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Serum proteomic spectral characteristics of acute myeloid leukemia and their clinical significance

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ABSTRACT. We investigated the differences between the serum proteomic spectral characteristics of acute myeloid leukemia (AML) patients and those of healthy people. We collected peripheral blood serum samples from 62 AML patients and 15 healthy controls. After removing high-abundance proteins, low-abundance serum proteins were separated using two-dimensional gel electrophoresis to identify differences between AML patients and healthy people. We investigated the different protein dots by mass fingerprint analysis, and evaluated the results using the Masort retrieval program provided by the MSDB protein bank. To further investigate the relationship between standard chemotherapy treatment efficacy and differences in protein patterns, we divided 21 patients into two groups (A and B) according to the efficacy of standard chemotherapy. Compared with the healthy cases, the AML patients demonstrated significant abnormal expression in 14 proteins ($P < 0.05$); α1-trypsin inhibitor ($P < 0.01$), prealbumin ($P < 0.01$), apolipoprotein E ($P < 0.010$), and apolipoprotein A-IV ($P < 0.01$) expression decreased, whereas haptoglobin HP2 ($P < 0.05$), serum

exogenous lectin ($P < 0.05$), H factor homologue protein ($P < 0.05$), and serum amyloid A1 ($P < 0.01$) expression increased. Further stratified analysis revealed that patients with high serum lectin expression had poor outcomes. The study revealed various proteins with differential expression levels in the peripheral blood of AML patients, and the difference in serum lectin expression is related to the efficiency of standard chemotherapy. Therefore, these proteins are potential diagnosis markers or prognostic indicators of AML.

Key words: Acute myeloid leukemia; Proteomics; AML biomarkers; Mass spectrometry; Lectin

INTRODUCTION

Acute myeloid leukemia (AML) is a widespread disorder that is characterized by the rapid clonal proliferation of immature myeloblasts (hematopoietic progenitors), and different degrees of myeloid differentiation in the bone marrow, peripheral blood, and extramedullary tissues (Vardiman et al., 2008). The French-American-British classification, which includes eight subgroups (M0-M7) according to the degree of AML differentiation and morphology, is still widely used in clinical settings.

The pathogeny of AML is responsible for cellular and genetic abnormality. The mutation of oncogenes contributes to changes in some protein kinases, and the survival and proliferation of hematopoietic stem cells or myeloblasts; deactivation of tumor suppressor genes and gene rearrangement leads to malignant transformation, the prevention of cell differentiation, and apoptosis disruption in hematopoietic stem cells or myeloblasts (Gilliland, 2001). Of approximately 12,330 people diagnosed with AML, 8950 died. Therapeutic decisions regarding targeted therapy for AML patients in clinical settings are crucially dependent on the characterization of the individual subtype of AML. At present, such decisions are mainly based on the cytogenetic and molecular alterations of blast cells, or morphology and the immunophenotype, without reference to a specific genetic marker. The analysis of genetic lesions, including gene expression changes, is particularly crucial for risk stratification, and to facilitate effective treatment design for patients with cytogenetically normal AML. Genes carry genetic information and proteins execute biological activities; therefore, further study into the mechanisms underlying AML at the protein level could significantly improve prognosis and targeted therapy in AML patients.

Proteomics technology has been widely applied to study hematological malignancies in some previous studies because it involves lager samples, is rapid, and has high sensitivity and specificity (Conrads et al., 2004; Petricoin and Liotta, 2004). For example, Zou et al. (2005), Albitar et al. (2006), and Mohamedali et al. (2009) have identified leukemia-related markers including Rho-GDP dissociation inhibitor auto-antibodies, alpha enolase, and aldolase enzyme A (Cui et al., 2005).

In the current study, we used a combination of traditional two-dimensional gel electrophoresis (2-DE)-based separation technology and matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF-MS) to compare the protein expression profiles of AML patients with those of healthy controls. Our objectives were to identify diagnostic markers to predict the prognosis of hematologic malignancies, explore the mechanisms underlying AML, and develop molecular targeted drugs.

MATERIAL AND METHODS

Subjects

AML was histologically confirmed in 62 subjects who had received no prior treatment. The subjects received chemotherapy in the hematological department of Zhangzhou Affiliated Hospital at Fujian Medical University between 2009 and 2011. The 62 subjects comprised 34 males and 28 females aged 21 to 73 years old; 13 had acute lymphoblastic leukemia (ALL), 48 had acute myeloid leukemia (5 M_1 cases, 19 M_2 cases, 4 M_3 cases, 4 M_4 cases, 14 M_5 cases, and 2 M_7 cases), and 1 patient had biphenotypic acute myeloid leukemia (BAL). We recruited 15 healthy people as controls (9 males and 6 females aged 19 to 54 years).

To further investigate the relationship between treatment efficacy and differences in protein patterns, we divided 21 patients into two groups (A and B) according to efficacy. Group A comprised 11 cases (5 M_2 cases, 4 M_3 cases, and 2 ALL cases) including 4 males and 7 females aged 22 to 56; all were in complete remission following a standard chemotherapy scheme of two courses, and a median follow-up time of 10 months (6-20 months). Group B comprised 10 cases (2 M_2 cases, 3 M_3 cases, and 5 ALL cases) including 7 males and 3 females aged 21 to 50; they were either not in complete remission or had experienced recurrence less than half a year after three courses of treatment. The diagnosis and efficacy standard of AML were both according to the reference (Zhang and Shen, 2007). The study was approved by the Ethics Committee, and written informed consent was obtained from all participants.

Reagents

Dithiothreitol, iodoacetamide, ammonium persulfate, urea, thiourea, and pancreatin were purchased from Sigma-Aldrich Co., Ltd.; an immobilized pH gradient (IPG) Drystrip (24 cm), pH 3-10, and IPG buffer, pH 3-10, were bought from GE Healthcare; and a ProteoMiner kit was obtained from Bio-Rad.

Instruments

Ettan IPGphor 3, ImageScanner III, and Ettan DALTsix were all from GE Healthcare life Sciences; Auto flex III MALDI TOF/TOF was from the Bruker Corporation.

Methods

Sample collection

In the morning, 5 mL intravenous blood was collected from all fasting subjects and centrifuged at 3000 r/min for 5 min. The supernatant was stored at -80°C. The Bradford method was used to measure protein concentration; high-abundance proteins were removed according the instructions provided with the Bio-Rad ProteoMiner kit and prepared for two-dimensional gel electrophoresis (2-DE).

Two-dimensional gel electrophoresis

The low-abundance serum proteins (125 μg) were separated by 2-DE and stained with

silver. The stained gel was scanned by Image Scanner III, and the results were analyzed using Image Master 2D Platinum 6.01 software to determine differences and obtain the molecular weight, isoelectric point (pI), and relative content of each protein.

Protein pattern of dimensional electrophoresis

Various protein dots were selected, cut from the gel, and digested using special enzymes. Peptide mass fingerprinting (PMF) was carried out by MALDI TOF/TOF-MS, and the results were evaluated using the Masort retrieval program provided by the MSDB protein bank. The mass spectrometry results of the proteins were verified by sequencing some peptides, and the reliability of the results was evaluated by the Mascore peptide matching rate.

Statistical analysis

SPSS version 13.0 was used for statistical analysis, and the data are reported as averages ± standard deviations. The *t*-test was carried out, and a P value < 0.05 was considered statistically significant.

RESULTS

Detection of different proteins in the two groups

There were differences in the expression levels of serum proteins between the AML and control groups. The Vol parameter of the Image Master software package was used to compare these differences. Initially, we calculated the OD values of the different protein dots and the Vol values. The results showed that there were 14 abnormal protein dots, in which D represented the expression of the protein dot and decreased, whereas U increased in the AML patients (Table 1).

Table 1. Vol parameter of various proteins in the acute myeloid leukemia (AML) and normal control groups.

	AML group	Normal control group
D1	0	7684.55 ± 1520.23
D2	0	8620.88 ± 1351.48
D3	0	7909.97 ± 2942.35
D4	0	2809.76 ± 2163.22
D5	0	4665.43 ± 1320.74
D6	0	5186.89 ± 5231.80
D7	0	9172.12 ± 1625.93
D8	0	4928.04 ± 5836.67
U1	3132.07 ± 1460.63	2112.77 ± 914.94
U2	4345.06 ± 2126.52	4460.77 ± 2313.72
U3	3180.90 ± 1460.70	4345.30 ± 1373.27
U4	5315.05 ± 3430.71	4402.67 ± 1116.94
U5	7891.03 ± 4169.80	2089.90 ± 238.06
U6	6730.86 ± 4096.02	1976.57 ± 261.57

Mass spectrometry identification of different proteins

Various protein dots were selected, cut, and subjected to in-gel enzymatic hydrolysis digestion. The extracted proteins were analyzed by MALDI TOF/TOF two-stage mass

spectrometry to obtain the peptide sequences, and then investigated using Masort to identify possible abnormal protein dots (shown in Table 2). The protein expression levels of α1-trypsin inhibitor, prealbumin (PA), apolipoprotein E, and apolipoprotein A-IV decreased, whereas those of haptoglobin HP2, serum exogenous lectin, H factor homologue protein, and serum amyloid A1 increased in the serum of the AML patients.

Table 2. Molecular weight, isoelectric point (pI), and relative content of the protein spectrum dots.

	Protein	Accession No.	Molecular weight (Da)	pI	Matching sequence number	Sequence overcast (%)
D1-D3	α1-trypsin inhibitor	gi|28637	22871	6.11	4	29%
D4	α1-trypsin inhibitor	gi|28637	22814	6.11	2	13%
D5-D6	prealbumin	gi|219978	15909	5.52	2	25%
D7	Apolipoprotein E	gi|178849	36188	5.65	3	10%
D8	Apolipoprotein A-IV	gi|17857	45353	5.33	2	4%
U1	Haptoglobin HP2	gi|223976	42344	6.23	3	15%
U2	Serum exogenous lectin	gi|1369904	33998	6.09	2	9%
U3-U4	H factor homologue protein	gi|183763	37637	7.75	2	7%
U5-U6	serum amyloid A1	gi|40316910	13524	6.28	3	40%

Relationship between differences in protein patterns and treatment efficiency

As shown in Table 3, group A achieved complete remission after 1-2 courses of standard chemotherapy; the median follow-up was 10 months (6-20 months), and the patients in the group remained in remission. We did not observe complete remission or relapse after remission in group B less than half a year after treatment with more than three courses of standard chemotherapy.

Table 3. Vol parameter values of the various proteins with different outcomes in acute myeloid leukemia (AML).

	Poor outcome	Good outcome
U1	3276.08 ± 1436.71	2976.03 ± 1533.59
U2	3922.13 ± 1538.71	4767.98 ± 2581.53
U3	3183.99 ± 1555.54	3178.58 ± 1455.87
U4	4409.62 ± 1998.37	6290.12 ± 4380.46a
U5	8746.68 ± 4349.91	6964.08 ± 3935.0
U6	8175.40 ± 4158.21	5154.99 ± 3557.35a

DISCUSSION

AML is a heterogeneous malignancy of the hematopoietic system that is induced by genetic and environmental factors. It involves a series of complex biological behaviors and is regulated by various factors. Recently, there have been significant improvements in the diagnosis and treatment of AML; many special fusion genes related to AML have been discovered, and their targeted drugs have been widely applied in clinical settings with satisfactory results (Zhang and Shen, 2007). However, there is also a lack of rapid, simple, and effective early diagnosis resources for clinical settings, especially serum tumor markers for AML.

The rapid development of proteomic technologies and methods has led to their widespread use for the detection of proteins in normal and tumor tissues to identify special tumor markers (Clarkson et al., 1975). MALDI-TOF-MS has become one of the core technologies in proteomics research; it has the merit of requiring small samples to determine

peptide sequences. Therefore, it is widely used in the early detection of cancer biomarkers, the development of therapeutic targets, and the investigation of the pathogenesis of the disease (Zhang et al., 2005; Omenn, 2006). Schwamborn et al. (2009) used MALDI-TOF-MS to establish a model for the diagnosis of bladder cancer. Orvisky et al. (2006) used MALDI-TOF to investigate 20 patients with primary liver cancer and 20 age-matched healthy people by analyzing serum proteins, removing high-abundance proteins, and concentrating them by denaturing ultrafiltration. There were 332 significant differential expression peaks, and 45 proteins were identified. Kawakami et al. (2005) used MALDI-TOF-MS to compare the serum of patients with hepatocellular carcinoma before and after radiofrequency ablation. After treatment, they found that several proteins, including pre-apoprotein, apolipoprotein A-4 precursor, and carboxyl terminal protein fragment of albumin, were expressed at low levels; and seven kinds of protein, including leucine α2 glycoprotein and α1-trypsin inhibitor, were expressed at high levels. Bai et al. (2013) used the method to identify serum candidate peptides for monitoring adult AML. Moreover, studies have shown that the alignment-based label-free quantitation of LC-MS/MS data sets can distinguish various types of leukemia (Foss et al., 2012; Kornblau et al., 2013; Elo et al., 2014).

In the current study, we analyzed the low-abundance serum proteins in the two groups using 2-DE combined with MALDI-TOF-MS after removing the high-abundance proteins. There were 14 abnormal protein expression spots. We discovered that the expression levels of alpha 1-trypsin inhibitor, PA, apolipoprotein E, and apolipoprotein A-IV decreased in the patient group, whereas those of haptoglobin HP2, serum exogenous lectin, and H factor homologue increased. Further stratified analysis showed that the expression of serum lectin was high and the corresponding treatment effect was poor in patients with AML. These differentially expressed proteins are involved in the body's nutritional metabolism, immune response, and cell adhesion. D8 and D9 were attributable to serum prealbumin (PA), which is synthesized by liver cells; its name derives from the fact that it precedes albumin on an electrophoresis gel. The metabolism of PA is relatively rapid. Therefore, there is a change in the level of PA in the early stages of liver function damage (Birkenmeier et al., 1984). When tumor cells infiltrate the liver and cause liver damage, they can cause PA to decrease to undetectable levels. The more serious the illness, the lower the level of PA expression. Chemotherapy drugs have some effect on the liver, but PA and liver function can be returned to normal in patients with AML after complete remission.

The decrease in the expression of PA might be a consequence of the body's reaction to AML, and may not be useful as a diagnostic marker for the disease. Moreover, further research is required into the relationships between the various proteins identified and AML.

CONCLUSIONS

It is crucial to confirm the individual subtype of AML for prognosis and therapy in clinical practice. Currently, without a specific genetic marker, the main approach depends on detecting the cytogenetic and molecular alterations of blast cells, or morphology and the immunophenotype. In the current study, we discovered various proteins with differential expression levels in the peripheral blood of patients with AML, and the differences in serum lectin expression were related to the efficiency of standard chemotherapy. Therefore, these proteins might be considered new diagnosis markers or prognostic indicators of acute myeloid leukemia.

Conflicts of interest

The authors declare no conflict of interest.

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