community in diagnosing and treating the disease [176].

Abbreviations

AFP	Alpha-fetoprotein
AUC	Area under the curve
Bclaf1	B-cell lymphoma-2–associated transcription factor 1
CA	Cholic Acid
CA 125	Cancer antigen 125
CMA	Chaperone-mediated autophagy
CDC	Complement-dependent cytotoxicity

CEA	Carcinoembryonic antigen
CDCA	Chenodeoxycholic acid
DIA	Data-independent acquisition
DIGE	Difference gel electrophoresis
eIF4E	Eukaryotic Initiation Factor 4E
EMT	Epithelial–mesenchymal transition
EN01	Alpha-enolase
ER	Endoplasmic reticulum
FDA	U.S. Food and Drug Administration
GC	Gas Chromatography
GC–MS	Gas Chromatography–Mass Spectrometry
GCA	Glycocholic acid
GSCs	Glioblastoma stem cells
HCC	Hepatocellular carcinoma
HIF-1	Hypoxia-inducible factor 1
HPLC	High-performance liquid chromatography
HPLC–MS	High-Performance Liquid Chromatography–Mass Spectrometry
HR-MS	High-Resolution Mass Spectrometry
HSPCs	Hematopoietic Stem/Progenitor Cells
HSP	Heat Shock Protein
IEF	Isoelectric Focusing
iPSCs	Induced pluripotent stem cells
ITIH2	Inter-Alpha-Trypsin Inhibitor Heavy Chain H2
KRT8	Keratin 8
LC	Liquid chromatography
LC–MS	Liquid chromatography–mass spectrometry
LC-QTOF-MS	Liquid Chromatography Quadrupole Time-of-Flight Mass Spectrometry
LCM	Laser capture microdissection
M2-PK	M2-type pyruvate kinase
MALDI-TOF–MS	Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry
MAST1	Microtubule-Associated Serine/Threonine Kinase 1
MNK1/2	MAP Kinase-Interacting Kinases 1 and 2
MS	Mass spectrometry
NMR	Nuclear magnetic resonance
OXPHOS	Oxidative phosphorylation
PCNA	Proliferating cell nuclear antigen
PE	Phosphatidylethanolamine
PFKP	Phosphofructokinase, platelet type
PTMs	Post-translational modifications
RCN1	Reticulocalbin-1
SELDI-TOF MS	Surface-enhanced laser desorption/ionization time-of-flight mass spectrometry
SDS	Sodium dodecyl sulfate
SWATH-MS	Sequential window acquisition of all theoretical mass spectra
TCA cycle	Tricarboxylic acid cycle
TCA	Taurocholic acid
TCDCA	Taurochenodeoxycholic acid
TNBC	Triple-negative breast cancer
UPR	Unfolded protein response
UHPLC	Ultra-high-performance liquid chromatography
UQCRC1	Ubiquinol-cytochrome c reductase core protein 1
VEGF	Vascular endothelial growth factor

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Author details
[1]School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
[2]College of Dentistry, University of Al Maarif, Al Anbar 31001, Iraq
[3]Student Research Committee, School of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
[4]College of Medicine , Al-Ayen Iraqi University, AUIQ, An Nasiriyah, Iraq
[5]Department of Surgical Diseases No.2, Samarkand State Medical University, Samarkand, Uzbekistan
[6]Department of Medicine, Termez University of Economics and Service, Termez, Uzbekistan
[7]Department of Research and Innovation, Saveetha School of Engineering, SIMATS, Chennai 602105, Tamilnadu, India
[8]Department of General Professional Subjects, Mamun University, Khiva, Uzbekistan
[9]Neurosciences Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

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