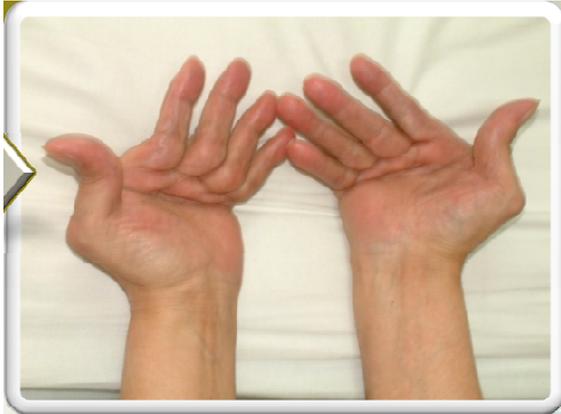


Identification of Potential Serum Biomarkers for Rheumatoid Arthritis by High Resolution Quantitative Proteomic Analysis

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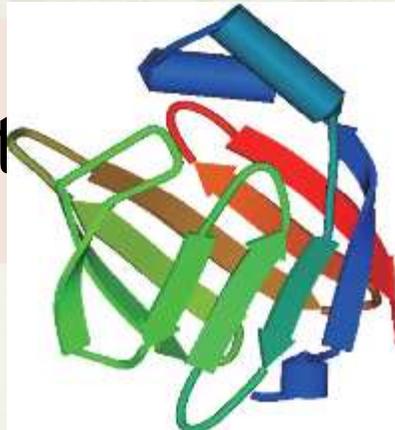
Background



RA 3.4% of world's population

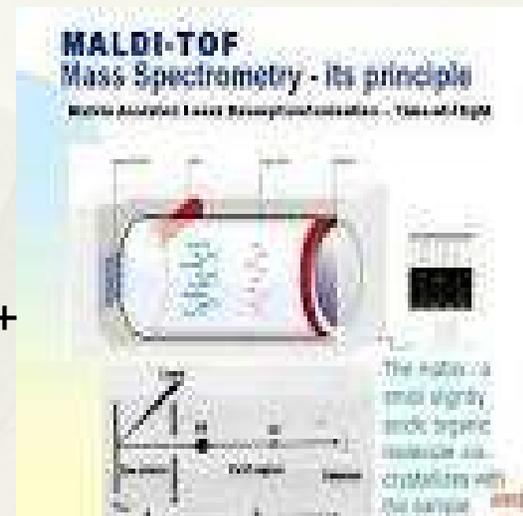
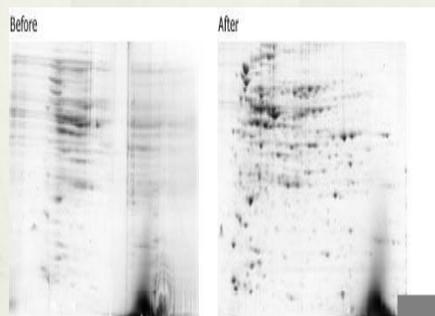
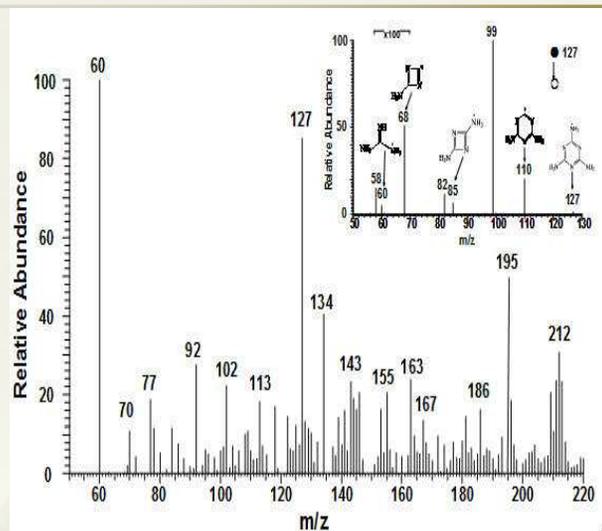
Atypical/ early RA often suffering
From delayed diagnosis and treatment

It is urgent to



biomarkers of RA

Background

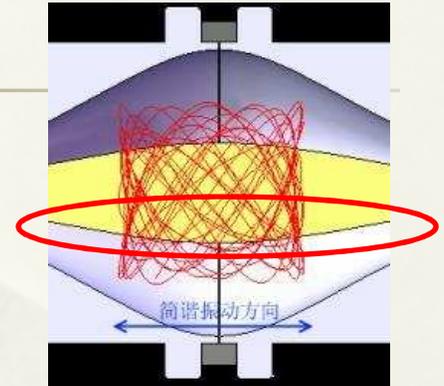
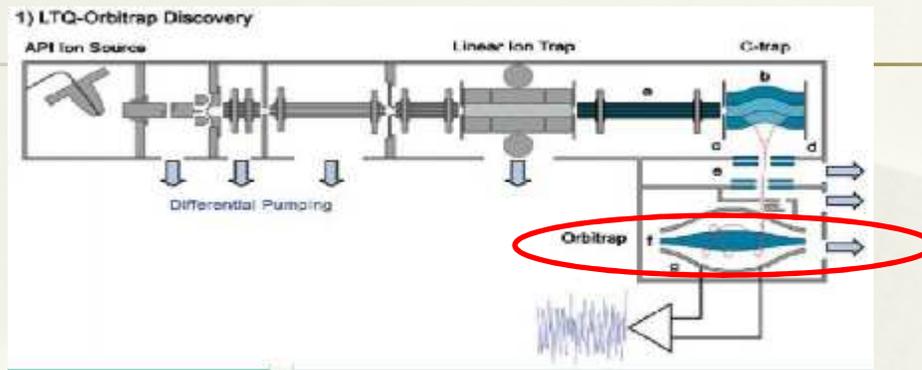


Low resolution, inadequacy for quantitative analysis
limited the efficiency of biomarker screening

?

It is important to find a better way for biomarker discovery

Background



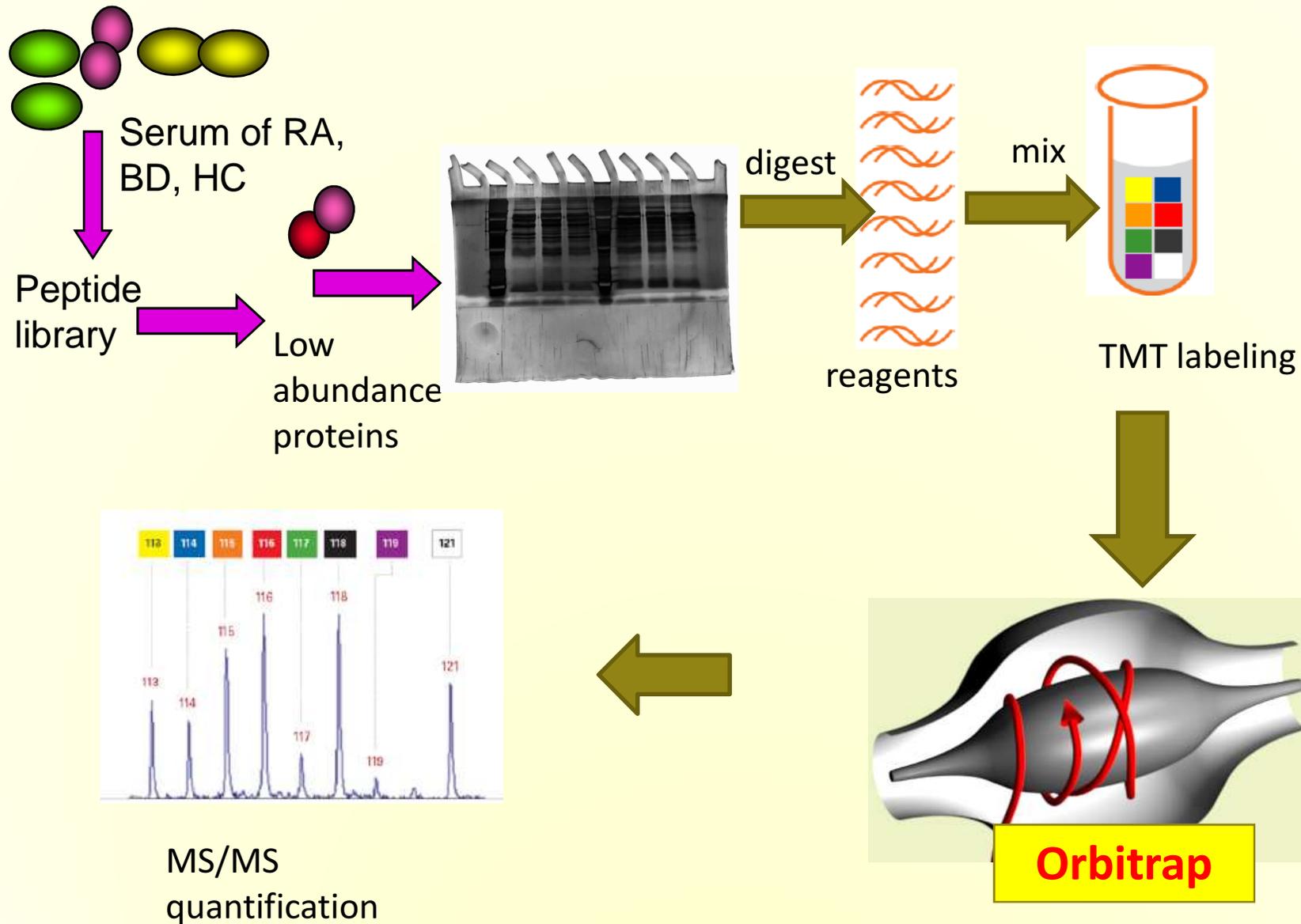
- * Orbitrap, use Fourier Transformation technology
Higher resolution power (up to 100000)
- * accurate mass measurement (2-5 ppm)
- * may provide a novel accurate platform for biomarker exploration

Objective

- * High resolution Mass spectrum based on Orbitrap to screen RA associated proteins
- * differentially expressed between RA patients and healthy controls.



Methods



Results

- * According to fold change ≥ 1.5 and $p < 0.05$ criteria, 18 proteins were up-regulated in RA patients ($RA/HC \geq 1.5$, $p < 0.05$), 8 proteins were down-regulated ($RA/HC \leq 0.67$, $p < 0.05$).

Serum biomarkers elevated in RA, BD

Figure 2.1.1 18 upregulated proteins screened out of RA

IPI number	PROTEIN name	RA/HC ratio	Amino acid	Molecular weight
IPI:IPI00022389.1	Isoform 1 of C-reactive protein	3.41±2.70	224	25
IPI:IPI00975939.1	SAA2-SAA2 protein	6.53±8.99	208	23.3
IPI:00968182.1	Uncharacterized protein	1.54±0.46	348	38.8
IPI:IPI00479723.5	Isoform 10 of Fibronectin	2.87±2.38	2176	240
IPI:00552578.2	Serum amyloid A protein	1.79±0.23	122	13.5
IPI:IPI00296099.6	Thrombospondin-1	1.83±0.20	1170	129
IPI:IPI00025052.2	Isoform of Ficolin-2	1.918±0.225	275	30.2
IPI:IPI00021842.1	Apolipoprotein E	2.28±0.05	317	36.1
IPI:IPI00022391.1	Serum amyloid P-component	2.29±1.01	223	25.4
IPI:IPI00291262.3	Isoform 1 of Clusterin	2.25±0.51	449	52.5
IPI:00026199.2	Glutathione peroxidase 3	1.75±0.48	226	25.5
IPI:00843942.1	Complement factor H-related protein	1.50±0.08	569	64.4
IPI:00883722.1	Complement factor H-related 1	1.61±0.10	271	30.8
IPI:00006662.1	Apolipoprotein D	1.63±0.04	189	21.3
IPI:00021857.1	Apolipoprotein C-III	2.65±0.16	99	10.8
IPI:00022731.1	Apolipoprotein C-IV	1.90±0.12	127	14.5
IPI:00304273.2	Apolipoprotein A-IV	1.53±1.43	396	45.4
IPI00022229.2	Apolipoprotein B-100	1.56±0.37	4563	515

Serum biomarkers screened in BD patients

IPI number	Name of protein	RA/HC ratio	Amino acid	MW (kD)
IPI00296099	Complement component C7	1.50±0.15	843	93.5
IPI00879709	Complement component C6 precursor	1.57±0.05	943	105.7
IPI00401213	cDNA FLJ46365 fis, clone TEST14051054	1.57±0.14	190	21.2
IPI01814270	Mutant Apo B 100	1.59±0.58	4344	489.5
IPI00896380	Isoform 2 of Ig mu chain C region	1.56±0.70	473	51.8
IPI00304273	Apolipoprotein A-IV	1.63±0.54	396	45.4
IPI00021857	Apolipoprotein C-III	2.68±2.64	99	10.8
IPI00022389	Isoform 1 of C-reactive protein	3.45±1.62	223	25.0
IPI00552578	Serum amyloid A protein	3.8±1.43	449	13.5
IPI00006146	Serum amyloid A2 isoform a	11.84±11.08	122	13.5

Serum biomarkers downregulated in RA, BD

Figure 2.1.2 8 downregulated proteins screened out of RA

IPI number	PROTEIN name	RA/HC ratio	Amino acid	Molecular weight
IPI:IPI00032220.3	Angiotensinogen	0.43±0.29	485	53.1
IPI:IPI00019580.1	Plasminogen	0.56±0.45	810	90.5
IPI:00656111.1	Isoform E of Proteoglycan 4	0.45±0.15	933	102
IPI:00923551.1	cDNA FLJ54318, highly similar to Complement C1r subcomponent	0.24±0.19	599	68.5
IPI:00478003.3	Alpha-2-macroglobulin	0.47±0.33	355	163
IPI00645038.1	Inter-alpha (Globulin) inhibitor H2	0.52±0.37	935	105
IPI:00385294.5	58 kDa protein	0.25±0.34	521	57.5
IPI:IPI00218732.4	Serum paraoxonase/arylesterase 1	0.46±0.32	355	39.7

Serum biomarkers screened in BD patients

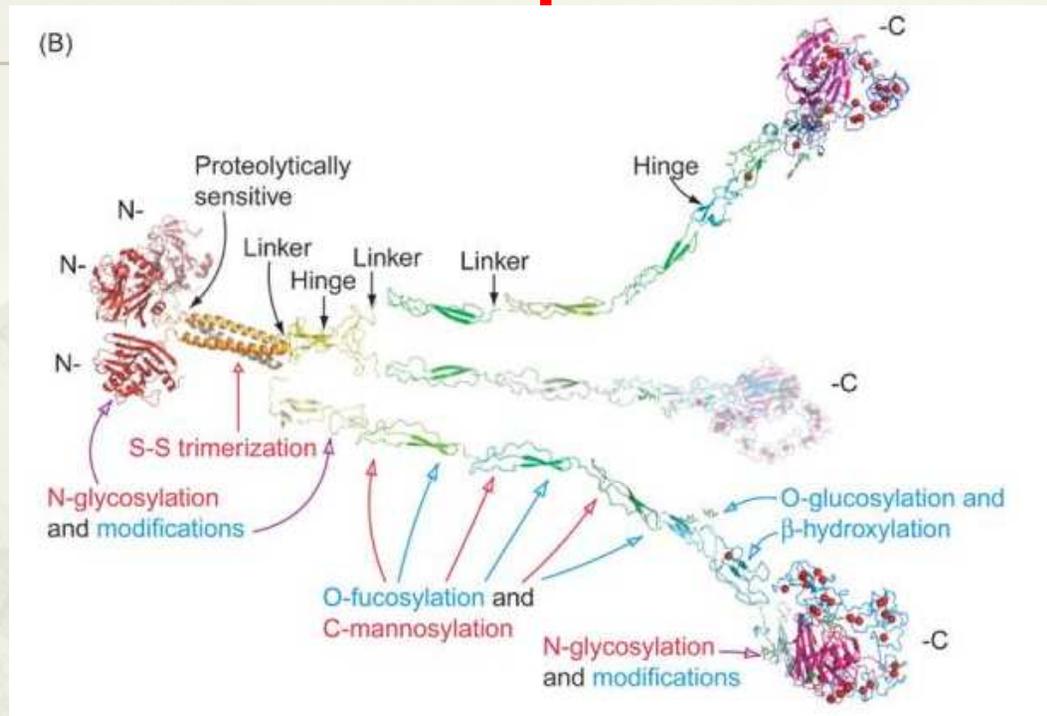
IPI number	Name of protein	RA/HC ratio	Amino acid	MW (kD)
IPI00022392	Complement C1q subcomponent subunit A	0.68±0.11	245	26.0
IPI00017601	Ceruloplasmin	0.67±0.04	1065	122.1
IPI00656111	Isoform E of Proteoglycan 4	0.62±0.34	933	102.4
IPI00984004	Similar to Ig kappa chain V-III region WOL	0.55±0.07	95	10.5
IPI00021854	Apolipoprotein A-II	0.48±0.14	100	11.2
IPI00923551	cDNA FLJ54318, highly similar to Complement C1r subcomponent	0.30±0.09	599	68.5
IPI00385294	58 kDa protein	0.33±0.07	521	57.5
IPI00645038	Inter-alpha (Globulin) inhibitor H2	0.38±0.17	485	105.2

Biomarker candidates of RA

Figure 2.1.1 18 upregulated proteins screened out of RA

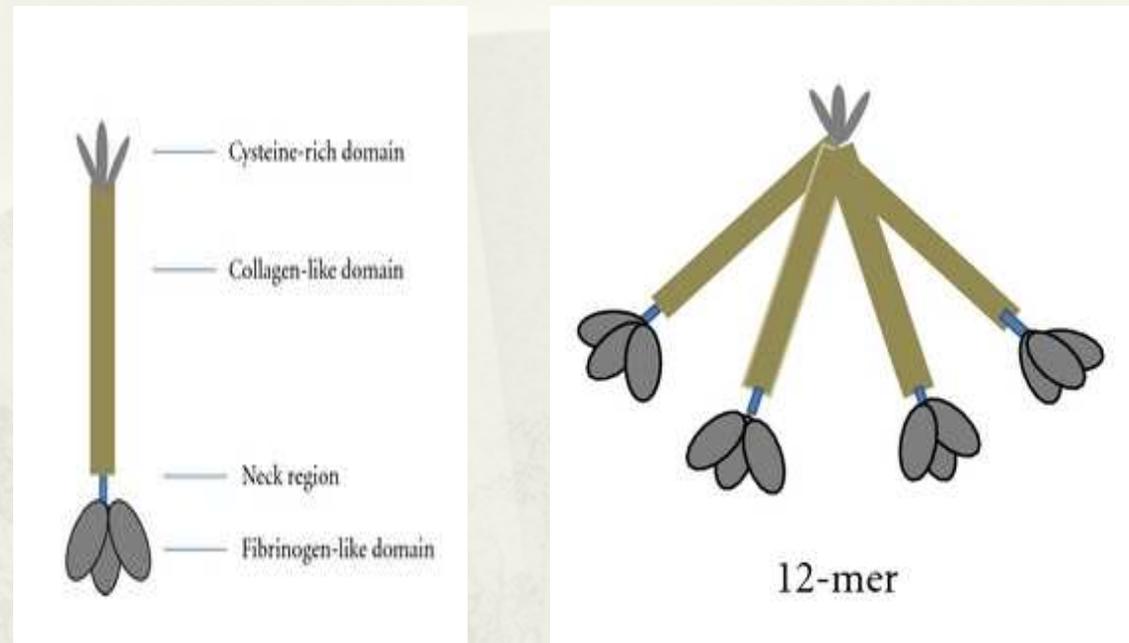
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Tsp-1



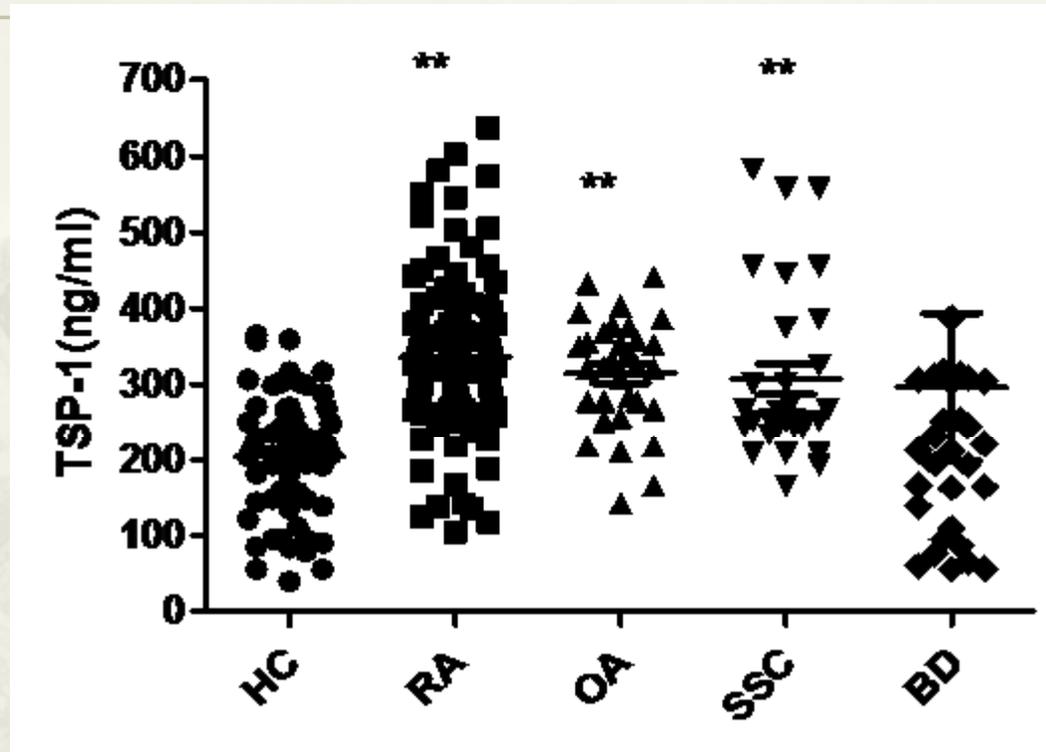
TSP-1 might acts as a chemotactic agent, which has an inhibitory effect on neovascularization, and might be a suppressor of Rheumatoid Arthritis

Ficolin-2



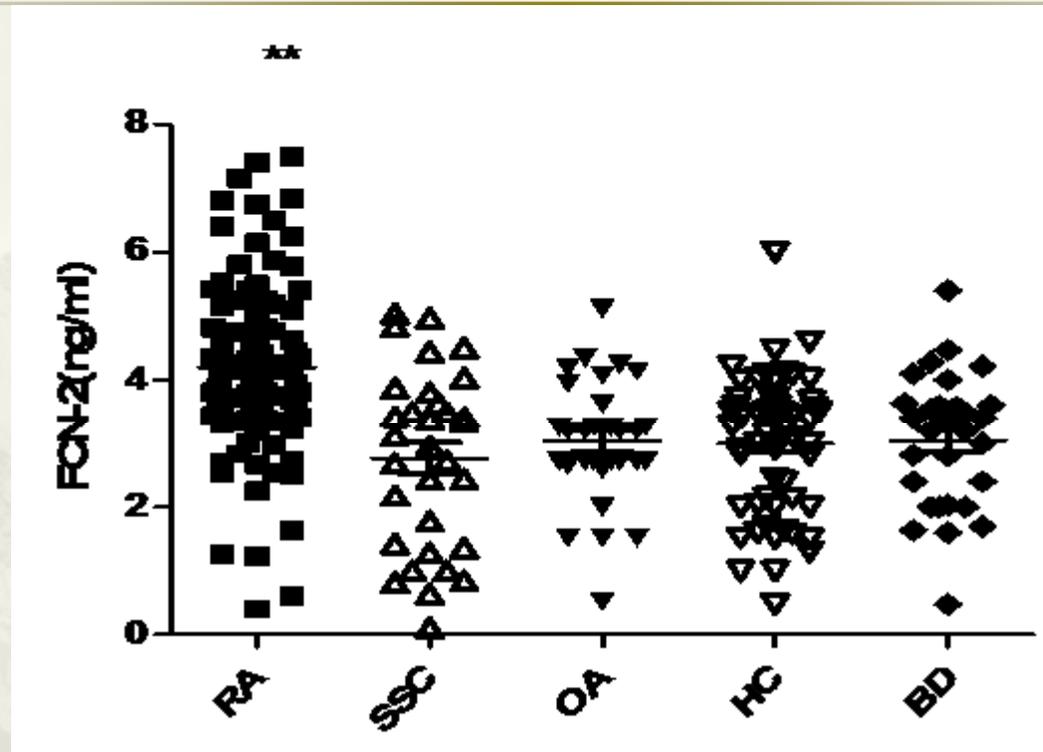
Ficolin-2 participates in the lectin complement pathway; as an important regulatory molecule in innate immunity , it may have been implicated in RA

TSP-1 levels in Autoimmune diseases



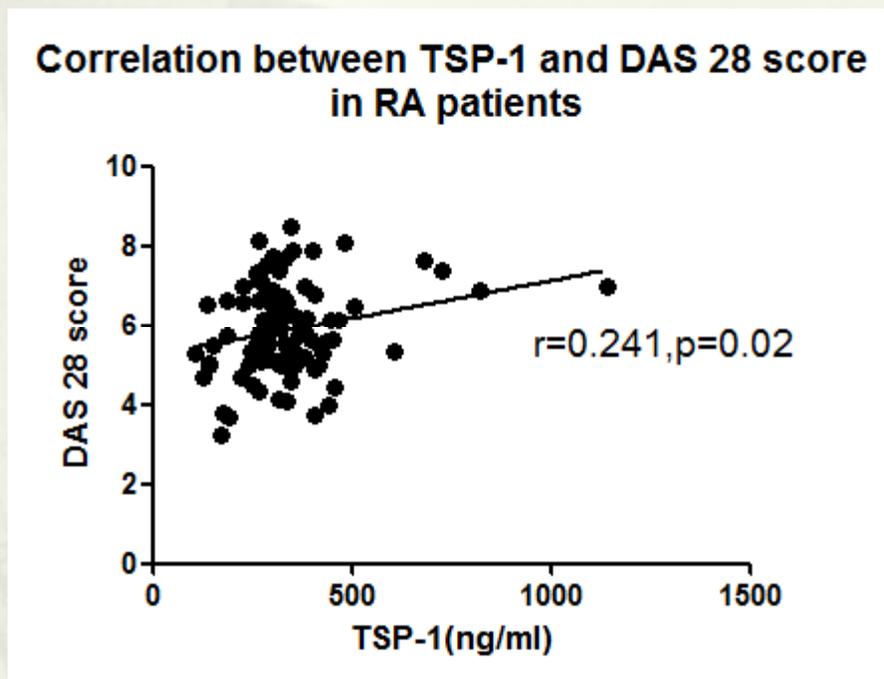
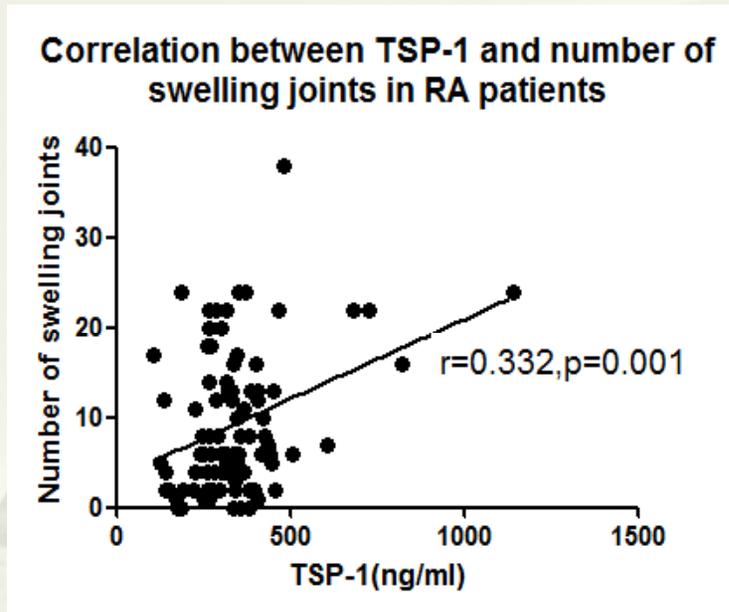
Level of TSP-1 was significantly elevated in 98 RA patients than in 58 healthy controls (** $p < 0.01$).

FCN-2 levels in autoimmune diseases



Level of FCN-2 was significantly elevated in 98 RA patients than in 58 healthy controls (** $p < 0.01$).

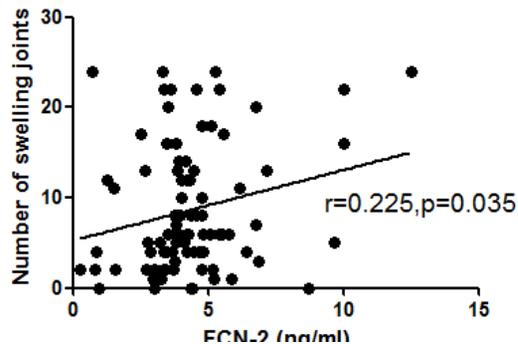
Correlation of serum TSP-1 with clinical manifestation of RA patients



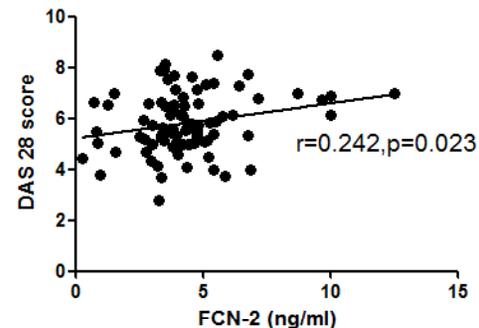
We can see from the figure that serum level of TSP-1 was positively correlated with the number of swelling joints and DAS28.

Correlation of FCN-2 with clinical manifestations in RA

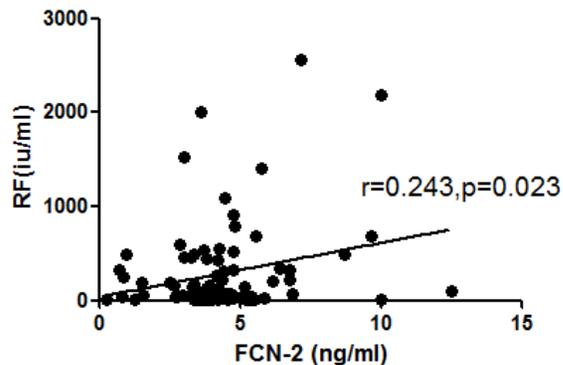
Correlation between level of FCN-2 and number of swelling joints RA patients



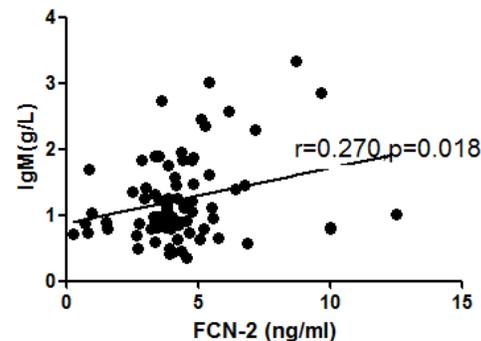
Correlation between level of FCN-2 and DAS 28 score in RA patients



Correlation between level of FCN-2 and RF in RA patients

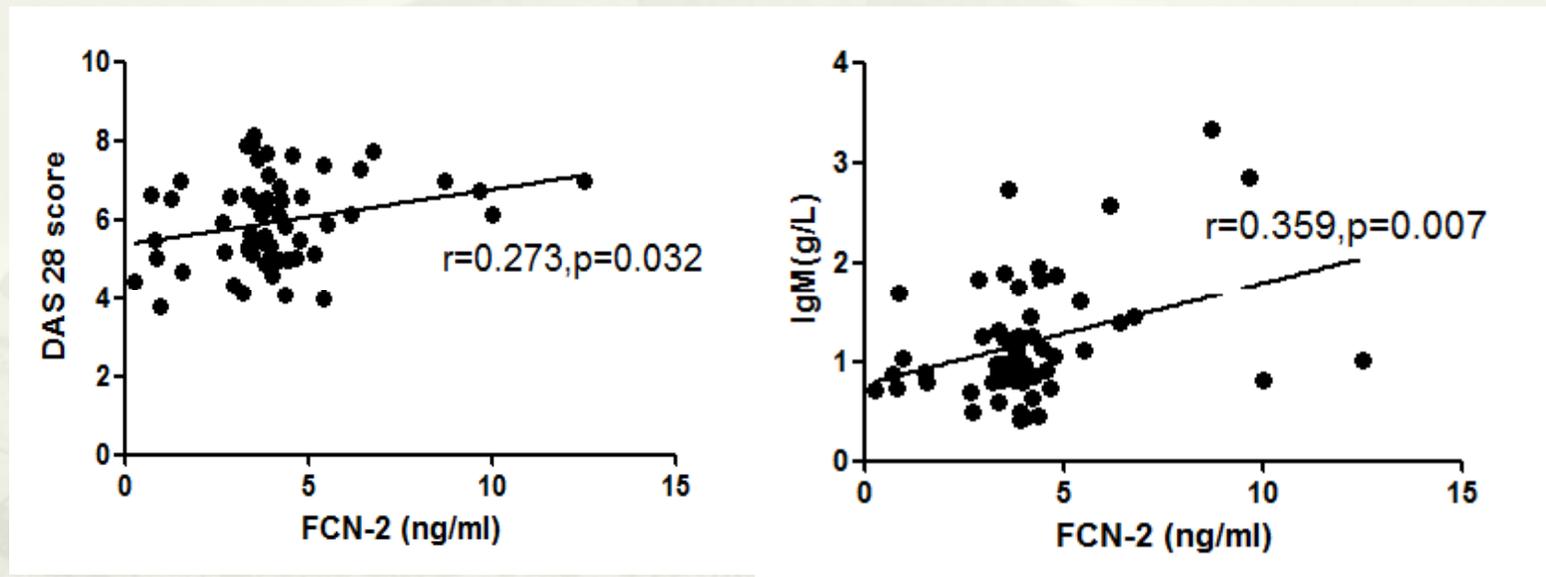


Correlation between level of FCN-2 and IgM in RA patients



Meanwhile, it was observed that serum level of ficolin was positively correlated with the number of swelling joints, DAS28, level of RF and IgM.

Clinical relevance of serum FCN-2 in early RA patients



Conclusion

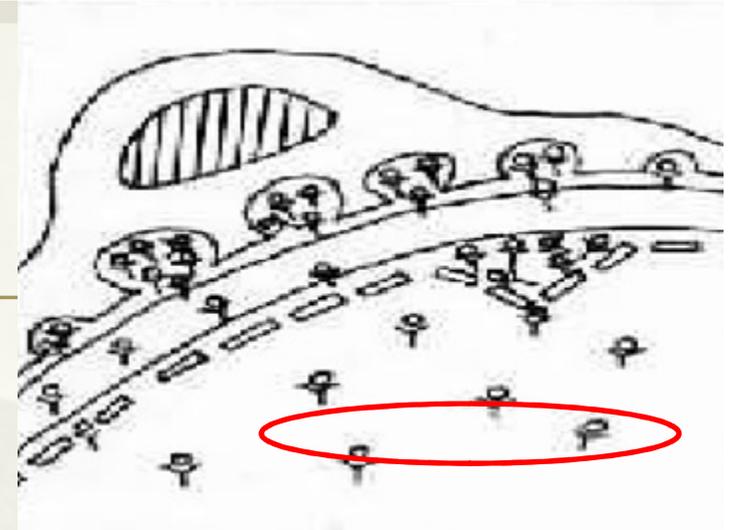
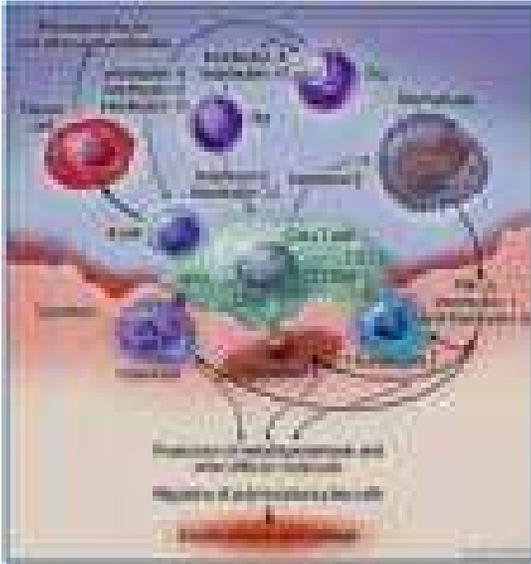
26 proteins, 18 elevated, 8 downregulated

TSP-1, FCN-2 were further proved to be correlated with disease activity indicator

Biomarkers screened may offer the potential to serve as diagnostic or disease evaluation tools for RA.

Limits

?



Most of the important disease indicators
Cytokine? Difficult to be detected

CIC relevant biomarkers?
what we should do next time

Thank you for your attention

- * Thanks for Professor Zhanguo LI
- * My collaborates: Xiaolin SUN
- * Guiye LI
- * Professor Haiteng DENG
- * Yuling CHENG
- * Tsinghua University
- * Professor Cibo HUANG