Talin is cleaved and expressed as a short form predominantly in patients with rheumatoid arthritis

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RESULTS

Fig.1

Fig.2

Fig.3

Fig.4

Fig.5

Table 1. Sensitivity and specificity of short-talin in RA patient PBMC

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<th>Pathology</th>
<th>RA 32kD short-talin</th>
<th>15kD short-talin</th>
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** p<0.01

CONCLUSION

Our findings suggest that the intracellular talin in RA patients is cleaved into short-talin and expressed predominantly in RA patient PBMC and plasma. This short-talin might be related to the pathogenesis of RA.

PURPOSE

Talin has been known as a cytoskeletal protein, which, by binding to integrin/beta-subunit, enhances the inside-out signaling from intracellular to extracellular of integrins, cell adhesion, cell migration, and causes chronic inflammation and angiogenesis. Last ACR meeting, we reported plasma talin was expressed predominantly in rheumatoid arthritis (RA) patients and it could be a candidate marker for the diagnosis and monitoring RA treatment. In this paper, we investigated the intracellular talin expression in RA patients.

METHODS

1) Patients and controls

RA patients (fulfilled 2010 ACR/EULAR Criteria) n=42
SLE patients n=30
Normal healthy controls n=31

2) Western blot

Antigen: cell lysis from peripheral blood mononuclear cells (PBMCs)

1st: anti-talin antibody (H-18)
2nd: HRP-conjugated anti-goat IgG
3rd: HRP-conjugated anti-mouse IgG

3) GST-fusion protein

Talin expressed as GST-fusion protein

Normal+RA1
Normal+RA2
Normal+RA3
Normal+RA4

Western Blot using H-18

Western Blot using TA205

RESULTS

Fig.1 32kD short-talin is detected predominantly in RA patients. PBMC lysate was incubated with calpain1 and was electrophoresed on a SDS-PAGE gel. Membrane was blotted with anti-talin antibody, H18. Red arrows show the N-terminal 32kDa short calpain1 fragment of talin. Blue arrows show the wild-type talin of calpain1 fragment.

Fig.2 19kD short-talin is detected predominantly in RA patients. Membrane was blotted with anti-talin antibody, TA205. Pink arrows show the C-terminal 15kDa short calpain1 fragment of talin. Blue arrows show the wild-type talin of calpain1 fragment.

Fig.4 Talin-fusion protein is cleaved by PBMC lysate from RA patients. GST-talin fusion protein was incubated with each of 4 RA patient PBMC lysates. GST-fusion protein was isolated using Glutathione Sepharose 4B beads. The wild-type talin of calpain1 fragment (green arrow) was degraded into 32 kDa short talin (orange arrow) after incubating with each RA patient PBMC lysate (RA1-RA3). These observations might suggest the production of short-talin by the cleavage with a protease.

Fig.5 Talin could be cleaved by PBMC lysate from RA patients. PBMC lysates obtained from a normal control was incubated with each of 4 RA patient PBMC lysate, incubated with calpain1, and were electrophoresed on a SDS-PAGE gel. Membrane was blotted with anti-talin antibody, H18. The wild-type talin fragment (blue arrow) was degraded into 32 kDa short (red arrows) talin after incubating with each RA patient PBMC lysate (RA1 - RA4). These observations might suggest the production of short-talin by the cleavage with a protease.

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Fig.5 Talin could be cleaved into short-talin by a protease.