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Title: Advances in Mass Spectrometry for the Screening and Validation of Colorectal Cancer Biomarkers: A Review

Ruiyang Shi*

Institute of Pharmacy, Nantong University, Nantong, China

**Corresponding author: Ruiyang Shi.*

Abstract

Colorectal cancer is a malignant tumor with persistently high incidence and mortality rates around the world. Early detection of colorectal cancer is essential to improving survival, yet current diagnostic tools show limited performance. Traditional screening methods, such as fecal occult blood testing, serum biomarker detection, and colonoscopy, have limitations in sensitivity, specificity, or patient compliance, making it difficult to meet the demands of large-scale early screening. Colorectal cancer, due to its high throughput and high sensitivity, enables the acquisition of much information in a single analysis, offering a new technological approach for the screening and validation of colorectal cancer biomarkers. The text systematically reviews the research progress on colorectal cancer markers based on mass spectrometric techniques. It concludes that the markers with potential research value in current studies include ROBO4, VCAM-1, phenylalanine, indoline, L-carnitine, and PSMD7. Although existing studies have demonstrated the great potential of mass spectrometry for marker discovery, most candidate markers remain in the exploratory stage, facing challenges such as small sample sizes, limited multicenter validation, and non-standardized detection workflows. In the future, the integration of mass spectrometry with multi-omics analysis is expected to advance colorectal cancer markers from research to clinical translation, enabling truly noninvasive early screening.

Keywords

colorectal cancer, data-independent acquisition (DIA), two-dimensional liquid chromatography-tandem mass spectrometry (2D LC-MS/MS), marker screening

1. Introduction

Colorectal cancer (CRC) is the second leading cause of cancer-related mortality worldwide, accounting for approximately 904,000 deaths in 2022, representing 9.4% of the total global cancer deaths [1]. In terms of disease burden, the disability-adjusted life years (DALY) associated with CRC continue to rise globally, imposing substantial impacts on social and economic development. Studies have shown that regions undergoing rapid economic growth and lifestyle transitions tend to experience rising CRC mortality and disease burden [2]. Although previous research has provided an overview of pan-cancer detection, biomarkers specifically for colorectal cancer still require systematic synthesis. Currently, CRC is characterized by a slow progression, making early diagnosis critically important for patient treatment and prevention. A variety of

screening options are available for CRC, including stool-based blood tests such as guaiac-based fecal occult blood testing (gFOBT) and the more sensitive fecal immunochemical test (FIT). Endoscopic methods that directly examine the rectum and colon using optical techniques include sigmoidoscopy and colonoscopy [3]. However, these methods have limitations in sensitivity and specificity, making it difficult to meet the screening needs of large-scale populations. Although colonoscopy offers significant advantages in detecting intestinal lesions, its invasiveness and operational complexity limit its widespread adoption as a first-line screening method. Against this backdrop, the introduction of high-sensitivity, high-specificity mass spectrometry technology has become particularly necessary. By analyzing proteomic and metabolomic profiles in body fluids, this technology holds promise for identifying more stable early-warning molecules, thereby offering a breakthrough for non-invasive, precise early diagnosis. In current research on CRC markers, mass spectrometry, leveraging its high sensitivity and resolution, has emerged as a key driver for deciphering tumor-associated molecular characteristics.

2. Mass Spectrometry Techniques for the Screening of Colorectal Cancer Markers

2.1 Data-independent Acquisition

Data-independent acquisition (DIA) is a type of mass spectrometry technology that collects data without bias. DIA scans all parent ions in the MS1 spectrum and collects signals from their fragments, whereas data-dependent acquisition (DDA) collects only peptide fragments [4]. DIA systematically and unbiasedly acquires fragment information from all precursor ions, thereby fundamentally avoiding the problems that arise from random precursor-ion selection in DDA, such as poor sampling reproducibility and the omission of low-abundance peptides. DIA uses all-ion fragmentation scanning, alternating between low- and high-energy spectra at set intervals. Moreover, DIA uses a process called differential processing to create a combined spectrum that holds fragment information from all parent ions. The other method, called segmental scanning, divides the full scan range into several windows based on mass-to-charge ratio, then breaks down and detects fragments from all parent ions in each window, one by one. The data collected by DIA is much more complex than DDA data, so it requires specialized algorithms for analysis. In the early days, scientists used spectral libraries to match and identify data. Still, in recent years, they have developed methods to analyze data directly without relying on existing spectral libraries, thereby improving both the depth and speed of identification. Because DIA has excellent repeatability, accurate quantification, and the ability to detect a wide range of molecules, it has become a key tool in advanced research areas such as large clinical studies, single-cell proteomics, and spatial proteomics.

2.2 Two-dimensional Chromatography Hyphenation

Two-dimensional liquid chromatography(2D-LC) is a typical example of multidimensional liquid chromatography. Two columns with different separation methods are connected in series via an interface. The first dimension can be one or more columns with the same separation method; the second dimension can be one or more columns with either the same or different separation methods. After the sample is separated in the first dimension, it passes through the interface into the second-dimension column for further separation. This reduces overlap between components, effectively increases peak capacity, and solves the co-elution problem that occurs in one-dimensional chromatography [5]. Combining two-dimensional chromatography with mass spectrometry combines the efficient separation of chromatography with the high-sensitivity identification of mass spectrometry, greatly improving the depth of analysis of complex samples. Based on how the interface works, there are two types. Heart-cutting mode selectively transfers only the target components, which is good for targeted analysis. The comprehensive two-dimensional mode continuously transfers all material from the column, providing a full picture of the sample. Based on how the interface works, there are two types. Heart-cutting mode selectively transfers only the target components, which is good for targeted analysis. The comprehensive two-dimensional mode continuously transfers all material from the column, providing a full picture of the sample.

3. Potential Biomarkers in the Blood or Tissues of Patients with Colorectal Cancer

3.1 Potential Biomarkers in the Blood or Tissues of Colorectal Cancer

Mass spectrometry has become a major supporting technology in the study of biological macromolecules, playing a key role in the research and analysis of protein structures [6]. Against this background, important progress has been made in the study of blood markers for colorectal cancer. Many researchers have used different mass spectrometry platforms to identify key markers with potential for clinical use.

Zhang Bizhu used data-independent acquisition (DIA) quantitative proteomics to analyze serum samples and identified indicators closely related to CRC. ROBO4 and VCAM-1 are two markers for colorectal cancer [7]. From a biological mechanism perspective, ROBO4 is a member of the neural guidance protein family and primarily regulates vascular endothelial cell migration and angiogenesis. VCAM-1 is a cell adhesion molecule that plays a key role in inflammation and tumor metastasis. The two markers abnormally expressed in the serum of colorectal cancer patients may be closely related to tumor-induced vascular remodeling and systemic inflammatory responses. This study used DIA quantitative proteomics to analyze serum samples from colorectal cancer patients and healthy controls and identified differentially expressed proteins. The results were then verified in a large sample using enzyme-linked immunosorbent assay (ELISA), confirming that the expression levels of ROBO4 and VCAM-1 in the serum of colorectal cancer patients were significantly increased. The combined detection of these two markers showed good diagnostic value for colorectal cancer.

Liu Kejian used UPLC-QTOFMS to build a serum metabolomics model. Through ROC curve analysis, he found that three metabolites—phenylalanine, indoline, and L-carnitine—have the potential to serve as diagnostic markers for CRC [8]. Consistently, a GC-MS-based metabolomics study by Ni et al. also identified phenylalanine as a significantly dysregulated circulating metabolite in colorectal cancer, further supporting the role of alterations in amino acid metabolism in CRC progression [9]. From a biological mechanism perspective, phenylalanine is an aromatic amino acid, and its abnormal metabolism may be related to the increased amino acid demand of tumor cells. Indoline, as a metabolite of tryptophan, can have its levels altered by disorders in gut microbiota metabolism. L-carnitine is involved in fatty acid oxidation, and changes in its levels may reflect the reprogramming of energy metabolism in the tumor state. This study included colorectal cancer patients and healthy controls. The researchers used ultra-performance liquid chromatography-quadrupole time-of-flight mass spectrometry to perform metabolomics analysis on serum samples. Combined with multivariate statistical analysis, they identified differential metabolites. The diagnostic performance of the three metabolites was evaluated using ROC curves, confirming their value as potential markers for colorectal cancer.

These studies have not only proven that it is possible to detect potential colorectal cancer markers in serum but also further strengthened the foundation of early screening technology based on liquid biopsy. At the same time, they have made significant contributions to the discovery of serum colorectal cancer markers, laying a foundation for future application in testing. If this continues to develop, we may be able to determine whether a person has colorectal cancer with just one blood test in the future. This also makes it possible to accurately identify high-risk groups for colorectal cancer and provide early warning.

3.2 Potential Biomarkers in the Tissues of Patients with Colorectal Cancer

In addition, regarding markers for colorectal cancer patients, although serum markers have advantages such as being non-invasive and convenient and are favored by colorectal cancer patients, using only serum markers will impose major limitations on practical application. For example, they cannot reflect the local microenvironment of colorectal tumors or the differences in tumors between individuals. Therefore, the study and in-depth exploration of markers in colorectal cancer patients' tissues are equally important.

Kang Yunxia and her colleagues used two-dimensional chromatography-mass spectrometry to analyze cancer tissues and adjacent normal tissues, along with western blotting. They confirmed that PSMD7 was highly expressed in colorectal cancer tissues. It hints that PSMD7 may be a new biomarker for colorectal cancer [10]. From a biological mechanism perspective, PSMD7 is one of the non-ATPase subunits of the proteasome 19S regulatory particle. It participates in the regulation of the ubiquitin-proteasome pathway and is responsible for protein degradation and turnover. Its high expression in colorectal cancer tissues may be related to the increased need of tumor cells to remove abnormal proteins to maintain rapid proliferation. At the same time, it may also contribute to tumor development by altering the stability of cell cycle regulators. This

study used two-dimensional chromatography-mass spectrometry to perform proteomics analysis of colorectal cancer tissues and matched adjacent normal tissue samples, identifying differentially expressed proteins. The results were verified by western blotting, confirming that PSMD7 expression in colorectal cancer tissues was significantly higher than in adjacent normal tissues, suggesting that it may be closely related to the occurrence and development of colorectal cancer.

This study discovered markers present in colorectal cancer tissues, which may be worth further in-depth research in the future and could serve as key markers for the diagnosis of colorectal cancer. All studies have successfully identified markers for colorectal cancer by combining mass spectrometry with other technologies, providing a breakthrough in its detection and diagnosis. This also provides a breakthrough for prognosis assessment and the development of personalized treatment targets.

4. Challenges and Prospects of Screening Colorectal Cancer Markers Using Mass Spectrometry Technology

At present, colorectal cancer lacks ideal tumor markers. Existing tumor markers generally have low sensitivity and cannot meet the clinical need for early tumor detection. Therefore, finding new tumor markers that can be used for early diagnosis is especially important in the diagnosis and treatment of colorectal cancer [11]. Although the studies mentioned above have shown several promising markers, there are still some problems. For example, new tumor markers such as ROBO4, VCAM-1, circulating tumor cells, CK20, and MSI have great potential for application in CRC diagnosis and treatment. However, there is currently little research on them, and some existing results remain controversial. Moreover, the detection technology still needs improvement; there is currently no ideal tumor marker for CRC diagnosis and treatment [7]. Therefore, the application of mass spectrometry technology is extremely important. In recent years, mass spectrometry technology has shown unique advantages in tumor marker screening. It can detect and quantify multiple biomolecules simultaneously, which is very helpful for discovering new markers. These findings indicate that integrating serum thermogram information with routine clinical data can modestly strengthen diagnostic assessment and help identify biologically meaningful patient subgroups, offering a promising non-invasive colorectal cancer evaluation [1]. However, relying solely on mass spectrometry technology makes it difficult to fully address the challenges of marker screening. Future researchers will also need to combine mass spectrometry technology with multi-omics analysis (such as genomics, transcriptomics, and metabolomics). By integrating multidimensional biological information, they can improve the accuracy and reliability of marker screening. At the same time, big data and artificial intelligence algorithms can be further combined to promote the discovery and eventual application of markers. In the end, through the combined use of multiple technologies and the application of emerging technologies, we will certainly be able to promote the widespread adoption of early screening for colorectal cancer and provide real protection for patient safety.

5. Conclusion

Significant progress has been made in the screening for colorectal cancer markers using mass spectrometry. Through techniques such as DIA quantitative proteomics, differential protein analysis, and two-dimensional chromatography-mass spectrometry, many researchers have successfully identified multiple markers with potential diagnostic value at both the blood and tissue levels. These markers include ROBO4, VCAM-1, phenylalanine, indoline, L-carnitine, and PSMD7, among others. These findings confirm that the unique advantages of mass spectrometry technology for high-throughput, high-sensitivity detection lay a scientific foundation for the development of multi-marker combined diagnostic models. However, although the markers mentioned above all have strong application potential, most candidate markers are still in the research stage. They all face problems such as limited sample sizes, lack of multi-center validation, and non-standardized detection methods. Therefore, the clinical value of some of these markers remains controversial, and there is a significant gap before they can be used in clinical practice.

Nevertheless, the screening of colorectal cancer markers using mass spectrometry technology still has great prospects for future development. With ongoing advances in technology and the gradual accumulation of clinical evidence, the transformation of mass spectrometry from laboratory research to routine clinical testing holds great promise. Early screening and personalized treatment for colorectal cancer will ultimately benefit a large number of patients.

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Conflicts of Interest

The authors declare no conflict of interest.

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