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Analysis of serum proteome profiles of non-Hodgkin lymphoma for biomarker identification

Ming Zhi Zhang, Zhen Chang Sun, Xiao Rui Fu, Fei Fei Nan, Qing Xia Fan, Xing Ai Wu, Li Geng, Wang Ma, Rui Lin Wang*

Department of Medical Oncology, First Affiliated Hospital, Zhengzhou University, 450052, Zhengzhou, Henan, China

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ABSTRACT

Serum samples from non-Hodgkin lymphoma (NHL) patients who had not undergone chemotherapy, lymphnoditis patients, and healthy adults were analyzed using surface-enhanced laser desorption–ionization time-of-flight mass spectrometry (SELDI-TOF MS) to detect the differentially expressed serum proteins. Models were developed to distinguish between the healthy adult group and the NHL group, with a sensitivity of 69% and specificity of 90%, and between the lymphnoditis group and the NHL group with a sensitivity of 74% and specificity of 84%. A protein with the *m/z* of M10 197.91 u was expressed at a significantly higher level in the NHL group, compared to the other groups. Furthermore, differences were also significant among different stages of NHL and among samples with different International Prognosis Index (IPI) scores or lactase dehydrogenase (LDH) levels. The three identified proteins may offer a new serological approach for early diagnosis, differential diagnosis, and pathogenic investigation of NHL. And the protein with the *m/z* of M10 197.91 u may be a new serological biomarker for monitoring treatment response and evaluating the prognosis of patients with NHL.

1. Introduction

Non-Hodgkin lymphoma (NHL) originates primarily from lymph node and other lymph tissue. It is one of the common malignant tumors in China and its incidence is increasing [1]. NHL is a malignant clonal disease with multiple molecular abnormalities, some of which can be detected in the blood [2]. Investigations into the molecular parameters of NHL have yet not discovered clinically useful biomarkers with high sensitivity and specificity. Moreover, NHL is a heterogeneous group of diseases resulting from multiple factors, which makes it difficult to use a single biological marker for evaluation. Therefore, discovery of a highly sensitive and specific NHL marker is still a primary goal for the research in the differential diagnosis, pathogenesis, treatment response monitoring, and prognostic evaluation of NHL.

Surface-enhanced laser desorption–ionization time-of-flight mass spectrometry (SELDI-TOF MS) is a new technology which allows high-throughput detection of proteins. The surface of the protein chips bind to specific proteins and thereby measure tumor markers in biological samples [3], which makes it widely used in clinical oncology research of cancers such as breast cancer [4], prostate cancer [5], malignant tumors in alimentary system [6,7], and ovarian cancer [8]. In this study, we aimed to detect differentially expressed proteins in serum of the healthy adults, lymphnoditis patients and non-Hodgkin lymphoma patients with this assay, so as to identify proteins differentially expressed proteins in NHL. Furthermore, we aimed to analyze the relationship between these proteins and the staging, International Prognosis Index (IPI) score, and lactase dehydrogenase (LDH) level and to determine their significance in the early

* *Corresponding author*. Tel.: +86 13838565629.
E-mail address: mingzhi_zhang@126.com (M.Z. Zhang).

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diagnosis, differential diagnosis, pathogenesis research, treatment response monitoring, and prognosis evaluation of NHL.

2. Materials and methods

2.1. Sample sources

Samples were obtained from 35 patients with NHL (20 men and 15 women) ranging in age from 16 to 73 years (median, 41). Types of NHL included diffuse large B cell lymphoma ($n=18$), mantle cell lymphoma ($n=5$), and peripheral T cell lymphoma ($n=12$). Bone marrow involvement (BMI) was positive in 18 cases. Serum LDH level was normal in 19 cases, and was elevated in 16 cases (normal, 75–240 U/L).

Clinical stages were as follows: stage I ($n=11$), stage II ($n=4$), stage III ($n=9$), and stage IV ($n=11$), according to the World Health Organization tumor classification (2001). Risk was classified according to IPI scoring: low risk ($n=15$), low-intermediate risk ($n=12$), high-intermediate risk ($n=8$); and high risk cases ($n=0$). IPI scoring method assigns one point for each of the following risk factors: age greater than 60 years; Stage III or IV disease; elevated serum LDH; ECOG/Zubrod performance status of 2, 3, or 4; more than 1 extranodal site. Risk is determined by point total: low risk (0–1 points), low-intermediate risk (2 points), high-intermediate risk (3 points), high risk (4–5 points).

The 32 lymphnoditis patients ranged in age from 19 to 70 years (median, 38). In all cases, tuberculous lymphnoditis and NHL were ruled out and inflammatory lymphnoditis was diagnosed. In both NHL patients and lymphnoditis patients, diagnosis was established using pathomorphological and immunohistochemical evidence and was confirmed by at least two pathologists.

The 29 healthy adults, with no previous lymphnoditis (during the last two years) or history of non-Hodgkin lymphoma, were recruited from the staff of our department and from graduate student volunteers. In all three groups, conditions which could influence serum protein expression were ruled out (e.g., acute infection, hypersensitiveness, autoimmune disease, HIV, hepatitis C). The hospital ethics committee reviewed and approved the study procedures.

2.2. Sample collection

Fasting venous blood sample (5 mL) was obtained, held for 30 min at room temperature, and then centrifuged for 20 min at 2000 rpm (centrifuge radius 6 cm). Aliquots of the supernatant were frozen (100 μL per tube) in liquid nitrogen or −80 °C freezer.

2.3. Protein detection

2.3.1. Reagents and equipment

Urea, acetonitrile (ACN), trifluoroacetic acid (TFA), 3-(Cyclohexylamino)-1-propanesulfonic acid (CHAPS) and 1,4-Dithiothreitol (DTT) were purchased from Sigma (USA); saturated sinapinic acid (SPA) was purchased from Ciphergen Biosystems (USA); Tris–Cl and sodium acetate (NaAc) were purchased from Shanghai Bioengineering Company (China); IMAC-Cu chip and array reader BPS were purchased from Ciphergen Biosystems (USA).

2.3.2. Sample preparation

Thawed samples (50 μL) were centrifuged for 5 min at 4 °C (centrifuge radius 6 cm, 10,000 rpm); 10 μL aliquots were diluted with 20 μL U9 buffer (9 mol/L urea, 2%CHAPS, 2 mmol/L DTT, and 50 mmol/L Tris–HCl, pH 9), and then agitated for 30 min at 4 °C. The supernatant (10 μL) was mixed with 110 μL binding buffer (50 mmol/L NaAC, pH 3.5), and the mixture was agitated for 5 min and preserved for subsequent use.

2.3.3. Pre-treatment of IMAC-Cu chip

The IMAC-Cu chip was placed on the operation platform and 50 μL of 100 mmol/L copper sulfate was added to each well. The IMAC-Cu chip was agitated for 5 min, and the copper sulfate was removed. The chips were washed with deionized water 5 times, air-dried, and then 50 μL of 100 mmol/L NaAC

Table 1 – Comparison of protein expression levels between the healthy adult group and the lymphnoditis group.

m/z	P	Mean — B	SD — B	Mean — normal	SD — normal
11367.01	4.99E−08	0.276318	0.26273	1.774807	1.287772
9069.605	2.56E−07	2.655808	1.310218	0.728386	0.516501
8877.146	6.25E−07	4.003676	2.537409	1.011595	1.069748
15876.69	8.68E−07	0.500379	0.28912	2.239525	1.994412
15132.14	9.68E−07	0.082527	0.126036	1.420464	2.724015
4218.389	3.82E−06	2.59557	3.337851	9.461398	4.522528
14042.78	3.82E−06	0.511901	0.722359	2.230107	1.64605
7806.736	8.16E−05	5.897931	3.451715	2.421102	2.062709
15334.74	0.000116	0.075554	0.135964	0.601358	1.003298
9285.411	0.00015	11.07983	5.534165	4.389976	2.485554
10178.27	0.000249	1.079031	1.295938	0.258427	0.308371
5905.252	0.000294	17.86736	8.793137	8.187633	4.082968
4595.754	0.000376	4.323531	4.683008	1.233094	1.741125
5833.399	0.000408	5.955121	4.520935	1.210735	1.20743
4529.958	0.001508	2.114289	2.285382	4.282039	3.237387
9343.528	0.003777	4.622486	3.712924	1.817981	0.905535
8932.98	0.005275	5.531962	3.020207	3.205655	2.327105
8520.353	0.006413	1.724179	2.34908	0.306013	0.548848
7564.629	0.00729	0.920167	1.057982	2.309978	2.589552
6431.209	0.011974	4.149754	2.860421	6.510395	3.388883
9407.223	0.013499	5.746216	2.993109	3.548133	2.314138
9187.992	0.047645	15.66859	18.97568	2.547505	3.317214
6629.908	0.052632	9.458952	6.346274	12.83245	5.556674
7933.693	0.105558	2.374022	1.243267	3.494185	2.372889
7764.836	0.193927	18.62955	11.39222	13.43508	7.836218
7666.889	0.259032	0.872118	0.576735	0.759674	1.074597
5504.915	0.306685	1.617903	1.697922	0.88312	0.864683
5703.476	0.394305	4.688573	5.852617	2.043327	2.701845
7964.069	0.418375	3.345798	2.931347	2.218996	1.299533
11718.58	0.495575	2.749743	2.439982	1.837655	1.220688
6665.556	0.550985	2.880348	2.881966	2.034839	3.560278
6006.292	0.701478	5.752342	2.294344	5.913547	2.861117
6331.805	0.798298	1.92646	1.759933	2.005877	2.143924
8766.581	0.798298	1.517858	1.34044	1.199757	0.72599
8141.282	0.881497	4.276354	3.324264	3.598778	2.010122
5339.595	0.915202	4.26495	4.046816	3.145977	2.205661

A total of 36 proteins were identified and the difference between groups was significant for 22 mass spectra peaks ($P<0.05$).