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Proteomics-Based Screening and Validation of Potential Drug Targets for Early Cancer Diagnosis: Pharmacological Implications

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Abstract: Early cancer diagnosis and targeted therapy are crucial for improving patient outcomes. Proteomics provides a promising approach for discovering drug targets by analyzing differential protein expression. This study employs Surface-Enhanced Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (SELDI-TOF-MS) technology to identify and validate protein biomarkers in colorectal cancer patients, highlighting their potential as pharmacological targets. SELDI-TOF-MS was utilized to compare the serum protein profiles of colorectal cancer patients and healthy controls. Differentially expressed proteins were identified and analyzed using Biomarkers Wizard software, with an emphasis on their potential role in drug sensitivity and therapeutic applications. Fifteen significant protein peaks were identified, with six showing substantial expression changes pre- and post-surgery. These proteins may serve as drug targets, offering insights for personalized cancer therapy. The identified protein markers not only aid in early cancer diagnosis but also have potential as therapeutic targets, paving the way for novel drug development and individualized treatment strategies.

Keywords: Proteomics, Drug Target Discovery, SELDI-TOF-MS, Early Cancer Diagnosis, Pharmacological Applications, Personalized Medicine

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INTRODUCTION

Proteomics, the comprehensive study of the composition, structure, function, and interactions of proteins within an organism, has emerged as a powerful high-throughput research technology. It provides valuable insights into the functions and regulatory mechanisms of proteins by systematically analyzing protein expression levels, post-translational modifications, and other related information (Alabert *et al*., 2014; Zubarev, 2013). Techniques such as protein isolation, quantification, and identification are integral to proteomics, which has been increasingly utilized to enhance cancer research by identifying potential protein markers (Huibo *et al*., 2023; Martens & Vizcaíno, 2017).

Cancer, characterized by uncontrolled cell proliferation, remains a significant health concern. Historically, cancer research has primarily focused on genetic alterations. However, with technological advancements, proteomics has become a focal point in cancer research due to its ability to provide more comprehensive and in-depth information (Astles, 2023; Yuzhalin, 2024). Proteomics not only aids in understanding cancer at a molecular level but also facilitates the discovery of new therapeutic approaches and cancer markers, thereby improving diagnostic accuracy and treatment efficacy (Kuruma, 2017; Tanase *et al*., 2017).

Recent studies have highlighted the potential of proteomics in identifying biomarkers for various cancers. For instance, proteomics has been applied to identify markers for early diagnosis of colorectal cancer (Steinert *et al*., 2016), lung cancer (Xie *et al*., 2015; Gasparri *et al*., 2020), and cervical cancer (Kontostathi *et al*., 2016). These studies underscore the importance of proteomics in detecting cancer at an early stage, although challenges such as limited sensitivity and specificity of conventional diagnostic techniques persist (Hosseini & Khamesee, 2021; Shukla *et al*., 2015).

In the context of colorectal cancer, proteomics offers a promising avenue for early detection and targeted therapy. SELDI-TOF-MS (Surface-Enhanced Laser Desorption/Ionization Time-of-Flight Mass Spectrometry) technology, known for its speed, accuracy, high-throughput, and sensitivity, has been effectively utilized for early cancer diagnosis and tumor marker screening (Schlichtemeier *et al*., 2019; Nardone *et al*., 2021). This study employs SELDI-TOF-MS to analyze serum proteins from colorectal cancer patients, comparing them with healthy controls to identify differential protein markers. The identified markers were further evaluated for their potential as drug targets, aiming to contribute to precision medicine (Ardito *et al*., 2016; Ramzan *et al*., 2023).

MATERIALS AND METHODS

SELDI-TOF MS Technique

Basic Principles

Tumor development is a complex and progressive biological process influenced by various environmental carcinogenic factors. To understand the overall metabolic changes and disease progression at the molecular level,

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serum proteomics technology has been increasingly utilized. This approach allows for the comprehensive analysis of protein expression profiles, which can reveal the impact of environmental factors on cellular genetic material. These studies have shown significant theoretical value and broad clinical application prospects, particularly in the early detection of tumor risks (Schlichtemeier *et al*., 2019).

Differential proteomics is a method used to identify, quantify, and characterize proteins that are differentially expressed between normal and cancerous tissue cells. This approach helps in screening for protein markers associated with cancer, which can serve as the basis for effective early diagnosis. Surface-Enhanced Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (SELDI-TOF MS) is a powerful technique in differential proteomics, combining chromatography and mass spectrometry. The core of SELDI-TOF MS technology is a chip with a wide range of binding properties, which can be chemically or biochemically modified to detect various proteins (Ardito *et al*., 2016).

In this study, SELDI-TOF-MS was employed to profile serum proteins from colorectal cancer patients and healthy controls. The high-throughput and sensitivity of this technology make it ideal for identifying low-abundance proteins that could serve as potential drug targets. The identified proteins were further analyzed to assess their relevance in drug discovery.

Time-of-Flight Mass Spectrometry (TOF-MS) is a method used to analyze the composition or structure of substances by measuring the time it takes for ions with the same kinetic energy but different mass-to-charge ratios to travel a constant distance in a vacuum tube. The basic principle involves ionizing the sample and accelerating it with an electric field to give the sample ions the same kinetic energy. The flight time of each ion is determined by its mass-to-charge ratio, allowing for the measurement of individual samples (Nardone *et al*., 2021).

The kinetic energy carried by the sample after ionisation by the ion source and acceleration by the electric field is:

$$zeV = mv^2 / 2 \qquad (1)$$

Where m represents the sample ion mass and v is the potential difference of the accelerating electric field.

The flight time of the sample ion can be expressed as:

$$t = L / v = \sqrt{m / 2zeV} \qquad (2)$$

Where L is the distance of the flight tube.

From Equation (2), it is evident that the time of flight of a sample ion is determined by the ion's mass-to-charge ratio. Ions with a smaller mass-to-charge ratio travel faster, while those with a larger mass-to-charge ratio travel slower and take longer to reach the detector. This principle forms the basis for mass analysis of ions in TOF-MS.

SELDI-TOF MS technology integrates TOF-MS with surface-enhanced laser desorption/ionization for protein screening and detection. Its primary advantage is the ability to rapidly generate protein expression profiles from untreated biological samples, facilitating the identification of differentially expressed proteins in cancerous cells compared to normal cells. These proteins can serve as markers for early cancer diagnosis (Ardito *et al*., 2016).

Analytical Steps

SELDI-TOF MS combines chromatography and mass spectrometry to bind proteins in biological samples to a chromatographic matrix on the chip surface. The appropriate chip is selected based on the protein's nature, and samples (e.g., cell lysate, serum) are added to the protein-binding chip. Proteins bind to the chip based on their intrinsic amino acid sequences. After binding, the chip is washed to remove unbound proteins, leaving only specifically bound proteins (Ramzan *et al*., 2023).

Energy-absorbing molecules (EAM) are then added to form mixed crystals with the proteins, facilitating desorption and ionization during mass spectrometry detection. The chip is placed in a reader, irradiated by a laser, and the resulting ions are detected in a vacuum tube. The flight time of these ions, determined by their mass-to-charge ratio, is used to plot mass spectra. The software processes the detection results, displaying the relative molecular mass and content of the proteins, with a detection range of 0 to 5×10^5^ (Nardone *et al*., 2021).

Serum Samples and Instrumentation

Serum Sample Collection

Serum samples were processed to ensure the integrity of protein markers. Protein-binding chips and mass spectrometry conditions were optimized for detecting proteins relevant to pharmacological studies.

Human serum samples were collected from SH Changzheng Hospital and YF Hospital of NJ Medical University, with approval from all donors and the local ethics committee. Blood was drawn intravenously from donors in the morning after an overnight fast. The first 2-3 ml of blood was discarded, and the remaining blood was collected in EDTA tubes containing K2EDTA anticoagulant. Samples were centrifuged at 2000 r/min for 10 minutes to remove cellular debris and platelets. The light yellow serum layer was aspirated, centrifuged

again, and stored at -80°C.

SH Changzheng Hospital provided samples from 20 healthy controls, 20 newly diagnosed colorectal cancer (NDCC) patients, and 20 recurrent refractory colorectal cancer (RRCC) patients. Samples were pooled and divided into technical replicates for proteomics experiments. Similarly, YF Hospital provided samples from 40 healthy controls and 40 colorectal cancer patients before and after surgery. These samples were also pooled and divided into technical replicates. All samples were stored at -85°C after labeling.

Instrument and Equipment Selection

The following instruments and equipment were used:

- Peptide captrap columns and C18 reversed-phase analytical columns (0.2 mm i.d. × 180 mm, 4 μm, 250 Å) from Company M (Auburn, USA)
- Ultrafiltration tubes (2 kDa pore size) and disposable syringe filters (0.2 μm pore size) from Company N (Billerica, USA)
- Cell culture dishes, centrifuge tubes (10 mL and 60 mL), 1 mL centrifuge tubes, 1.5 mL spiral-mouth centrifuge tubes, micropipettes, and 384-well plates from Company C (Corning, USA) and Company A (Union City, USA)
- Centrifuge and UV spectrophotometer from BC (Brea, USA)
- pH meter from S (Hamburg, Germany)
- Micropipettes from G (Middleton, USA)
- Vortex oscillator from SI (Bohemia, USA)
- Analytical balance from M-T (Anaheim, USA)
- Comfort Mixer from E (Westbury, USA)
- Centrifugal Concentration System from L (Kansas City, USA)
- Milli-Q deionized water system, HPLC system, nanolitre electrospray ionization source, and 2D Ion Trap Mass Spectrometer LTQXL from M and TS (Rockford, USA)
- Real-time fluorescence quantitative PCR instrument from ABI (Foster City, USA)
- Ultra-clean bench from SH Purification Equipment Co. Ltd
- Optical microscope from C Instrument Factory
- Super-constant water bath from SH Laboratory Equipment Co. Ltd
- 360° rotary silent mixer from JG Industries Ltd

Experimental Methodology Design

Processing of Serum Samples

Serum samples were processed as follows:

1. Remove serum samples from a deep cryogenic refrigerator at -85°C and place on an ice box to thaw.
2. Centrifuge at 5000 rpm/min at 5°C for 3 minutes.
3. For serum protein microarray preparation, dilute 2 μL of serum with three times the volume of U9 buffer.
4. Add 8 μL of the diluted sample to 100 μL of binding buffer to achieve a total dilution of approximately 54-fold, avoiding air bubbles.
5. Store the processed serum samples for subsequent microarray preparation.

Serum Protein Microarray Preparation

Protein mass spectrometry was performed using a protein chip biomarker system with CM cation exchange and Q10 anion exchange chips. The chip was mounted on a Bioprocessor and equilibrated with LHEEPES binding/washing buffer (pH=7.1) for 5 times, each for 4 minutes at 5°C. Diluted serum samples were added to the Bioprocessor wells, incubated with shaking for 60 minutes, washed with LHEEPES buffer 5 times, rinsed with MilliQ water, and dried. A saturated solution of CHCA was applied to each well twice, and the chip was dried before detection.

SELDI-TOF-MS Technique for Screening Serum Samples

The chip was loaded into the Bioprocessor, and 250 μL of NaAC (120 mmol/L, pH 4.2) was added to each well, oscillated at 500 r/min for 3 minutes, and the procedure was repeated twice. The treated 96-well plate was placed on an ice box, 180 μL of NaAC solution was added, and the plate was oscillated at 500 r/min for 3 minutes at 5°C. The treated sample (120 μL) was added to the protein chip, shaken at 500 r/min for 60 minutes at 5°C, and the residual liquid was removed. The chip was washed with NaAC solution and deionized water, dried, and 1.5 μL of 55% saturated SPA solution was applied twice.

The setup parameters were:

- Molecular weight range: 1000 Da to 25000 Da
- Highest molecular weight: 30000 Da

Raw data were corrected using Ciphergen Biosystems software to homogenize the total ionic strength and molecular weight. Biomarker Wizard and ZUCI-Protein Chip Data software packages were used for noise filtering and baseline removal. The protein chip plate was analyzed using MELDI-TOF-MS, and m/z peaks (mass-to-charge ratios) were obtained. Peaks with <0.25% variation were considered the same protein.

STATISTICAL METHODS

Statistical analysis was conducted using SPSS and Biomarker Wizard software. Differential expression analysis identified proteins with significant changes, potential drug targets for therapeutic intervention. One-way ANOVA was performed on protein content data with the same mass-to-charge ratio in different groups.

Paired t-tests analyzed changes in mass spectra before and after treatment. Ciphergen Protein Chip software read the protein chip data, and Biomarker Wizard software performed variance analysis to establish and validate the diagnostic decision-making model.

RESULTS

SELDI-TOF-MS technology, integrating protein microarray and mass spectrometry, is characterized by its high sensitivity and throughput. It is capable of detecting low-abundance and low-molecular-weight proteins, making it a powerful tool for identifying cancer-related proteins. The distinct mass spectra of cancer gene proteins and the similarity of protein profiles from the same genes form the basis for rapid and accurate cancer identification. This section presents the results of screening human serum samples for differentially expressed cancer gene proteins using SELDI-TOF-MS, aiming to provide reliable support for early cancer diagnosis.

Mass Spectrometry and Differential Expression Protein Screening

Comparative Results of Mass Spectra

The processed colorectal cancer serum protein samples were analyzed using SELDI-TOF-MS, with three replicate experiments yielding consistent mass spectra. Therefore, the results of one experiment are presented here. The protein mass spectra of the blank control group (Control) and newly diagnosed colorectal cancer (NDCC) patients are shown in Figure 1. The horizontal axis represents the mass-to-charge ratio (m/z), and the vertical axis represents protein abundance.

As illustrated in Figure 1, the serum protein fingerprint profile of NDCC patients exhibited 15 statistically significant protein peaks that differed from those of the control group. The distinct differences in protein expression between the NDCC and control groups highlight the potential of SELDI-TOF-MS for effective early-stage cancer screening.

Differential Expression Protein Screening

A total of 47 protein peaks were detected in the molecular weight range of 1000 to 25000 Da. Comparative analysis using Biomarkers Wizard software identified 15 protein peaks with significant differences between the colorectal cancer and control groups. The screening results are summarized in Table 1.

Differential expression protein screening

(1) Screening of differential proteins in serum of colorectal cancer patients

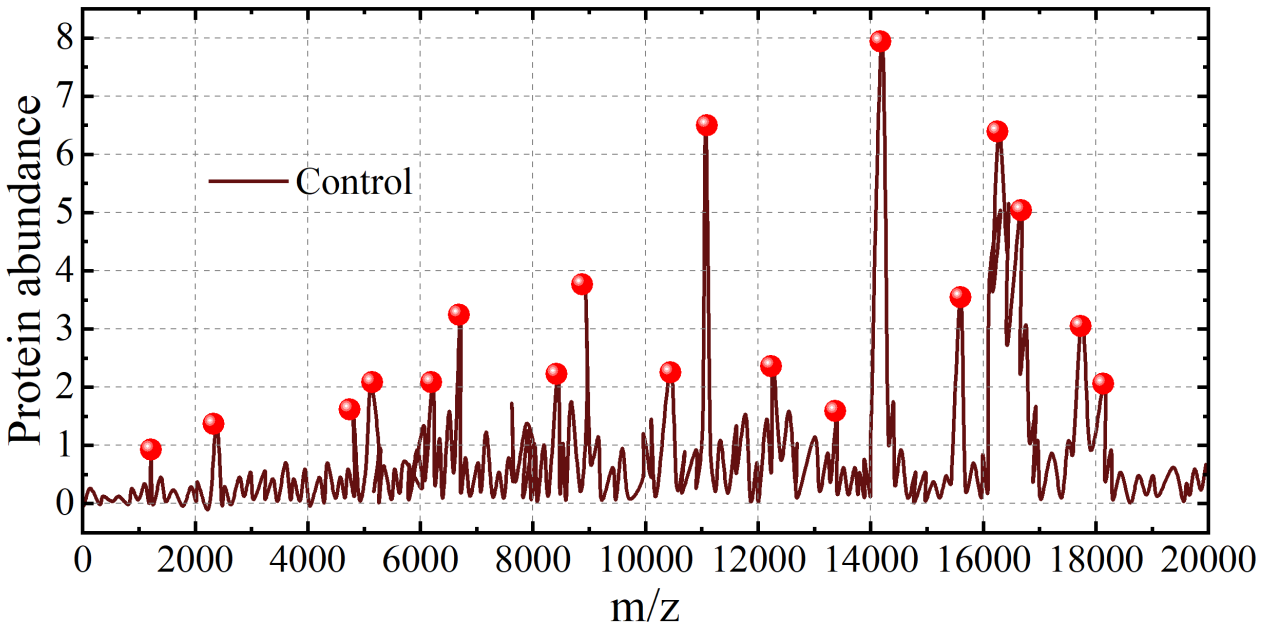
A total of 47 protein peaks were detected in the molecular weight range of 1000~25000 Da. Comparing the mass spectra of serum proteins in the colorectal cancer group and the blank control group with Biomarkers Wizard software, it was found that there existed 15 protein peaks with obvious differences. The screening results of specific differential proteins are shown in Table 1.

Through the analysis of the protein fingerprint mass spectra obtained in the previous section by Biomarkers Wizard software, there was no significant difference in the expression of most protein peaks in the serum protein samples of colorectal cancer patients and the blank control group, but there were still 47 protein peaks in the mass spectra of the two groups that had a certain judgemental difference in their expression, among which 15 protein peaks of the colorectal cancer patients had significant differences when comparing them with those of the blank control group. Among the 15 serum proteins with expression differences, there were 7 proteins with up-regulated expression in the serum of colorectal cancer patients (i.e. the bolded part in the table), and their average molecular weights were 2,384.86 Da, 5,029.64 Da, 5,914.27 Da, 6,484.26 Da, and 8,563.61 Da. The mean molecular weights of 8,987.68 Da and 8,987.68 Da were the same as those of the blank control group, and the mean molecular weights of the two proteins were the same as those of the blank control group, 8987.68 Da and 14286.37 Da. A total of eight protein peaks showed down-regulation of protein expression when compared with the blank control group. The data show that the SELDI-TOF-MS technique of proteomics can be used to effectively screen serum proteins from patients with early stage of cancer, and can also effectively differentiate the up-regulation and down-regulation of different protein molecular weights from that of normal serum.

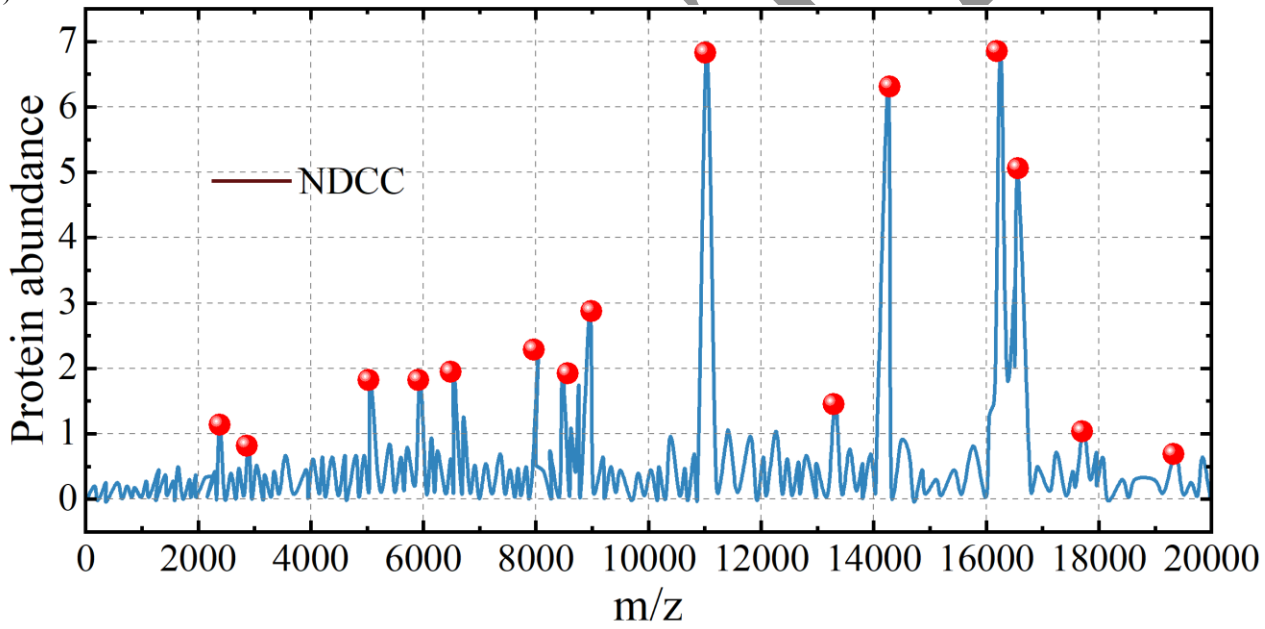
Among the 47 detected protein peaks, 15 showed significant differential expression between colorectal cancer patients and healthy controls. Of these, six proteins exhibited marked changes between pre- and post-operative samples, suggesting their potential as drug targets. These findings support the use of proteomics in identifying proteins that could be exploited for therapeutic purposes.

(2) Screening of differential proteins in serum of preoperative and postoperative groups of colorectal cancer patients

Under the same conditions and parameters, IMA3 protein microarray and Biomarkers Wizard software were applied to analyse the serum of postoperative colorectal cancer patients and compare the protein profiles with those of the preoperative group and normal control group. Table 2 shows the statistical results of differential protein expression in the serum protein mass spectra of the preoperative and postoperative groups. As can be seen from the table, the average molecular weights of serum proteins expressed in the postoperative group were 2384.86 Da, 5029.64 Da, 5914.27 Da, 6484.26 Da, 8563.61 Da and 8987.68 Da.



(a) Control



(b) NDCC

Fig. 1: Comparison of mass spectra

The protein expression of the postoperative group was decreased, and the intensity of the protein peaks was comparable to that of the blank control group without any significant difference, but the difference was significant in comparison with that of the pre-operative group ($P < 0.05$). In addition, the protein profiles of the postoperative group also changed when compared with the preoperative group and the blank control group, in which the expression of proteins with relatively large molecular weights, such as 16,192.43 Da, 16,561.79 Da, 17,701.78 Da, and 19,325.13 Da, was lower than that of the preoperative group.

Establishment and validation of the serological diagnostic model

Diagnostic modelling

The differential protein peaks obtained from Biomarker Wizard software analysis were set up as a database, imported into Biomarker Pattern statistical analysis software, selected the corresponding conditions, and grouped into preoperative and postoperative groups, so as to obtain the specific protein markers that could be correctly grouped and to draw a tree-node map. Figure 2 shows the diagnostic decision-making model for colorectal cancer serum protein markers.

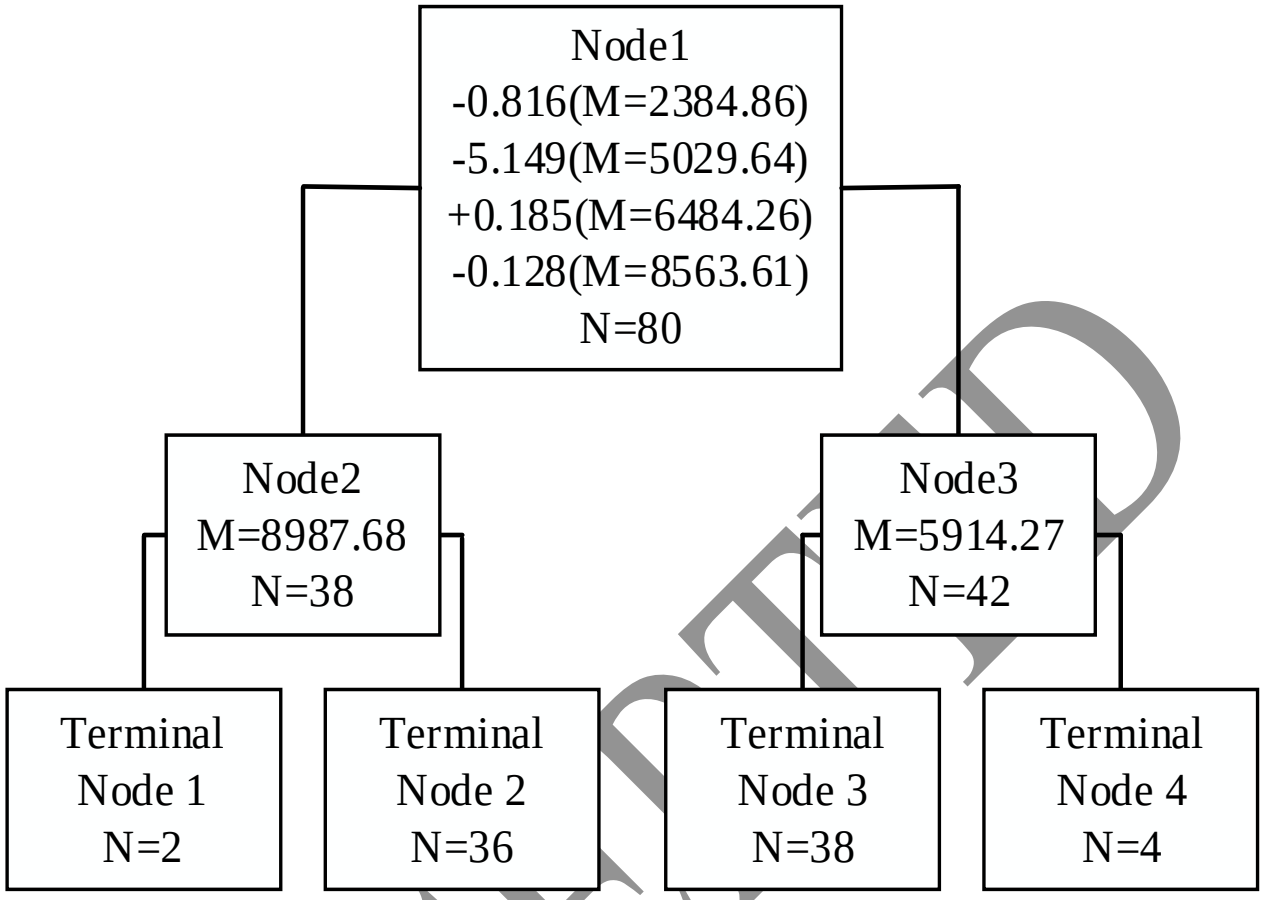


Fig. 2: Serum protein marker diagnostic decision model

It was found that preoperative and postoperative colorectal cancer samples could be correctly grouped using the diagnostic decision model consisting of six proteins with average relative molecular masses of 2384.86 Da, 5029.64 Da, 5914.27 Da, 6484.26 Da, 8563.61 Da, and 8987.68 Da, respectively, and that two out of 40 preoperative samples were misclassified as postoperative samples, while four out of 40 postoperative samples were misclassified as postoperative samples. Four of the 40 postoperative samples were misclassified in the preoperative group, with a sensitivity and specificity of 95.00% (38/40) and 90.00% (36/40), respectively. The diagnostic decision model built using these markers produced a total of 4 end nodes, 80 samples were divided into 2 groups at the root node (Node1) by the 4 marker proteins 2384.86Da, 5029.64Da, 6484.26Da, and 8563.61Da, and the 38 samples with peaks ≤-2.115 were classified within the left branching node Node2, and the 42 samples with peaks > -The 38 samples within Node2 continued to be divided by the 8987.68Da marker protein, the 2 samples with peaks ≤ 23.062 were divided into the left end node Node1, and the 14 samples with peaks > 23.062 were divided into the right end node Node2. 31 samples within Node3 continued to be divided by the 5914.27 marker protein. Samples continued to be divided by 5914.27 flagged proteins, 38 samples with peaks ≤ 6.748 were divided to the left end node Node3, and 4 samples with peaks 6.748 were divided to the right end node Node4.

Validation of the diagnostic model

Based on the established serum protein diagnostic decision-making model for colorectal cancer, another 52 colorectal cancer patients with 40 normal serum samples were analysed by double-blind method using this model. Its specific results are shown in Table 3.

As can be seen from the table, 87 cases of 92 serum specimens were judged correctly, and only 5 cases were judged incorrectly. Among them, 48 cases of colorectal cancer patients and 38 cases of normal serum were judged correctly, with a positive detection rate (sensitivity) of 92.31% and a negative detection rate (specificity) of 97.50%. This shows that SELDI-TOF-MS technology can achieve effective screening of serum proteins in the early stage of cancer, and the diagnostic decision model established based on the molecular weight data of the screened proteins can achieve effective classification of cancer in the early stage of cancer, help doctors to better grasp the cancer status of the patients, and provide data support for the timely adoption of therapeutic measures.

Table 1: The screening results of the specific difference protein

m/z	P value	Average expression intensity of protein peak	
		NDCC	Control
2384.86	0.00022	1.1358	0.9248
2868.21	0.00009	1.1553	1.3765
5029.64	0.00071	1.7187	1.5003
5914.27	0.00125	1.8448	1.7261
6484.26	0.00138	2.1161	2.0828
7966.25	0.00027	2.0003	2.9081
8563.61	0.00079	4.1595	2.3874
8987.68	0.00104	6.5207	2.9939
11012.31	0.00031	4.3415	5.1168
13287.73	0.00087	1.4838	2.8853
14286.37	0.00115	6.3693	1.6392
16192.43	0.00042	6.7137	6.9077
16561.79	0.00106	3.8503	4.0629
17701.78	0.00018	0.9492	4.5722
19325.13	0.00032	0.6911	2.0562

Table 2: The expression of the difference protein before and after surgery

m/z	P value	Average expression intensity of protein peak	
		Before	After
2384.86	0.00048	1.1358	0.9306
2868.21	0.00064	1.1553	1.1542
5029.64	0.00047	1.7187	1.5015
5914.27	0.00072	1.8448	1.7237
6484.26	0.00041	2.1161	2.0651
7966.25	0.00066	2.0003	2.0002
8563.61	0.00013	4.1595	2.3738
8987.68	0.00038	6.5207	2.9941
11012.31	0.00071	4.3415	4.3379
13287.73	0.00076	1.4838	1.4826
14286.37	0.00023	6.3693	6.3513
16192.43	0.00074	6.7137	6.7124
16561.79	0.00019	3.8503	3.8518
17701.78	0.00076	0.9492	0.9493
19325.13	0.00013	0.6911	0.6908

Table 3: Diagnostic model validation results

Actual Class	Colorectal cancer	Normal serum
Total Cases	52	40
Percent Correct	92.31%	97.50%
Colorectal cancer (N=50)	48	2
Normal serum (N=42)	3	39

DISCUSSION

China is a high-incidence area for colorectal cancer, with the number of cases rising year by year. The incidence rate of colorectal cancer in China accounts for about 42.17% of the global incidence rate (Hosseini & Khamesee, 2021). It has been reported that 8.6%-16.3% of newly diagnosed colorectal cancer patients are associated with carcinomatous ascites, and the incidence of metastasis in patients with advanced colorectal cancer is even higher at 42.2%-44.5% (Yuzhalin, 2024). Colorectal cancer cell metastasis often leads to complications such as abdominal infection, malnutrition, renal insufficiency, and intestinal obstruction, which are significant causes of poor prognosis in colorectal cancer patients. Therefore, early diagnosis of colorectal cancer is crucial for improving survival rates and prognosis.

Patients with colorectal cancer cell metastasis are often difficult to diagnose early and have a very poor prognosis. Improving the early diagnosis of colorectal cancer cell metastasis and identifying new specific tumor markers to predict metastasis are of great importance. In this study, we applied SELDI-TOF-MS technology to identify specific proteins predicting colorectal cancer and cancer cell metastasis by comparing the differences in serum protein fingerprints between colorectal cancer patients and normal controls (Steinert *et al*., 2016).

Colorectal cancer metastasis is a complex process involving multiple genetic and proteomic alterations. The success of metastatic cancer treatment largely depends on early diagnosis and understanding the molecular mechanisms of tumor infiltration and metastasis. Although the treatment of colorectal cancer has improved in recent years, patient prognosis has not significantly improved. The occurrence of metastasis after radical resection is a major factor affecting prognosis and leading to death (Kuruma, 2017). Using SELDI-TOF-MS technology to study serum proteomics in patients with metastatic colorectal cancer is an effective method for early cancer diagnosis. This study identified six protein peaks with average molecular weights of 2,384.86 Da, 5,029.64 Da, 5,914.27 Da, 6,484.26 Da, 8,563.61 Da, and 8,987.68 Da that significantly differed between preoperative and postoperative patients. These proteins were significantly downregulated in the colorectal cancer metastasis group and may serve as metastasis-associated proteins (Ardito *et al*., 2016).

SELDI-TOF-MS technology is an effective technique for detecting neoplastic biomarkers. The screened protein markers can sensitively diagnose colorectal cancer at an early stage, monitor metastasis, and help determine prognosis and provide new treatment options. Follow-up work will further purify and identify the screened protein markers to determine their nature and sequence. Additionally, other tumors will be added as controls to improve the specificity of the protein diagnostic model for colorectal cancer (Nardone *et al*., 2021).

The differential protein markers identified in this study hold promise as therapeutic targets for colorectal cancer. Proteins with altered expression patterns may be involved in pathways critical for tumor progression and could be targeted to enhance treatment efficacy. Our findings align with the growing trend of using proteomics for precision medicine, offering potential for developing targeted therapies (Tanase *et al*., 2017, Jia Z *et al*, 2025).

While our study provides a strong foundation for identifying drug targets, further in vivo and in vitro studies are needed to confirm the biological functions of these proteins. Future research should also explore the mechanisms by which these targets influence drug sensitivity and resistance (Huibo *et al*., 2023, Liang J *et al*, 2025).

CONCLUSION

This study underscores the efficacy of SELDI-TOF-MS technology in pinpointing protein markers for early cancer diagnosis and drug development. The identification of six significant protein markers lays the groundwork for future research into targeted cancer therapies, thereby accentuating the potential of proteomics to advance pharmacological science. Future work will focus on further characterizing these markers to elucidate their roles in cancer progression and to explore their therapeutic potential.

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