Am J Transl Res 2019;11(10):6522-6533
www.ajtr.org /ISSN:1943-8141/AJTR0088650

Original Article

Proteomic analysis for identifying the differences in molecular profiling between fanconi anaemia and aplastic anaemia

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Received November 19, 2018; Accepted April 30, 2019; Epub October 15, 2019; Published October 30, 2019

Abstract: Treatment and prognosis of Fanconi anaemia (FA) and acquired aplastic anaemia (AA) differ. However, delayed and inappropriate treatments are administered in FA due to its similarities to AA in presentation. The objective of the current study was to elucidate differences between the molecular mechanisms underlying FA and AA as well as to identify biomarkers and pathways associated with FA via bioinformatics analyses. Proteomic data were obtained from bone marrow samples of patients with FA and AA. Gene ontology analysis was performed using a Database for Annotation, Visualization and Integrated Discovery. KEGG pathway enrichment analyses were conducted using the ClueGO plug-in in Cytoscape. A DEP-associated protein-protein interaction (PPI) network was constructed using STRING and visualized in Cytoscape. A total of 114 DEPs, including 71 upregulated proteins and 43 downregulated proteins, were present in the FA samples, compared with those in the AA samples. Upregulated proteins were enriched in the nucleosome assembly, canonical glycolysis, glycolytic process, and the glycolysis/gluconeogenesis pathway, whereas downregulated proteins were enriched in relation to immune response, negative regulation of apoptosis, proteolysis and CoA biosynthesis. Eight hub proteins with a high degree of connectivity were obtained as follows: alpha-enolase (ENO1), HSP90AA1, phosphoglycerate kinase 1 (PGK1), HSP90AB1, ACTC1, ACTBL2, EEF1A1 and CFL1. Upregulation of ENO1 and CFL1 in patients with FA was confirmed through a WB experiment, and substantiated by the results of data analyses. Bioinformatics analyses are useful for identification of biomarkers and pathways associated with FA and AA. Some crucial DEPs, such as ENO1, PGK1, ACTC1, ACTBL2, EEF1A1 and CFL1, may play an important role in FA and show potential as serological markers for its early diagnosis.

Keywords: Fanconi anemia, aplastic anemia, proteomics, bioinformatics analysis, biomarker

Introduction

Fanconi anaemia (FA) is a rare autosomal recessive genetic disease. Most patients are characterized by bone marrow failure as well as congenital malformations. However, some patients present with complex and diverse clinical manifestations, indicating the difficulties involved in differentiating between FA and AA [1-4]. Aplastic anaemia comprises a group of pathogenies leading to the bone marrow failure syndrome, bone marrow hematopoietic hypoplasia, and peripheral blood pancytopenia. It is characterized by clinical anaemia, bleeding, and infection [5, 6]. Valid methods for identifying biomarkers useful for the screening and diagnosis of FA as opposed to AA are very important. Proteomics focuses on the composition, content and signalling pathways of proteins in cells, tissues or organisms. Networks, pathways and protein-protein interactions (PPIs) involved in FA and AA can be elucidated using this new technology [7].

In this analysis, we performed a proteomic analysis of the marrow of patients with FA and AA to detect differentially expressed proteins (DEPs). We examined biological processes (BP), molecular functions (MF), and cellular components (CC) using the Database for Annotation, Vi-

sualization and Integrated Discovery (DAVID) [8, 9] and the Kyoto Encyclopaedia of Genes and Genomes (KEGG) pathways of DEPs via ClueGO [10]. We identified 5 key modules, established the PPI network of DEPs and selected 8 hub proteins with a high degree of connectivity as follows; alpha enolase (ENO1), HSP90AA1, phosphoglycerate kinase 1 (PGK1), HSP90AB1, ACTC1, ACTBL2, EEF1A1 and CFL1. These proteins may play an important role in FA, serve as new biomarkers for its diagnosis and guide its combination therapy.

Materials and methods

Patient samples

The study group was composed of a patient with FA and a patient with AA. The findings of chromosomal fracture experiments of FA patients examined at the Children's Hospital of Soochow University were negative. Information was obtained from primary pathological reports. All human bone marrow tissue samples used in this study were approved by the Hospital Research Ethics Committee.

Protein preparation and TMT labelling

Cells in the marrow and blood were harvested at 12,000 *g* for 10 min at 4°C. Collected sediment was washed thrice with PBS buffer (pH 7.4). The samples were ground into powder in liquid nitrogen, and proteins extracted using lysis buffer. The suspension was sonicated at 200 W for 15 min and centrifuged at 12,000 *g* for 15 min at 4°C. The supernatant was mixed well with 5 volumes of chilled acetone containing 10% (v/v) TCA, incubated overnight at 20°C, centrifuged at 12,000 *g* for 15 min at 4°C, and discarded. The precipitate was washed thrice with chilled acetone. The pellet was air dried and dissolved in lysis buffer. Next, in-gel digestion was performed.

Proteins were digested with trypsin at a protein:trypsin ratio of 30:1 at 37°C for 16 h. Peptides were dried via vacuum centrifugation following digestion by trypsin. The peptides were reconstituted and processed with a TMT label in accordance with the manufacturer's protocols. The labelled peptides were used for mass spectrometry and identification.

LC-MS/MS analysis using an Orbitrap elite hybrid mass spectrometer

Each fraction was resuspended using buffer A (5% CAN and 0.1% FA), and centrifuged at 12,000 *g* for 10 min. The final peptide concentration was approximately 0.5 μg/μL. Approximately 10 μL of the peptides were injected into nanoHPLC using an auto sampler on a 2 cm C18 trap column.

Identification of proteins through mass spectrometry

The largest dataset selected was Swiss-Prot (Human), which included 20,316 protein sequences. The results were exported to Microsoft Excel for further analysis. Quantitative protein ratios were weighted and normalized. Ratios with fold changes >1.50 were considered significant.

Bioinformatics analysis

For each gene list, pathway and process enrichment analyses were carried out using the following ontology sources: KEGG Pathway, GO Biological Processes, Reactome Gene Sets, Canonical Pathways, and CORUM. All genes in the genome were used as the enrichment background. Terms with a *p*-value <0.01, a minimum count of 3, and an enrichment factor (ratio of observed counts to expected counts due to chance) >1.5 were collected and grouped into clusters based on their membership similarities. The most statistically significant term within a cluster was chosen to represent that cluster. PPI enrichment analysis reflected the interactions of DEPs, wherein the interacting protein complex functionally affected the physiological process. The MCODE algorithm was used to identify densely connected network components. GO analysis was performed to detect molecular functions, biological processes and cellular components with DAVID (https://david.ncifcrf.gov/). ClueGo and MCODE plug-ins in Cytoscape were used to perform pathway and process enrichment analyses and PPI enrichment analysis.

Statistical analysis

Differences between groups were analysed using Student's t-test or the one-way ANOVA test. Statistical significance was set at $P<0.05$.

Table 1. General information of children with AA and FA

Number	Samples	Disease type	Chromosomal break test	Gene mutation sites	Parents carry genetic conditions
1	Marrow	FA	Negative	*BRCA2* gene with two heterozygous mutations: c.*T943A* (exon10), c.*T7469C* (exon15)	Each of the parents carry a mutant gene
2	Marrow	AA	Negative	Negative	

Statistical analyses and the drawing of plots were performed using GraphPad Prism 6.01 software (La Jolla, CA, USA).

Results

Sample information and gene test results of patients with FA

Experimental samples were obtained from the bone marrow of one patient with FA and one patient with AA. The patient diagnosed with FA exhibited defects as indicated by the FA genetic test, and carried 2 heterozygous mutations in *BRCA2*: *c.T943A* (exon10) and *c.T7469C* (exon15) (**Table 1**), inherited from the father and the mother, respectively.

Statistical analysis of FA and AA proteomics

The largest dataset selected was Swiss-Prot (Human). A total of 20,316 unique protein sequences were obtained and matched with 564 proteins in the bone marrow. The workflow of the proteomic analysis is shown in **Figure 1A**. Identification of the physicochemical properties of proteins generally reflect protein derivation in peptides and proteome sample identification, and therefore require technical and biological evaluation. In our study, the sequence coverage of most identified proteins was satisfactory (**Figure 1B**). In the sequence coverage of 350 proteins, 62% proteins were distributed within the 95% confidence interval (CI), and 17.9% proteins (101 proteins) were within the 80% CI. Therefore, a mass distribution was constructed (**Figure 1C**); the molecular weights of 90% of the proteins were between 10-90 kDa, whereas those of 5 proteins were less than 10 kDa, and those of 65 proteins were higher than 90 kDa. The number of PSMs that matched the proteins is shown in **Figure 1D**. The number of PSMs may be used to quantify map counts. Hence, this index provides an approximate indication of the distribution of proteins with different levels of abundance. The distribution of isoelectric points of identified proteins is displayed in **Figure 1E**. Sequence coverage and the number of PSMs matching the proteins showed that protein identification was credible. The distribution of molecular weights and isoelectric points of the identified proteins were reasonable, as well. A total of 114 DEPs, including 71 upregulated proteins and 43 downregulated proteins, were identified in the FA samples compared with those in the AA samples.

GO function and KEGG pathway enrichment analysis of DEPs

GO analysis of BP, MF, and CC of the bone marrow samples were performed using the DAVID website. The results of DAVID revealed that upregulated and downregulated DEPs had a total of 117 GO terms. GO analysis indicated that upregulated DEPs were associated with nucleosome assembly, canonical glycolysis, respiratory burst, cell or subcellular component movement and the glycolytic process, whereas downregulated genes were mainly involved in cellular protein metabolic processes, immune response, negative regulation of apoptosis, proteolysis and retinal homeostasis. Details of the top 5 GO terms of BP, MF, and CC are shown in **Table 2**.

KEGG pathway enrichment analysis was performed using ClueGO in Cytoscape. KEGG results indicated that upregulated DEPs were mainly associated with glycolysis/gluconeogenesis, leukocyte trans-endothelial migration, the estrogen signalling pathway, the pentose phosphate pathway, the glucagon signalling pathway, propanoate metabolism and pyruvate metabolism. Downregulated DEPs were associated with lysosomes, autophagy, phagosomes, pantothenate, CoA biosynthesis and the neurotrophin signalling pathway (**Figure 2A**, **2B** and **Table 3**).

PPI network of DEPs

PPI relationships of the 114 DEPs were obtained using STRING and visualized using Cytoscape. In STRING, active interaction sources included textming, experiments, databases, coexpression, neighbourhood, gene fusion, and

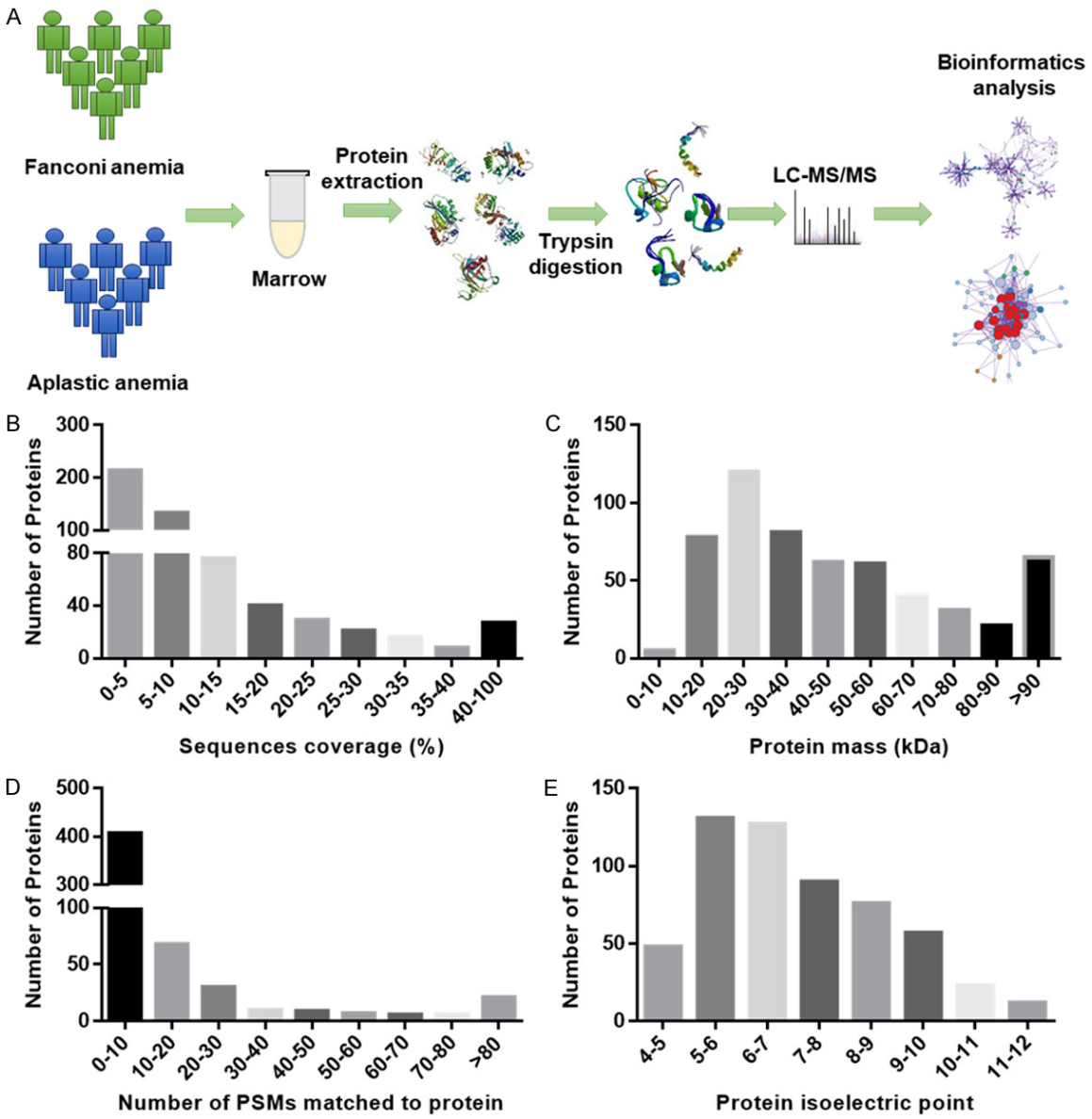

Figure 1. Statistical analysis of proteomic data of FA and AA. A. Sample processing and data analysis procedure used for proteomic analysis of FA and AA. B. Sequence coverage distribution of identified proteins. Results showed that protein coverage distribution was reasonable. C. The protein mass distribution of identified proteins. D. Number of PSMs matching the identified proteins. E. The distribution of protein isoelectric points of identified proteins.

co-occurrence. PPIs with an interaction score >0.4 (medium confidence) were selected, and a total of 415 PPI relationships were generated. The total PPI network of DEPs is shown in **Figure 3**. Based on the PPI network, the top 8 hub genes showing a high degree of connectivity were selected as the hub proteins (**Table 4**).

The hub proteins, which play an important role in FA progression, were alpha-enolase (ENO1, degree: 33), isoform 2 of heat shock protein HSP 90-alpha (HSP90AA1, degree: 29), phosphoglycerate kinase 1 (PGK1; degree: 23), heat shock protein HSP 90 beta (HSP90AB1, degree: 22), actin (degree: 21), beta-actin-like protein 2 (ACTBL2, degree: 21), elongation factor 1-alpha 1 (EEF1A1, degree: 20) and cofilin-1 (CFL1, degree: 20).

Module and hub protein analysis

A PPI enrichment analysis was conducted, and the 5 most significant sub-modules of DEPs were extracted from the PPI network [11]. Mo-

Table 2. Top five GO functions from the enrichment analyses of upregulated and downregulated DEPs

Expression	Category	Term	Count	%	*p* value
Upregulated	GOTERM_BP_DIRECT	Nucleosome assembly	8	12.3	2.70E-07
	GOTERM_BP_DIRECT	Canonical glycolysis	5	7.7	2.40E-06
	GOTERM_BP_DIRECT	Respiratory burst	4	6.2	1.30E-05
	GOTERM_BP_DIRECT	Movement of cell or subcellular component	6	9.2	1.60E-05
	GOTERM_BP_DIRECT	Glycolytic process	4	6.2	2.60E-04
	GOTERM_CC_DIRECT	Extracellular exosome	51	78.5	8.50E-29
	GOTERM_CC_DIRECT	Focal adhesion	17	26.2	2.80E-13
	GOTERM_CC_DIRECT	Membrane	27	41.5	6.90E-09
	GOTERM_CC_DIRECT	Cytosol	32	49.2	3.20E-08
	GOTERM_CC_DIRECT	Vesicle	8	12.3	3.20E-07
	GOTERM_MF_DIRECT	Poly(A) RNA binding	18	27.7	6.50E-07
	GOTERM_MF_DIRECT	Protein binding	52	80	4.20E-06
	GOTERM_MF_DIRECT	Nucleosomal DNA binding	5	7.7	2.70E-05
	GOTERM_MF_DIRECT	Structural constituent of cytoskeleton	6	9.2	6.00E-05
	GOTERM_MF_DIRECT	Superoxide-generating NADPH oxidase activity	3	4.6	7.60E-04
Downregulated	GOTERM_BP_DIRECT	Cellular protein metabolic process	5	14.3	8.10E-05
	GOTERM_BP_DIRECT	Immune response	6	17.1	1.30E-03
	GOTERM_BP_DIRECT	Negative regulation of apoptotic process	6	17.1	1.80E-03
	GOTERM_BP_DIRECT	Proteolysis	6	17.1	2.70E-03
	GOTERM_BP_DIRECT	Retina homeostasis	3	8.6	2.80E-03
	GOTERM_CC_DIRECT	Extracellular exosome	28	80	1.80E-16
	GOTERM_CC_DIRECT	Extracellular space	18	51.4	3.70E-11
	GOTERM_CC_DIRECT	Lysosome	7	20	3.40E-06
	GOTERM_CC_DIRECT	Melanosome	5	14.3	3.60E-05
	GOTERM_CC_DIRECT	Cell surface	7	20	4.50E-04
	GOTERM_MF_DIRECT	Serine-type endopeptidase activity	7	20	8.80E-06
	GOTERM_MF_DIRECT	Protein binding	25	71.4	1.30E-02
	GOTERM_MF_DIRECT	Histone binding	3	8.6	2.40E-02
	GOTERM_MF_DIRECT	Heparin binding	3	8.6	3.90E-02
	GOTERM_MF_DIRECT	Lysophospholipase activity	2	5.7	4.80E-02

dule 1 (Molecular Complex Detection [MCODE] score =7.263) was constructed with 20 nodes and 69 edges (**Figure 4A**); module 2 (MCODE score =6.5000) was constructed with 9 nodes and 26 edges (**Figure 4B**); module 3 (MCODE score =5.000) was constructed with 5 nodes and 10 edges (**Figure 4C**); module 4 (MCODE score =4.000) was constructed with 4 nodes and 6 edges (**Figure 4D**); and module 5 (MCODE score =4.000) was constructed with 4 nodes and 6 edges (**Figure 4E**). Further GO analysis of the MCODE 1 component mainly focused on translational initiation, SRP-dependent co-translational protein targeting the membrane, nuclear-transcribed mRNA catabolic process, nonsense-mediated decay, rRNA processing, translation and canonical glycolysis (**Table 5**). KEGG pathway enrichment analyses were associated with carbon metabolism, antibiotic biosynthesis, ribosomes, glycolysis/gluconeogenesis, amino acid biosynthesis, glucagon signalling pathway, pentose phosphate pathway, and the estrogen signalling pathway (**Table 6**). Furthermore, to verify results of the data analysis, we collected samples from patients with AA and FA, and investigated changes in key proteins using western blot analysis. The results showed that compared with AA, ENO1 and CFL1 were upregulated in FA, while WB results were consistent with those of the data analysis (**Figure 4F**).

Discussion

FA is an autosomal recessive disorder characterized by progressive bone marrow hematopoi-

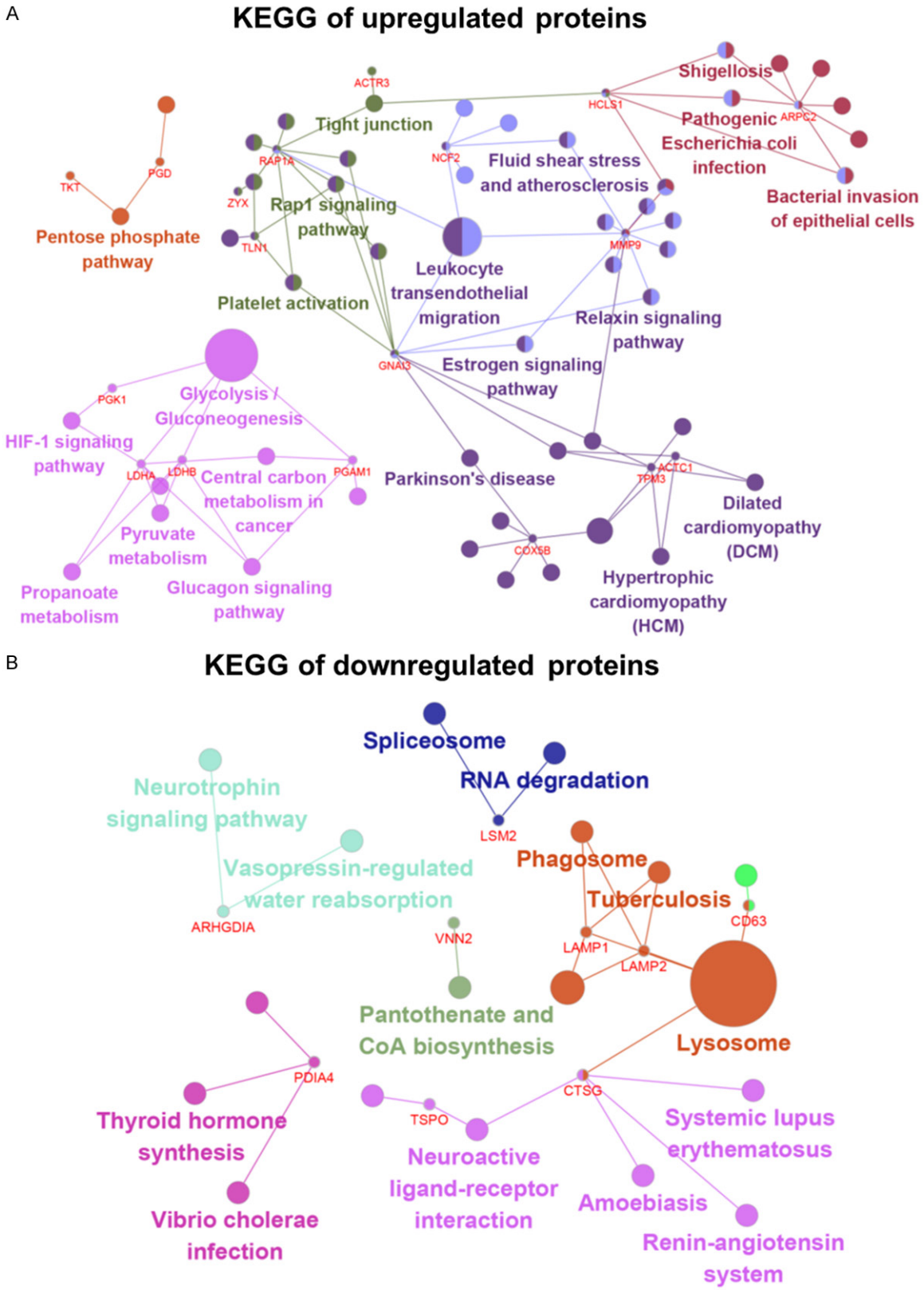

Figure 2. Signalling pathway enrichment analysis of DEP function in AA and FA. A. KEGG pathways of upregulated proteins. Upregulated DEPs were mainly associated with glycolysis/gluconeogenesis, leukocyte transendothelial migration, the estrogen signalling pathway and the pentose phosphate pathway. B. KEGG pathways of downregu-

lated proteins. Downregulated DEPs were associated with lysosomes, autophagy, phagosome, pantothenate and CoA biosynthesis and the neurotrophin signalling pathway. DEPs functional and signalling pathway enrichment was conducted using Cytoscape. DEPs, differentially expressed proteins; KEGG, Kyoto Encyclopaedia of Genes and Genomes.

Table 3. KEGG pathway analysis of DEPs associated with FA

Expression	Terms	Description	*p* Value	Number of proteins
Upregulated	KEGG:00010	Glycolysis/Gluconeogenesis	2.78435E-05	4
	KEGG:04670	Leukocyte trans-endothelial migration	0.000197977	4
	KEGG:04260	Cardiac muscle contraction	0.000943429	3
	KEGG:00030	Pentose phosphate pathway	0.002019859	2
	KEGG:04922	Glucagon signalling pathway	0.002117107	3
	KEGG:00640	Propanoate metabolism	0.00229856	2
	KEGG:00620	Pyruvate metabolism	0.00341019	2
	KEGG:04611	Platelet activation	0.003521256	3
Downregulated	KEGG:04142	Lysosome	4.65034E-06	4
	KEGG:04140	Autophagy	0.005296114	2
	KEGG:04145	Phagosome	0.007417878	2
	KEGG:05152	Tuberculosis	0.010204301	2
	KEGG:00770	Pantothenate and CoA biosynthesis	0.011122788	1
	KEGG:04614	Renin-angiotensin system	0.014211982	1
	KEGG:04080	Neuroactive ligand-receptor interaction	0.023839615	2
	KEGG:04962	Vasopressin-regulated water reabsorption	0.027181831	1
	KEGG:05110	Vibrio cholerae infection	0.030885554	1
	KEGG:04918	Thyroid hormone synthesis	0.045688094	1

etic failure, multiple congenital anomalies, and predisposition to neoplastic disease [12]. Some studies have shown that clinical manifestations of FA may not be typical, especially in those patients who present as chimeras, and easily lead to misdiagnoses or missed diagnoses [13]. Currently, only chromosomal aberrations induced by mitomycin C or diepoxybutane can be distinguished from other diseases, such as AA [14, 15]. To date, systematic studies comparing FA and AA, or comparative proteomic analyses of FA and AA have been rarely performed. As a part of this study, proteomics was conducted to examine the differences in the functions, signalling pathways and PPI in bone marrow samples of FA and AA patients. This study could be used for the identification of appropriate proteomic protocols and biomarkers as well as for the determination of FA and AA. A total of 114 DEPs, including 71 upregulated proteins and 43 downregulated proteins, were identified. GO function and KEGG pathway analyses of DEPs were performed to enhance the understanding of these DEPs. GO analysis indicated that the upregulated proteins were mainly associated with the following functions: nucleosome assembly, canonical glycolysis, respiratory burst, cell or subcellular component movement, and glycolytic process, whereas downregulated proteins were mainly associated with cellular protein metabolic processes, immune response, negative regulation of apoptosis, proteolysis, and retinal homeostasis. The hub proteins were analysed and selected through degree calculation. Our findings indicate that ENO1, HSP90AA1, PGK1, HSP90AB1, ACTC1, ACTBL2, EEF1A1 and CFL1 may play key roles in FA and also function as diagnostic markers.

ENO1 was the most significant hub protein. It is a multifunctional enzyme that participates in glycolysis and other processes, such as hypoxia tolerance, growth control and allergic responses [16-19]. It can activate plasminogen on cell surfaces and also functions in the intravascular and pericellular fibrinolytic system. It stimulates immunoglobulin production and participates in the immune system. ENO1 is upregulated in FA. Thus, its functional features may

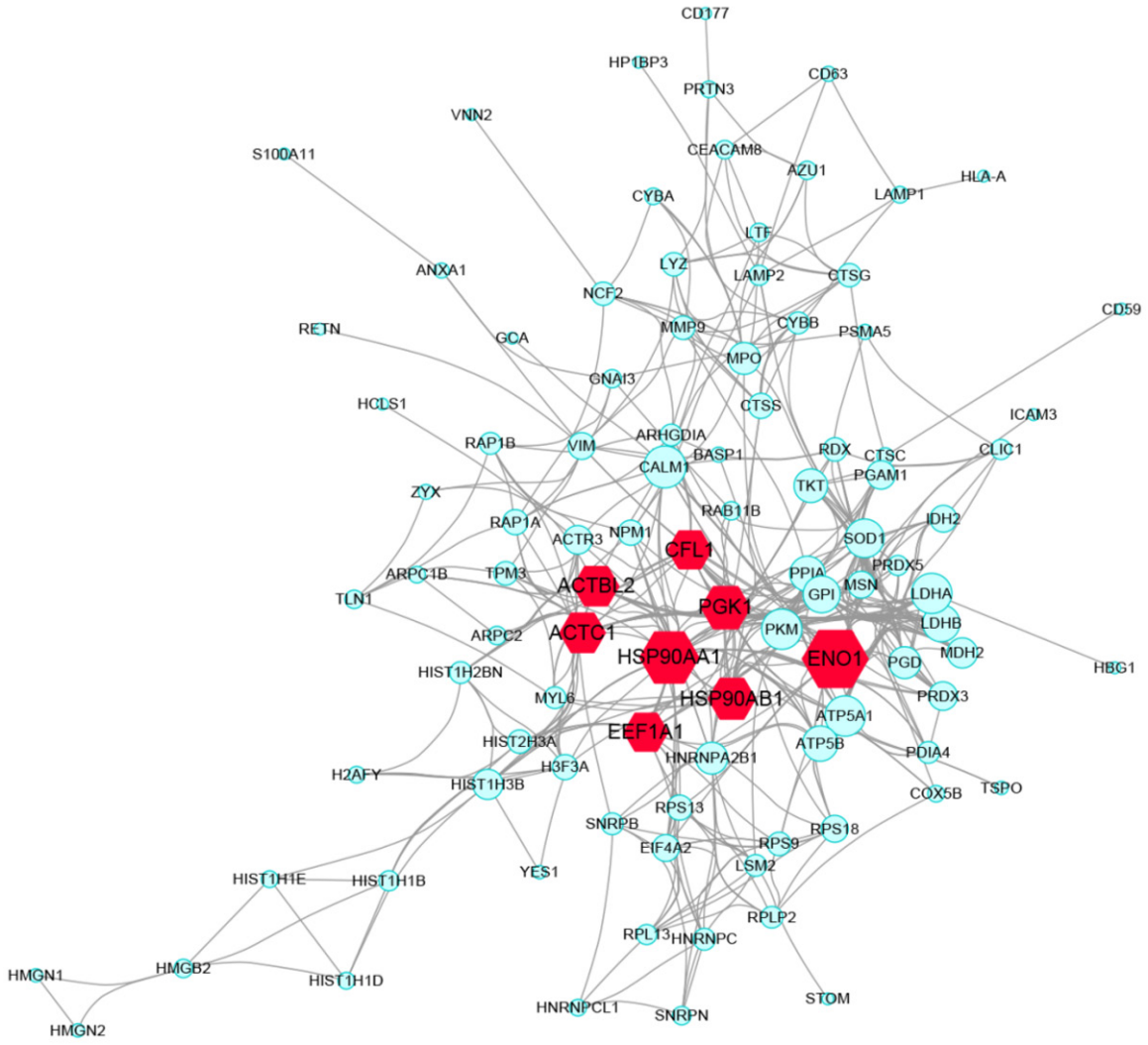

Figure 3. Protein-protein interaction between DEPs, (PPI) network complex and modular analysis. Using the STRING online database, total of 114 DEPs (including 71 upregulated proteins and 43 downregulated proteins) were filtered into the DEGs PPI network complex. Red labels represent hub genes. The size of the edge was determined on the basis of the degree score of proteins.

Table 4. Top hub proteins in the PPI network based on degree

Name	Degree	Eccentricity	Betweenness	Stress	Bridging	Centroid
ENO1	33	0.2	1496.064246	11498	15.15034088	0
HSP90AA1	29	0.25	1487.316057	14224	17.33456018	0
PGK1	23	0.2	244.8566235	3848	6.987651367	-26
HSP90AB1	22	0.25	696.3301408	9648	16.4548778	-12
ACTC1	21	0.25	546.361113	8242	12.90466075	-21
ACTBL2	21	0.25	546.361113	8242	12.90466075	-21
EEF1A1	20	0.25	512.4611003	6102	15.37382408	-25
CFL1	20	0.2	387.9301232	3856	11.66773024	-25

partially explain changes in the immune function of FA. PGK1 acts as a polymerase alpha cofactor protein and glycolytic enzyme [20-23]. It is involved in the synthesis of pyruvate from

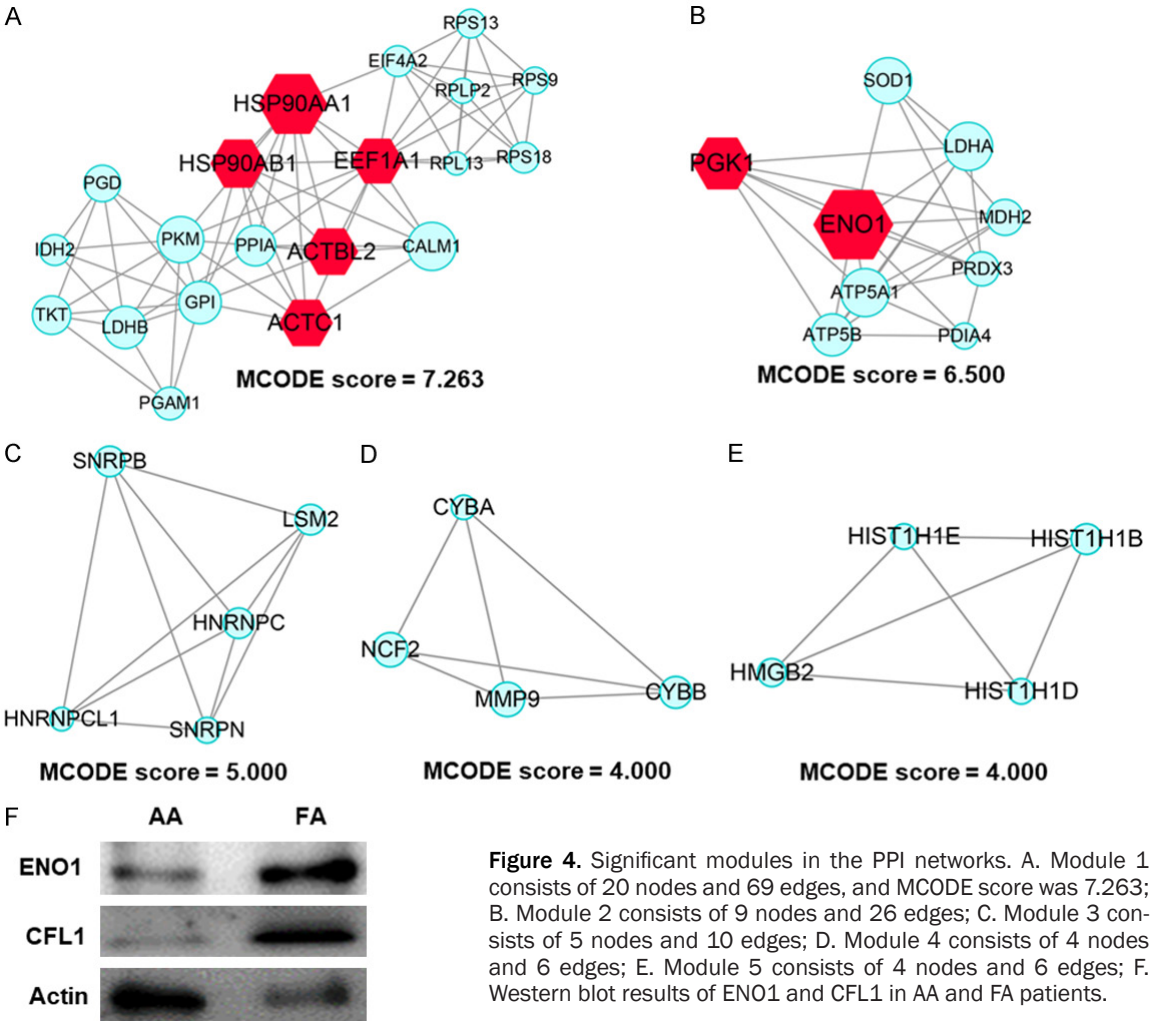

Figure 4. Significant modules in the PPI networks. A. Module 1 consists of 20 nodes and 69 edges, and MCODE score was 7.263; B. Module 2 consists of 9 nodes and 26 edges; C. Module 3 consists of 5 nodes and 10 edges; D. Module 4 consists of 4 nodes and 6 edges; E. Module 5 consists of 4 nodes and 6 edges; F. Western blot results of ENO1 and CFL1 in AA and FA patients.

Table 5. Top 10 GO functional enrichment analyses of the most significant DEP modules

Category	Term	Count	%	*p* Value
GOTERM_BP_DIRECT	Translational initiation	6	30	2.60E-07
GOTERM_BP_DIRECT	SRP-dependent cotranslational protein targeting to membrane	5	25	2.70E-06
GOTERM_BP_DIRECT	Viral transcription	5	25	5.30E-06
GOTERM_BP_DIRECT	Nuclear-transcribed mRNA catabolic process, nonsense-mediated decay	5	25	6.80E-06
GOTERM_BP_DIRECT	rRNA processing	5	25	6.80E-05
GOTERM_BP_DIRECT	Translation	5	25	1.30E-04
GOTERM_BP_DIRECT	Canonical glycolysis	3	15	3.50E-04
GOTERM_BP_DIRECT	Response to salt stress	2	10	9.60E-03
GOTERM_BP_DIRECT	Positive regulation of cell size	2	10	1.10E-02
GOTERM_BP_DIRECT	Positive regulation of protein import into nucleus, translocation	2	10	1.10E-02

D-glyceraldehyde-3-phosphate and is thereby related to energy metabolism. Molecular function analysis indicated that PGK1 is involved in ATP-binding as well as in phosphoglycerate kinase activities and protein-disulfide reductase activities. Biological process analysis revealed that PGK1 is associated with canonical glycolysis, cellular response to hypoxia, epithelial cell differentiation and gluconeogenesis.

GO and KEGG pathway analyses were performed to enhance our understanding of DEP

Table 6. Top 10 KEGG pathway enrichment analyses in the most significant DEP module

Category	Term	Description	Count	%	*p* Value
KEGG_PATHWAY	hsa01200	Carbon metabolism	6	30	5.70E-06
KEGG_PATHWAY	hsa01130	Biosynthesis of antibiotics	7	35	7.20E-06
KEGG_PATHWAY	hsa03010	Ribosome	5	25	2.80E-04
KEGG_PATHWAY	hsa00010	Glycolysis/Gluconeogenesis	4	20	5.40E-04
KEGG_PATHWAY	hsa01230	Biosynthesis of amino acids	4	20	7.20E-04
KEGG_PATHWAY	hsa04922	Glucagon signalling pathway	4	20	1.70E-03
KEGG_PATHWAY	hsa00030	Pentose phosphate pathway	3	15	2.20E-03
KEGG_PATHWAY	hsa04915	Estrogen signalling pathway	3	15	2.40E-02

interactions. Biological process analysis indicated that nucleosome assembly was mainly associated with upregulated proteins. Nucleosome subunits of eukaryotic chromatin were formed by spontaneous reactions between histones and DNA. The nucleosome assembly pathway has been studied in disease and disorder research, and was found to be associated with FA.

In marrow proteomics, differentially expressed networks and pathways of FA and AA were mainly involved in metabolic processes, monosaccharide bio-syntheses and gluconeogenesis, thereby reflecting energy metabolism differences between FA and AA. In addition, protein translation and replication differed between the two diseases. Moreover, the activation of DNA fragmentation factor, DNA fragmentation induced by apoptosis, formation of senescence-associated heterochromatin foci and the regulation of cell morphogenesis were enriched by DEP analysis. These pathways and functions, which are associated with leukaemia, provide useful information related to the treatment of FA.

FA related research has undergone a shift from chromosomal aberrations to DNA repair and oncology studies, which, via monitoring of pathogenesis and mediation of DNA hinge injury repair pathways, have made remarkable breakthroughs. High-tumour susceptibility studies have also made considerable progress. Proteomic enrichment has also revealed certain tumour-related functions and pathways. KEGG pathways for upregulated DEPs were mainly associated with glycolysis/gluconeogenesis, leukocyte trans-endothelial migration, the pentose phosphate pathway and the glucagon signalling pathway, indicating that energy metabolism of FA patients was stronger than that of AA patients. Downregulated DEPs were associated with lysosome, autophagy, phagosome, tuberculosis, pantothenate, and CoA biosynthesis. Cell function may be further reduced in FA patients compared to that of AA patients. Enrichment of these functions and pathways may provide insight into the conversion of FA to leukaemia [24].

To the best of our knowledge, this is the first study to use bioinformatics to compare differences between FA and AA. Our findings may also provide information related to the identification and differential diagnosis of FA and AA for follow-up studies.

Conclusions

The present study examined the DEPs of FA and AA through a systematic bioinformatics analysis and revealed that immunological processes and functions of FA patients exhibiting cellular macromolecule localization ability were reduced compared with that of AA. ENO1, PGK1, ACTC1, ACTBL2, EEF1A1 and CFL1 may be used as serologic markers for the early diagnosis of FA. These findings may remarkably improve our understanding of the differences between molecular mechanisms underlying FA and AA. Candidate hub proteins and pathways may be used as therapeutic targets.

Acknowledgements

The work was supported by the Natural Science Foundation of China (NO. 81770193), Jiangsu Province Projects (NO. BE2017659, BRA2017541, and CXTDA2017014), and Suzhou projects (SZS201615, SZZX201504, SS201809, and SYS201643).

Disclosure of conflict of interest

None.

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