Using protein microarray reveals clinical correlation between self-perception of patient and the apoptosis-related proteins in rheumatoid arthritis

Jian-ting Wen
Anhui University of Traditional Chinese Medicine
Jian Liu (liujianahzy@126.com)
Anhui University of Traditional Chinese Medicine
Hui Jiang
Anhui University of Traditional Chinese Medicine
Lei Wan
Anhui University of Traditional Chinese Medicine
Ling Xin
Anhui University of Traditional Chinese Medicine
Yue Sun
Anhui University of Traditional Chinese Medicine
Yan-qiu Sun
Anhui University of Traditional Chinese Medicine
Ying Zhang
Anhui University of Traditional Chinese Medicine
Xin-lei Du
Anhui University of Traditional Chinese Medicine
Xin Wang
Anhui University of Traditional Chinese Medicine
Jie Wang
Anhui University of Traditional Chinese Medicine

Research

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Abstract

Background: The most severe effects of rheumatoid arthritis (RA) are loss of physical function, which may have a significant impact on self-perception of patient (SPP). However, the inherent relationship between SPP and the key proteins is not clear. The aim of this study was to get an insight into SPP of RA in connection with the the apoptosis-related proteins.

Methods: We set out to investigate changes of the apoptosis-related proteins expression in the peripheral blood mononuclear cells (PBMCs) of RA. Additionally, we aimed to correlate the apoptosis-related proteins expression profiles with SPP and clinical indexes. To this end, we employed antibody microarrays of the the apoptosis-related proteins in PBMCs from four RA patients and seven healthy controls. We used bioinformatics to screen several the apoptosis-related proteins. To validate key protein candidates, we performed Enzyme linked immunosorbent assay (ELISA) on 30 RA patients and 30 healthy controls.

Results: We found the expression of ten the apoptosis-related proteins (caspase3, CD40, SMAC, HSP27, HTRA, IGFBP-1, IGFBP-6, sTNF-R1, sTNF-R2, TRAILR-3) were significantly altered in PBMCs of RA patients. Receiver operating characteristic (ROC) curve analysis suggested that these ten the apoptosis-related proteins are potential biomarkers of RA. Spearman Correlation analysis and Logistic-regression analysis revealed that the 10 selected the apoptosis-related proteins correlated with SPP and clinical indexes.

Conclusion: Therefore, we highlight some the apoptosis-related proteins may serve as potential biomarkers in prediction of SPP for RA patients, although the underlying mechanisms need to be further explored.

Background

RA is an autoimmune disease and the most common type of inflammatory arthritis that is a major cause of disability[1 2]. The overall world prevalence of rheumatoid arthritis (RA) ranges from 0.5-1.0%, women are 2–3 times more likely to experience major depression than men[3 4]. It was eventually lead to joints deformity and loss of function if not adequately treated[5]. RA patients not only suffer from physical pain, but also bear great psychological pressure[6 7]. A decrease in functionality is accompanied by deteriorating social and emotional well-being[8]. There, persistent psychological stress often leads to anxiety disorders, depression, and quality of life (QOL) decreased[9 10]. In particular, SPP has attracted more and more attention in the clinical which closely related to the therapeutic effect. Currently, the internationally recommended that SPP of RA is evaluated using DAS28, VAS, SAS, SDS, SF-36 and there has become the most accepted evaluation method by the majority of clinicians[11]. A previous study performed a cross-sectional study with RA patients in Ecuador, they found that the QOL of RA patients is severely affected, depression, fatigue, morning stiffness, pain, high disease activity, and disability have a negative effect on

QOL in RA, likewise, patients with more comorbidities and extraarticular manifestations show worse QOL[12].

The exact causes of in RA is unknown. Recently, the number of studies revealing the important role of epigenetics in the pathogenesis of RA has increased[13 14]. However, we do not know whether it is related to decreased SPP in RA. These days, although there are advances in the treatment of RA, we still need to discovery the pathogenesis of RA. The RA clinical diagnosis is mainly based on causes of RA, clinical symptoms, signs, laboratory tests, and clinical imaging[15]. Until now there is no gold standard for early diagnostic approach for RA, and patients are usually comprehensively evaluated by serological tests for rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPA) and for inflammatory markers such as hypersensitive C-reactive protein (CRP), an erythrocyte sedimentation rate (ESR) test, and an imaging test if symptoms appear[16 17]. However, RF-positive rate and ACPA-specificity can be detected in approximately 50–80% of RA patients, which suggesting that the overall detection efficiency is lower[18]. Apoptosis plays a key role in RA, and the inhibition of apoptosis may provide a novel treatment method for RA diseases[19 20]. Therefore, there is an increasingly growing interest in identifying biomarkers for diseases, in which apoptosis is involved. Studies toward novel diagnostic biomarker discovery, using protein microarray assay, have been increasing[21 22]. A proteomics approach using protein microarray assays can be used to determine the pattern of proteins and compare their absolute levels between diseased and healthy controls[23].

In present study, we obtained peripheral blood mononuclear cells (PBCMs) from four RA patients and tried to discover diagnostic biomarkers in informative blood samples using protein microarray assays. In the discovery and validation set, candidate biomarkers were selected and verified. Additionally, the relationship between these candidate biomarkers with SPP and clinical indexes of RA patients was also analyzed.

Materials And Methods

RA patients and healthy controls

Four patients (three females and one male, 45-66 years of age) with RA were recruited from the Department of Rheumatology and Immunology of First Affiliated hospital of Anhui University of Traditional Chinese Medicine from June 2019 to December 2019. Seven healthy control (five females and two males, 45-66 years of age) without previous history selected from the Physical Examination Center in First Affiliated hospital of Anhui University of Traditional Chinese Medicine. These RA patients and healthy controls samples (frst cohort) were used for the protein microarray and the associated ELISA verifcation.

We further recruited more RA patients and healthy controls samples per each group (second cohort) for the independent ELISA verifcation experiments (30 RA patients and 30 healthy controls). All RA patients fulfilled the 2010 ACR/EULAR (American College of Rheumatology/European League Against

Rheumatism) criteria for the classification of RA[24]. In addition, 30 age- and sex-matched healthy controls with no clinical history of tumors, trauma, infectious diseases or autoimmune diseases, who underwent routine Physical Examination Center in the same hospital during the same period. All samples from RA patients and healthy controls were obtained with written informed consent. This study was approved by the ethics committee of the First Affiliated hospital of Anhui University of Traditional Chinese Medicine.

Measurements

We collected demographic and clinical data from the subjects, including age, gender, disease duration, blood chemistry. All clinical measurements were performed by the clinical laboratory staff of our hospital. The clinical laboratory data such as erythrocyte sedimentation rate (ESR), high-sensitivity C-reactive protein (CRP), rheumatoid factor (RF), anti-cyclic citrullinated peptide antibody (CCP) and clinical characteristics are determined.

All of the participants enrolled were asked to fill in the DAS28, VAS, SDS, SAS, SF-36 under the guidance of clinical doctors, SF-36 consists of 8 dimensions, namely physical functioning (PF), role-physical (RP), body pain (BP), general health (GH), vitality (VT), social functioning (SF), role-remotional (RE), mental health (MH).

PBMCs preparation and total RNA extraction

An amount of 5 mL of whole blood was obtained from RA patients and healthy controls and PBMCs were isolated through Ficoll–Paque density gradient centrifugation (GE Healthcare, Uppsala, Sweden). The concentration of cells was adjusted to 5-7×10^6 cells per ml and reserved at − 80 °C until use.

Total RNA was extracted from PBMCs of all samples using Trizol reagent (Invitrogen Life Technologies, Carlsbad, CA, USA) according to the manufacturers instructions. The RNA was then reverse transcribed to single stranded cDNA, which was used as a template to synthesize the second cDNA strand. Aliquots of total RNA samples were used to determine the RNA concentration and purity using the Nano Drop ND-1000 spectral photometer (peqlab). The RNAs were selected on the basis of a combination of p-value, fold change, raw intensity and type. In addition, RNAs with miRNA response elements (MREs) related to RA reported in the literatures were selected preferentially. All qPCR assays were performed on the Viia7 Real-Time PCR System, each sample was replicated three times. Library quality was assessed on the Agilent Bioanalyzer 2100 system. The relative expression levels of RNAs were calculated using the $2^{-\Delta\Delta Ct}$ method.

The apoptosis-related protein microarray analysis

Capture antibodies were spotted onto a glass slide at a volume of 350 pL per spot at a pitch of 500 mm using a microarray printer^{+} . Biotinylated goat anti-bovine lgG antibody was also spotted as a detection control. Each capture antibody was printed in quadruplicate spots within a subarray. Each glass slide contained 16 identical subarrays separated by a 16-well gasketed hybridization chamber to prevent

sample cross-contamination. Antibody arrays were stored at -20 ℃ until use. The raw signal data was extracted using MapPix 6.0 software and quantitative data were extracted and analyzed with software for the multiplexed antibody array.

Bioinformatics Analysis

The raw data obtained by the chip scan was subjected to chip background removal and inter-chip normalization processing by Raybiotech software.2.3.2. After the raw data were normalized by the software, the resulting data were selected for analysis. DEPs with P<0.05 were first retained and then further screened by Foldchange (expression difference multiple). The selection conditions were as follows: ▯Foldchange≤0.83 or Foldchange ≥1.2; and ▯ Fluorescent signal > 150. For cluster analysis, the heatmap.2 function and gplots package from R/bioconductor were used. The distance between two samples was calculated as the Euclidean distance; the distance between the two clusters was calculated with the furthest neighbor method (complete), and the distance between classes was defined as the maximum distance. Fisher's exact test and the clusterProfiler package from R/bioconductor were used. For selection, the number of genes that differed on a certain GO term and KEGG pathway were ≥ 2, and P < 0.05. The normalized data were exported to SIMCA-p 11.5 for principal component analysis (PCA), partial least-squares discriminant analysis (PLSDA), and orthogonal partial least-squares discriminant analysis (OPLSDA).

Validation with ELISA

According to the manufacturers'instructions, the differentially expressed proteins levels in serum were quantified using ELISA kit (eBioscience, Inc., San Diego, CA, USA). Briefly, whole-blood samples were centrifuged at 4,000 g for 10 min to collect serum. After that, conditioned media or supernatants of lysed samples were collected and added to 96-well plates pre-coated with appropriate capture antibody, followed by incubation with the appropriate biotinylated detection antibody. Lastly, Streptavidin horseradish-peroxidase (HRP) was added to each well and incubated for 1 h at room temperature. The results were detected using an ELISA microplate reader (BioTek) at 450 nm.

Statistical Analysis

SPSS 23.0 software for Windows was used for statistical analysis. Continuous variables are expressed as means ± standard deviation and categorical variables are expressed as numerals. When normally distributed, independent samples will be compared for numerical variables between the two groups using Student's t-test. Mann-Whitney U-test was used for the data not conforming to a normal distribution. Categorical data were compared between groups using the Chi-square test. Correlations between the variables were performed using Spearman Correlation Analysis and Logistic-regression. A p value < 0.05 was statistically significant (*p < 0.05, **p < 0.01, ***p < 0.001).

Results

Basic characteristics of RA patients and healthy controls

Thirty RA patients (2 males, 28 females, mean age: 55.63 ± 11.41 years, course of disease: 15.67 ± 8.53 years) and thirty healthy controls (2 males, 28 females, mean age: 54.33 ± 12.59 years) were used as the study group. No significant differences in age or gender were identified between the two groups ($p > 0.05$).

Comparisons of SPP and clincial indexes between RA patients and healthy controls

As showed in Table 1, compared with healthy control, PF, RP, GH, MH of RA were significantly lower, while DAS28, VAS, SAS, SDS, BP were significantly higher than healthy control ($P < 0.05$). Meanwhile, ESR, CRP, RF, CCP of RA were significantly higher than HC ($P < 0.05$).

Table 1
Comparison of SPP and clincial data between the two groups

Index	RA (n = 30)	Control (n = 30)	P
DAS28 score	6.54 (6.07, 7.73)	1.47 (1.21, 1.66)	0.000
VAS score	7.00 (6.00, 8.00)	0.90 (0.78, 1.00)	0.000
SAS score	55.25 (54.36, 62.50)	29.80 (28.35, 31.10)	0.000
SDS score	55.00 (51.25, 65.00)	32.60 (30.39, 34.39)	0.000
PF score	50.00 (33.75, 55.00)	62.82 (59.75, 66.68)	0.000
RP score	0.00 (0.00, 25.00)	30.19 (25.33, 33.48)	0.000
BP score	50.00 (50.00, 62.00)	74.98 (71.47, 79.48)	0.000
GH score	44.97 ± 11.82	58.73 (54.68, 62.03)	0.000
VT score	40.00 (31.50, 44.25)	42.50 (39.52, 45.24)	0.071
SF score	55.00 (37.50, 62.50)	59.87 (57.58, 62.49)	0.439
RE score	33.33 (0.00, 66.66)	66.67 (33.33, 66.67)	0.301
MH score	45.00 (42.00, 61.00)	66.38 (63.98, 69.06)	0.000
ESR(mm/h)	41.5(27.75, 77.00)	2.63 (2.49, 2.71)	0.000
hs-CRP(mg/L)	28.43 (8.10, 60.77)	1.85 ± 0.99	0.000
RF(U/ml)	91.85 (23.45, 255.15)	4.56 (4.45, 4.64)	0.000
CCP(U/ml)	55 (15.45, 200.00)	2.50 ± 0.62	0.000

Expression Of The Apoptosis-related Differential Proteins In Ra Patients

To identify the apoptosis-related differential proteins in RA, we performed antibody microarrays analysis of the the apoptosis-related differential proteins in the PBMCs from four patients with RA and seven healthy people. After the raw data were normalized, the the apoptosis-related proteins were identified between the two groups. We identified 30 proteins (17 upregulated and 13 downregulated) that were differentially expressed between the two groups (Fig. 1A). Scatter plots showed 10 abundance of the apoptosis-related differential proteins by screening for log2-fold-changes greater than 1 and $P < 0.05$ (Fig. 1B). A heat map was constructed to group the the apoptosis-related differential proteins based on their expression levels among the samples (Fig. 1C). OPLS-DA clearly separated tested samples into two blocks according to their apoptosis-related differential proteins in both RA and healthy controls (Fig. 1D).

Function of the apoptosis-related differential proteins in RA patients

To explore the the apoptosis-related differential proteins, Gene Ontology (GO) terms, Kyoto Encyclopedia of Genes and Genomes (KEGG) and functional enrichment analyses were performed. We placed emphasis on GO analysis of proteins found that regulation of apoptotic process involved in morphogenesis, regulation of apoptotic process involved in deveiopment, positive regulation of proteolysis were in Biological Process (BP) (Fig. 2A), varicosity, receptor complex and neuronal cell body were in Cellular Component (CC) (Fig. 2B), TRAIL binding, death receptor binding, death receptor activity were in Molecular Function (MF) (Fig. 2C). Many pathways connected with functions of proteins in RA were defined by the KEGG analysis, TNF signaling pathway, MAPK signaling pathway, Apoptosis were in proteins (Fig. 2D).

Protein–protein interaction network describing RA

To better understand the cellular networks that are altered in the PBMCs of RA patients, the relationship between proteins was analyzed using different cutoff points with similar results (Fig. 3A). Additionally, we created protein–protein interaction network models of RA-specifc using the STRING database (Fig. 3B).

Verification of the apoptosis-related differential proteins in RA patients

A total of 10 the apoptosis-related differential proteins by screening for log2-fold-changes greater than 1 and $P < 0.05$ in RA (Table 2), including 7 upregulated and 3 downregulated proteins were selected.

Serum from 30 RA patients and 30 healthy controls were used for verification by ELISA. We selected 10 the apoptosis-related differential proteins from the most significant proteins for further verification. The expression profile of caspase3, CD40, SMAC ($p < 0.01$) in the RA group was apparently lower than in healthy control (Fig. 4A, B, G). The expression profile of HSP27, HTRA, IGFBP-1, IGFBP-6, sTNF-R1, sTNF-R2, TRAILR-3 ($P < 0.01$) in the RA group were apparently higher than healthy control (Fig. 4C, D, E, F, H, I, J).

Table 2
Basic characteristics of the 10 the apoptosis-related differential proteins in RA patients.

Protein name	Gene ID	Foldchange	p	Regulation
HSP27	3315	4.656	0.000	up
IGFBP-1	3484	3.693	0.000	up
TRAILR-3	8794	0.326	0.000	down
sTNF-R1	7132	0.390	0.000	down
CD40	958	1.664	0.003	up
sTNF-R2	7133	0.531	0.003	down
HTRA	27429	5.089	0.009	up
SMAC	56616	3.033	0.011	up
IGFBP-6	3489	2.065	0.013	up
caspase3	836	2.667	0.017	up

ROC curve analysis of the apoptosis-related differential proteins in RA patients

To further assess the biological functions, we performed ROC curve analysis to evaluate the diagnostic value of the 10 the apoptosis-related differential proteins. As showed in Fig. 5, ROC curves of confirmed proteins suggested that the levels of caspase3 (AUC = 0.9856), CD40 (AUC = 1.0000), HSP27 (AUC = 0.8889), HTRA (AUC = 0.9988), IGFBP-1 (AUC = 1.0000), IGFBP-6 (AUC = 1.0000), SMAC (AUC = 1.0000), sTNF-R1 (AUC = 0.9744), sTNF-R2 (AUC = 0.9946), TRAILR-3 (AUC = 0.9917), which indicated that these proteins can be used as potential molecular markers for the diagnosis in RA.

Spearman Correlation analysis of the apoptosis-related differential proteins with SPP and clinical indexes in RA patients

To clarify the relationship between the the apoptosis-related differential proteins with SPP and clinical indexes, we performed Spearman Correlation analysis. The results of Spearman Correlation analysis suggested that caspase3 was positively correlated with age, ESR, RF (Fig. 6A-C). HSP27 was negatively correlated with CCP (Fig. 6D). HTRA was positively correlated with VT (Fig. 6E). IGFBP-1 was positively correlated with SAS (Fig. 6F). IGFBP-6 was negatively correlated with VAS (Fig. 6G). SMAC was positively correlated with CRP, negatively correlated with RF (Fig. 6H-I). sTNF-R2 was positively correlated with ESR, negatively correlated with MH (Fig. 6J-K).

Logistic-regression analysis of SPP, clinical indexes related the apoptosis-related differential proteins

In order to identify risk factors of the the apoptosis-related differential proteins in RA patients, we conducted Logistic-regression analysis. Significant differences in caspase3 was found between RA

patients with ESR ($p = 0.002$), CCP ($p = 0.027$), SAS ($p = 0.012$), indicating that ESR, CCP, SAS were risk factors for caspase3. Significant differences in HPS27 was found between RA patients with VAS ($p = 0.034$), MH ($p = 0.038$), indicating that VAS and MH were risk factors for HPS27. Significant differences in SMAC was found between RA patients with RF ($p = 0.023$), DAS28 ($p = 0.050$), VT ($p = 0.001$), indicating that RF, DAS28, VT were risk factors for SMAC.

Discussion

The early diagnosis of RA is very challenging because there are no specifc diagnostic indicator. Antibody microarray assay-based proteomics is a state-of-the-art analytical technique that enables the diagnosis and treatment of diseases. Proteomics has been a subject of interest in recent research, there has been a recent expansion in proteomics research on a number of diferent rheumatic diseases. Many factors contributed to decreased SPP for those living with RA[25]. With the change of medical model from a biomedical to a bio-psycho-social one and with the development of value-based medicine, SPP should be considered in making clinical decision[26]. Therefore, medical workers are increasingly concerned about SPP of RA. However, it is necessary to seek out new biomarkers and explore their functions for details of the mechanisms of decreased SPP in RA remain ambiguous.

Protein chips play an important role in scientific research due to its richer detect target factors and smaller sample size requirement. It is widely used in the research of disease mechanisms. Recently, Mun S producted a analysis of changes in serum protein expression profiles of RA patients by SCIEX TripleTOF 560, which found that serum amyloid A4 and vitamin D binding protein could be potential biomarkers related to the inflammatory response and joint destruction that accompany RA. This finding provides an important reference for further research on RA[27]. To identify predictive biomarkers in patients with RA, 50 the apoptosis-related proteins profiling was conducted individually with 7 PBMCs from the normal group and 9 from the patient group with RA. Analysis of proteins profiling revealed the ten apoptosis-related proteins in the discovery set that could be considered as potential candidate biomarkers. The ten candidate biomarkers, namely cysteine aspastic acid-specific protease 3 (caspase3), heat shock protein 27 (HSP27), tumor necrosis factor receptor superfamily member 5 (TNFRSF5, CD40), second mitochondria-derived activator of caspase, SMAC), HtrA serine peptidase 1 (HTRA), insulin-like growth factor binding protein − 1 (IGFBP-1), insulin-like growth factor binding protein − 6 (IGFBP-6), tumor necrosis factor receptor superfamily member 1A (sTNF-R1), tumor necrosis factor receptor superfamily member 1B (sTNF-R2), tumor necrosis factor receptor superfamily member 10C (TRAILR-3) with > 1.5 of fold change in RA compared to healthy controls. Functional annotation of the the apoptosis-related proteins into GO terms and KEGG pathways was performed, which revealed that these the apoptosis-related proteins involved in many pathophysiological processes, including regulation of apoptotic process, receptor complex, death receptor binding. A PPI network was constructed, which showed that these the apoptosis-related proteins are closely related in terms of their functions.

ELISA experiments for ten the apoptosis-related proteins were performed to further verify the diferential expression level. The expression profile of caspase3, CD40, SMAC ($p < 0.01$) in the RA group was

apparently lower than in healthy control. While the expression profile of HSP27, HTRA, IGFBP-1, IGFBP-6, sTNF-R1, sTNF-R2, TRAILR-3 ($P < 0.01$) in the RA group were apparently higher than healthy control. ROC curve analysis revealed the 10 the apoptosis-related differential proteins as diagnostic predictors of RA (all AUC value is greater than 0.900). Caspase 3 is involved in signalling pathways leading to apoptosis, which plays significant roles in the pathogenesis of RA[28]. Hsps have been implicated in the RA[29]. Sedlackova L found that significantly increased Hsp27 and Hsp90a mRNA levels in RA synovial tissues, which indicated that Hsps could be new diagnostic approach to RA patients[30]. The anti-apoptotic activity of inhibitors of apoptosis proteins (IAP) proteins can be blocked by the SMAC, which is liberated into the cytoplasm in response to proapoptotic stimuli. Lattuada D found that SMAC was associated with caspase 8 and caspase 3 activities, and induced significant apoptosis in all RA-FLS samples. In addition, SMAC significantly upregulated IGFBP-5, a protein involved in differentiation, apoptosis, and osteoblastic activation, so they included that SMAC may represent a new therapeutic approach to RA treatment[31].

Finally, we would like to discuss our findings in regard of their SPP and clinical indexes. We performed Spearman Correlation analysis suggested that caspase3 was positively correlated with age, ESR, RF, HSP27 was negatively correlated with CCP, HTRA was positively correlated with VT, IGFBP-1 was positively correlated with SAS, IGFBP-6 was negatively correlated with VAS, SMAC was positively correlated with CRP, negatively correlated with RF, sTNF-R2 was positively correlated with ESR, negatively correlated with MH. Additionally, results from Regression Analysis indicated that ESR, CCP, SAS were risk factors for caspase3, VAS and MH were risk factors for HPS27, RF, DAS28, VT were risk factors for SMAC. Liao H used 2-dimensional liquid chromatography-coupled tandem mass spectrometry to identify protein biomarkers of disease severity in the synovial fluid and serum of RA patients, they concluded that CRP, S100A8, S100A9 and S100A12 proteins could be identified for prognosis of the erosive form of RA[32]. However, there were no analysis between potential proteins and clinical indicators, nor did it predict the risk factors affectted potential proteins.

This study addresses important questions in understanding the important problem area in persons with decreased SPP in RA. The novelty in this study is that we have used microarray analysis combined with SPP to investigate their relationship. The main limitation of this study is a small number and inhomogeneity of respondents. Further in vitro and animal studies should be performed to evaluate comprehension of the detailed mechanism and specific functions of genes in decreased QOL in RA. To sum up, the ten apoptosis-related differential proteins are of potentially significant prediction value in decreased SPP in RA patients, but there remains a need to further study the mechanisms of these proteins.

Conclusions

In this work, we focus on the relationship between self-perception of patient (SPP) and the apoptosis-related proteins in RA patients. We choosed four RA patients and seven healthy control for antibody array. Ten apoptosis-related proteins were verified by ELISA.We recruited 30 patients with RA and 30 healthy controls to fill in the five quentionnaires, including DAS28, VAS, SDS, SAS, SF-36. Finally, Spearman

Correlation Analysis and Logistic-regression Analysis revealed that the ten apoptosis-related proteins were closely associated with SPP. They may serve as potential biomarkers for RA patient diagnosis.

Abbreviations

RA: rheumatoid arthritis; SPP:self-perception of patient; PBMCs:peripheral blood mononuclear cells; ELISA:Enzyme linked immunosorbent assay; ROC:Receiver operating characteristic; QOL:quality of life; DAS28:the Disease Activity Score in 28 joints; VAS:Visual Analogue Scale ; SDS:Self-rating Depression Scale; SAS:Self-rating Anxiety Scale; SF-36:MOS 36-Item Short Form Survey; CRP:high-sensitivity C-reactive protein; ESR:erythrocyte sedimentation rate; RF:rheumatoid factor; CCP:anti-cyclic citrullinated peptide antibody; PF:physical functioning; RP:role-physical; BP:body pain; GH:general health; VT:vitality; SF:social functioning; RE:role-remotional; MH:mental health; caspase3:cysteine aspastic acid-specific protease 3; HSP27:heat shock protein 27; TNFRSF5:tumor necrosis factor receptor superfamily member 5; SMAC:second mitochondria-derived activator of caspase; HTRA:HtrA serine peptidase 1; IGFBP-1:insulin-like growth factor binding protein − 1; IGFBP-6:insulin-like growth factor binding protein − 6; sTNF-R1:tumor necrosis factor receptor superfamily member 1A; sTNF-R2:tumor necrosis factor receptor superfamily member 1B; TRAILR-3:tumor necrosis factor receptor superfamily member 10C.

Declarations

Authors’contributions

WJT, LJ, JH, WL, SY, and XL contributed to the study design. WJT contributed to data analysis, wrote the first draft, and revised the manuscript. SYQ, ZY, DXL, WX, and WJ contributed to the specimen and date collection. LJ was the supervisors of the project and contributed to the manuscript revision. All authors reviewed and accepted the content of the final manuscript.

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Availability of data and materials

The data used to support the findings of this study are available from the corresponding author upon request.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of the First Affiliated Hospital of Anhui University of Traditional Chinese Medicine and carried out under the Helsinki Declaration. Before participating in the study, the patients filled in a written informed consent forms. A written informed consent was obtained from all the study participants.

Consent for publication

Not applicable.

Conflicts of interest

The authors have no competing interests to declare.

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Figures

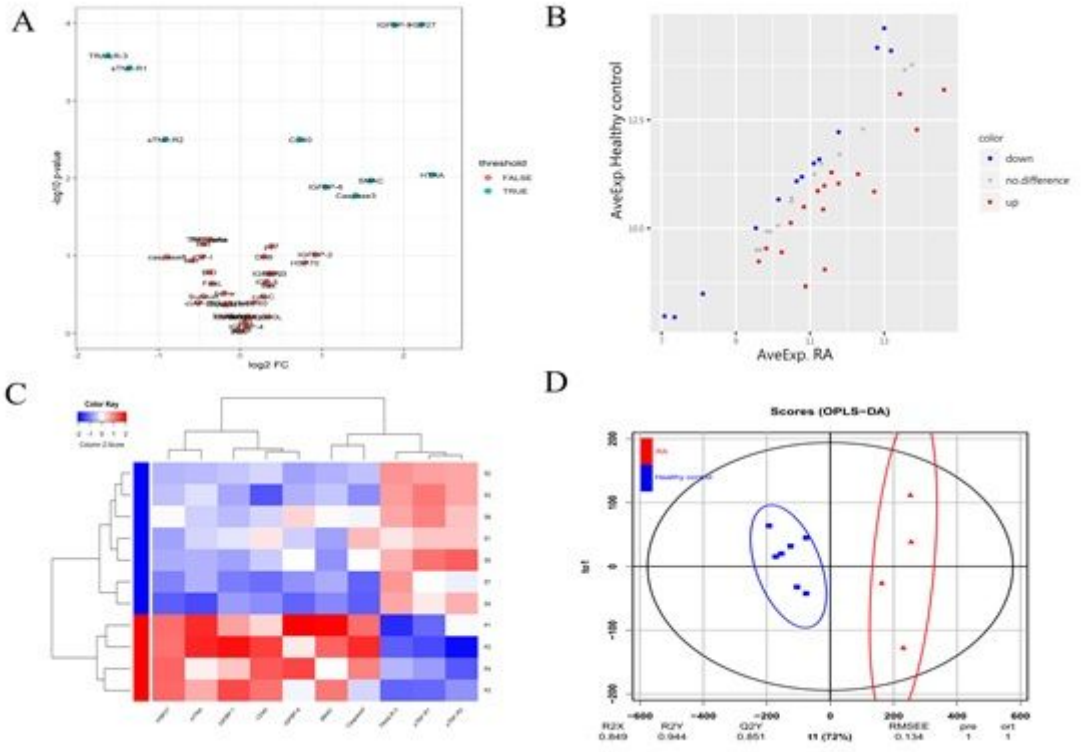

Figure 1

Antibody microarrays determining the apoptosis-related differential proteins expression profiles in RA patients. (A) Volcano plot of the apoptosis-related differential proteins: The vertical lines correspond to 1.5-fold up and down and the horizontal line represents a P-value of 0.05, blue: true; red: false. (B) Scatter plot of the apoptosis-related differential proteins: Red: up expression; blue: down expression; grey: no difference. (C) Heatmap of the apoptosis-related differential proteins: Red: high relative expression; blue: low relative expression. (D) Principal component analysis of the apoptosis-related differential proteins: Red: RA, blue: healthy controls.

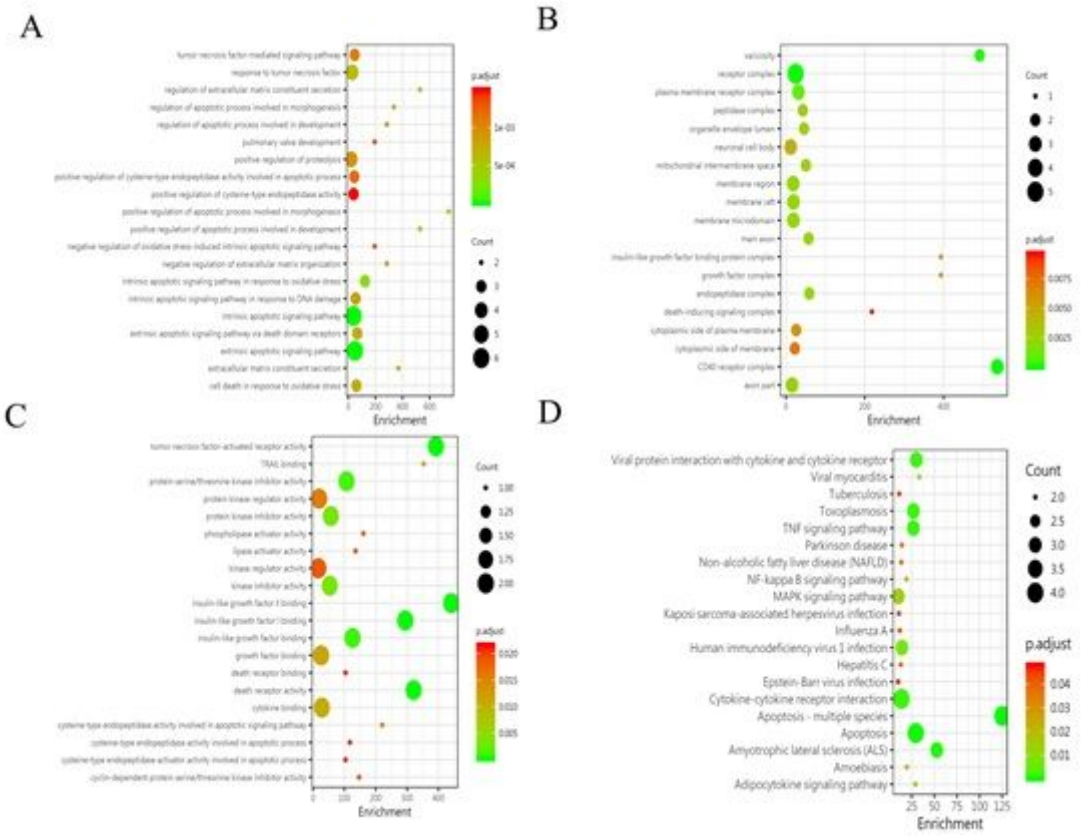

Figure 2

GO analysis and KEGG pathway annotation of the apoptosis-related differential proteins. (A) The top 20 significantly obvious changes of BP in GO biological process classification of proteins. (B) The top 19 significantly obvious change of CC in GO biological process classification of proteins. (C) The top 20 significantly obvious changes of MF in GO biological process classification of proteins. (D) The top 20 significant obvious changes in KEGG pathway classification of proteins. The bar plot shows the top Enrichment Score (−log10 (Pvalue)) value of the significant pathway. The color represents the difference, darker indicating a greater difference. The circle represents the relationship.

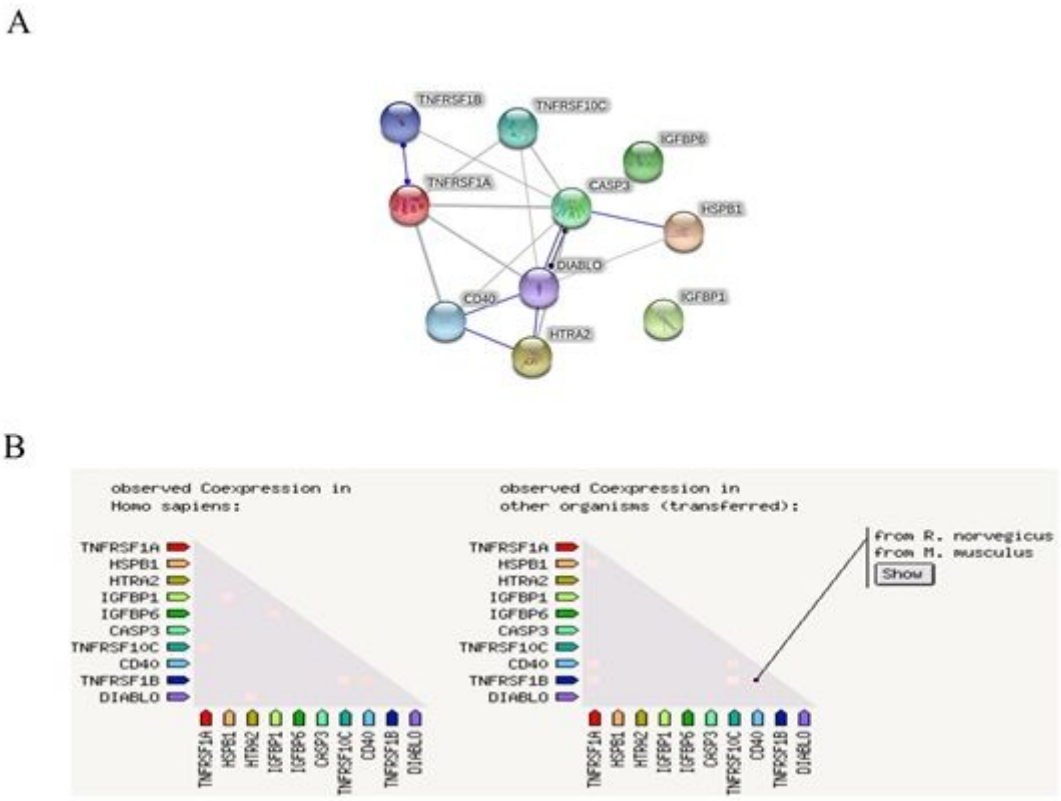

Figure 3

Interaction network of the the apoptosis-related differential proteins. (A) The relationship between differential proteins. (B) The PPI network of the the apoptosis-related differential proteins was constructed.

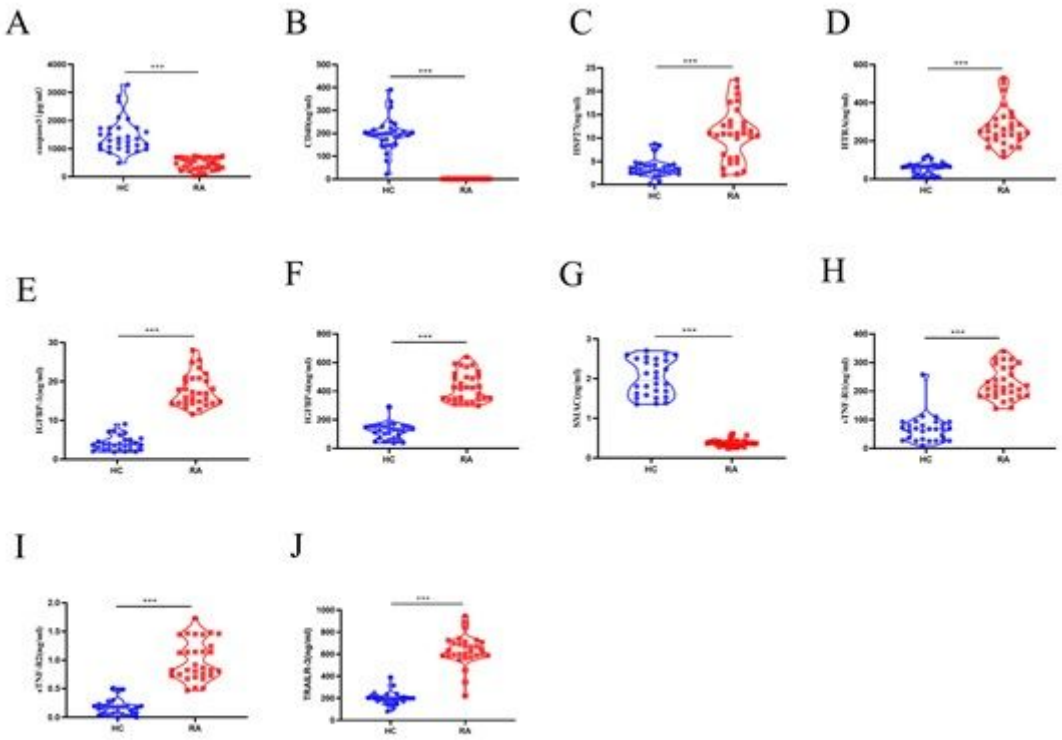

Figure 4

Serum levels of the the apoptosis-related differential proteins were measured by ELISA. (A, B, G) Caspase3, CD40, SMAC expression were significantly decreased (P <0 .01). (C, D, E, F, H, I, J) HSP27, HTRA, IGFBP-1, IGFBP-6,sTNF-R1, sTNF-R2, TRAILR-3 expression were significantly increased (P <0 .01).

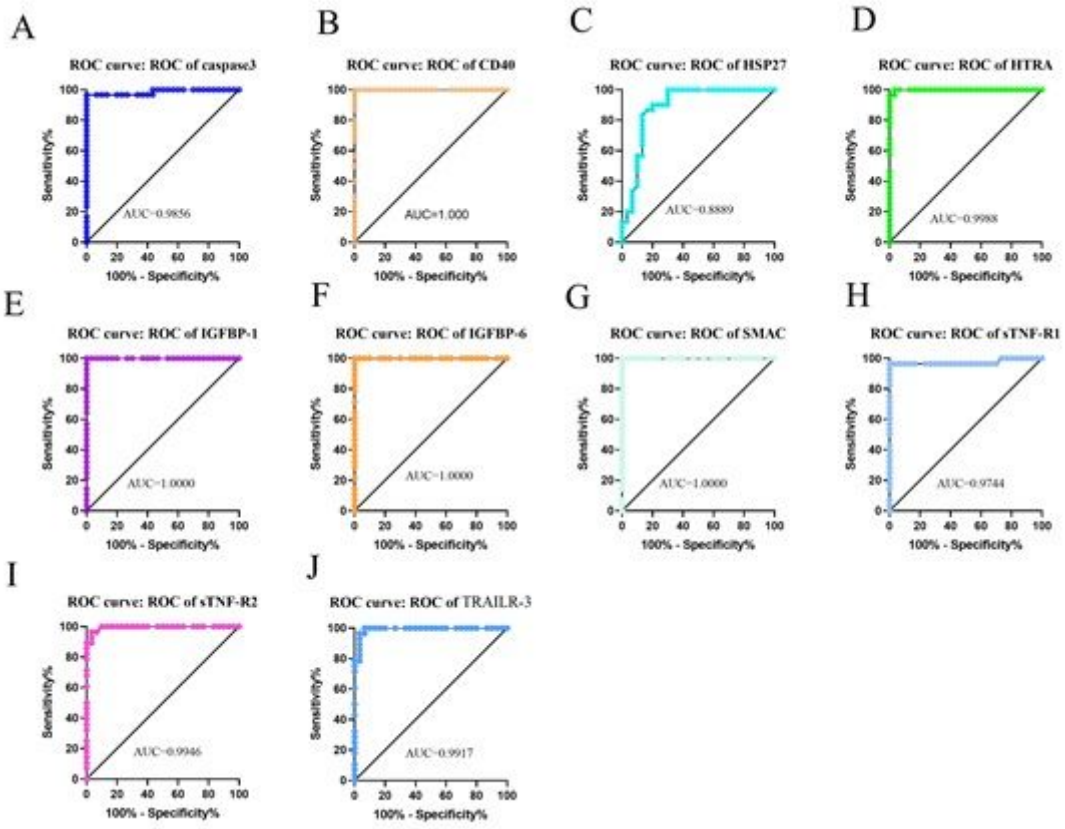

Figure 5

ROC curve analysis of the apoptosis-related differential proteins.

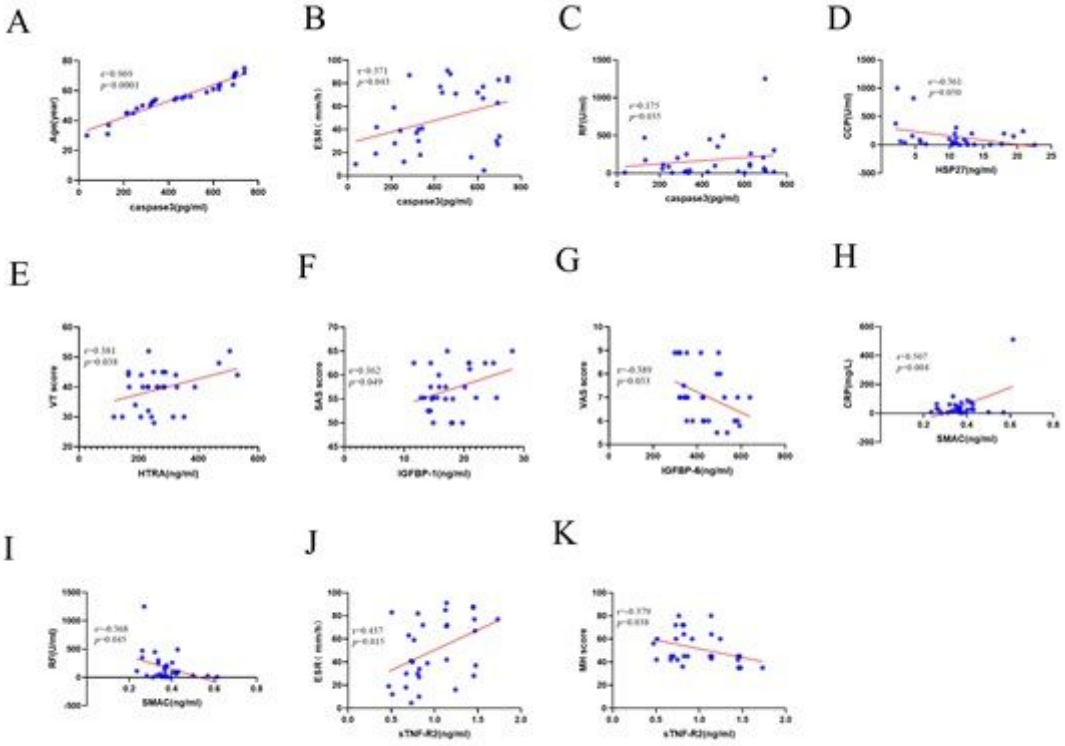

Figure 6

Correlation between the apoptosis-related differential proteins with SPP and clincial indexes. (A - C) There was a close positive correlation of the caspase3 with age, ESR, RF. (D) There was a close negative correlation of the HSP27 with CCP. (E) There was a close positive correlation of the HTRA with VT. (F) There was a close positive correlation of the IGFBP-1 with SAS. (G) There was a close negative correlation of the IGFBP-6 with VAS. (H-I) There was a close positive correlation of the SMAC with CRP, a close negative correlation of the SMAC with RF. (K) There was a close positive correlation of the sTNF-R2 with ESR, a close negative correlation of the sTNF-R2 with MH.

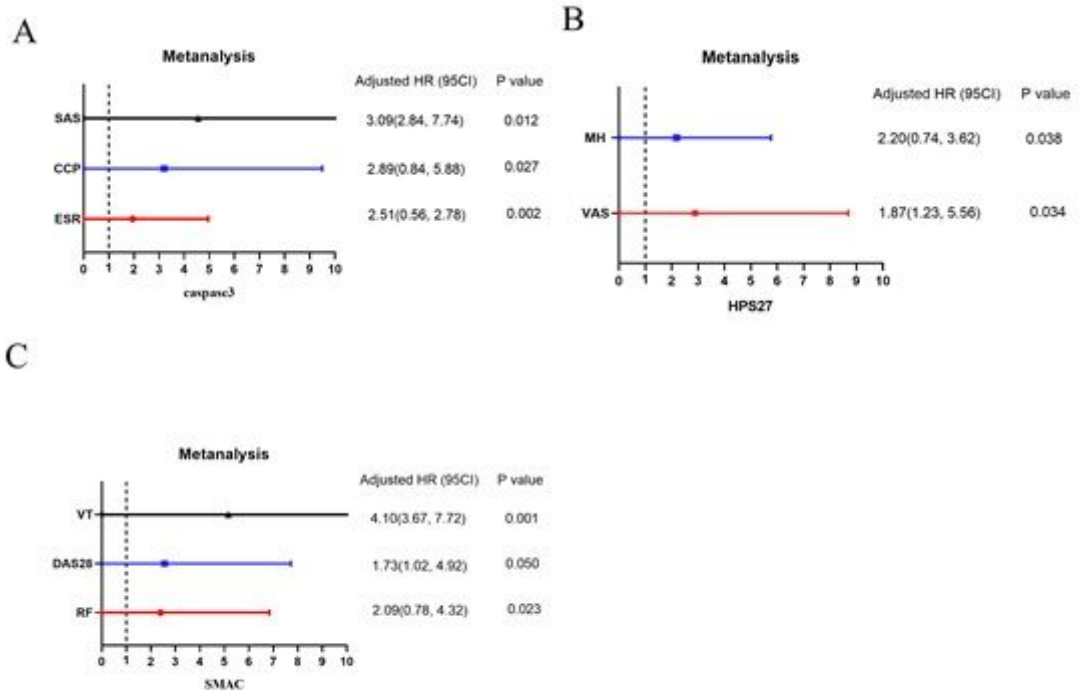

Figure 7

Logistic Regression analysis of the apoptosis-related differential proteins related factors. (A) ESR, CCP, SAS were risk factors for caspase3. (B) VAS and MH were risk factors for HPS27. (C) RF, DAS28, VT were risk factors for SMAC.