Arthritogenicity of annexin VII revealed by phosphoproteomics of rheumatoid synoviocytes

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INTRODUCTION

To identify novel molecules involved in the pathogenesis of rheumatoid arthritis (RA), we here applied differential phosphoproteomic analysis to articular synoviocytes between RA and osteoarthritis (OA). By the analysis, we identified multiple proteins differently phosphorylated between RA and OA. We focused on annexin VII (Anx7), one of the highly phosphorylated proteins in RA. We prepared Anx7-transgenic mice to evaluate their susceptibility to collagen-induced arthritis (CIA).

MATERIALS and METHODS

Synoviocytes, obtained from 5 patients with RA and 5 with OA, were cultured separately. Total proteins were extracted from the cultured synoviocytes and were then separated by 2-dimensional electrophoresis. The proteins were then used for phosphoproteomic analysis to articular synoviocytes between RA and OA. We identified multiple proteins with different phosphorylation levels between RA and OA. We focused on Anx7. To investigate roles of Anx7 in the pathology of RA, we prepared human Anx7 transgenic C57BL/6 (Anx7-Tg-B6) mice by using CAG promoter. To investigate roles of Anx7 in the pathology of RA, we immunized the Anx7-Tg-B6 mice with type II collagen (CII) to induce collagen induced arthritis (CIA). This experiment was approved by the institutional review board and informed consent.

RESULTS

Phosphoproteins were detected by phosphoprotein-specific staining of the 2-DE gels (Fig. 1). We detected 408 phosphoprotein spots in total, 287 of which were phosphorylated at similar levels between RA and OA groups (29±27 vs. 29±27). 56 out of the rest spots showed higher intensity in the RA group than in the OA group (OA/RA=2), and 51 out of the rest showed higher intensities in the OA group than in the RA group (RA/OA=0.5). In addition, 2 spots were detected only in the RA group and 12 spots were detected only in the OA group. Among the identified proteins, we focused on Anx7 of the spot c298 (Fig. 1), whose phosphorylation was 3.3 fold-higher in the RA group than in the OA group (Fig. 2A). Western blotting (WB) with anti-Anx7 monoclonal antibodies revealed that not only the intensity of phosphorylation but also whole amount of Anx7 were found 1.4-2.5 fold higher in the RA group than in the OA group (Fig. 2B). Immunohistochemistry (IHC) with anti-Anx7 monoclonal antibodies demonstrated that Anx7 was dominantly located in synovial lining cells of the patients with RA (Fig. 2C).

To investigate roles of Anx7 in the pathology of RA, we prepared Anx7-Tg-B6 mice, and then induced CIA to Anx7-Tg-B6 mice. Informatively the genetic background of C57BL/6 is known to be rather resistant to CIA induction. As a result, the Anx7-Tg-B6 mice, not the WT-B6 mice, showed severe redness and swelling of limbs (Fig. 3A), as is usually observed in CIA-sensitive mouse strains like wild type DBA/1J (WT-DBA/1J). 60% of the Anx7-Tg-B6 mice developed polyarthritis in 3 days after the 2nd immunization, although only 20% of the WT-B6 mice developed arthritis in the same time. The mean arthritis scores were much higher in the Anx7-Tg-B6 mice than in the WT-B6 mice (Fig. 3C). Accordingly, severe bone destruction in joints was observed radiographically in the Anx7-Tg-B6 mice (Fig. 3B). Further, destruction of bone and cartilage, proliferation of synovium, infiltration of inflammatory cells, and pannus-like formation were observed in the Anx7-Tg-B6 mice by HE staining (Fig. 3C). The Anx7-Tg-B6 mice showed significantly higher histological arthritis scores than the WT-B6 mice.

To confirm the contribution of Anx7 in the development of CIA, we examined effects of the anti-Anx7 antibodies on CIA in WT-DBA/1J mice, which are known to be susceptible to CIA. As a result, administration of the anti-Anx7 antibodies was found to suppress the severity of CIA in WT-DBA/1J mice (Fig. 3D). Serum concentrations of IL-6 and TNF-α elevated in CIA were not affected by the administration of the anti-Anx7 antibodies both in the Anx7-Tg-B6 mice and in the WT-DBA/1J mice.

DISCUSSION

We evidenced for the first time that phosphoproteome of the synoviocytes were considerably different between RA and OA. This may reflect different nature of synoviocytes like strong proliferative potential in RA.

Clinically, blocking of TNF-α and other inflammatory cytokines have been found effective in treatment of RA recently. However, considerable patients with RA still wait for other effective therapies because the blocking strategies can not be applied to them due to pre-existing complications like malignancy and severe infection. Further, the blocking therapy is sometimes causes adverse effects such as infection. In this context, efforts to establish a novel therapeutic target should be continued. Our data here suggest that Anx7 play a crucial role in the development and/or exacerbation in RA and that Anx7 is a potent therapeutic target independent from cytokines.