Role of synoviolin in rheumatoid arthritis: possible clinical relevance

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The symptoms of rheumatoid arthritis (RA) are derived from the process of chronic inflammation and the overgrowth of synovial cells. However, the mechanisms of RA flares are not fully understood. To clarify these mechanisms, the authors carried out immunoscreening using anti-rheumatoid synovial cell antibodies and identified and cloned synoviolin – an endoplasmic reticulum-associated degradation (ERAD) E3 ubiquitin ligase. This molecule is overexpressed in the rheumatoid synovium and approximately 30% of littermates of *synoviolin*-overexpressing mice developed spontaneous arthropathy. Moreover, *synoviolin*$^{+/-}$ mice were resistant to collagen-induced arthritis through enhanced apoptosis of synovial cells. Based on the gain- and loss-of-function, the authors consider synoviolin to play a critical role in the crisis of arthritis, and propose that RA is a hyper-ERAD disease. These findings provide a new pathogenetic model of RA, and suggest that synoviolin could be targeted as a therapeutic strategy for RA.

Keywords: apoptosis, endoplasmic reticulum-associated degradation, endoplasmic reticulum stress, hyper-endoplasmic reticulum-associated degredation disease, rheumatoid arthritis, synovial cell, synoviolin

Rheumatoid arthritis (RA) is a disease associated not only with painful joints, but also generalized symptoms related to the whole body such as febricula, malaise and anorexia. RA affects approximately 1% of the population worldwide [1,2]. Although RA is a serious condition, a specific cure that leads to the improvement of quality of life is not yet available, mainly because the exact etiology of RA is still poorly understood.

The pathological features of RA include the chronic inflammation of systemic joints associated with overgrowth of synovial cells, which eventually causes cartilage and bone destruction in the affected joint [3,4]. It is thought that inflammation results from the activation of the cytokine system regulated by inflammatory cells [5]. During the course of inflammation, activated macrophages produce tumor necrosis factor (TNF)-α, interleukin (IL)-1 and IL-6. These cytokines, in turn, stimulate overgrowth of synovial cells to form a mass of synovial tissue called pannus, which invades the bone and cartilage through osteoclast activation and protease production [6–9]. As RA is considered to be an autoimmune disease, medical treatment targeting inflammation has been applied. However, nearly 25% of RA patients do not respond to anticytokine or anti-inflammatory therapies [10–12]. This article will focus on synovial cells and discuss the mechanisms of hyperplasia of rheumatoid synovial cells (RSCs).

Molecular cloning of synoviolin

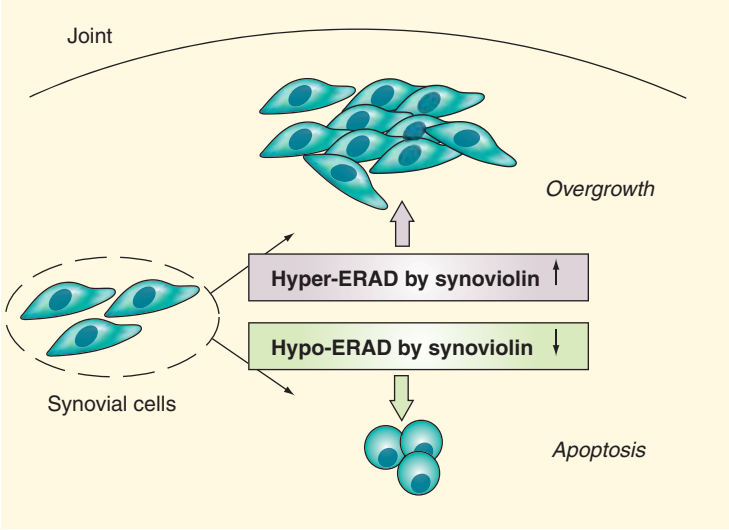
The authors' laboratory first cloned RSCs and found that these cells bear autonomous proliferation properties with aberrant cytokine production in a culture system [13,14]. Moreover, it was found that human T-cell leukemia virus type I (HTLV-I), one of the epidemic human retroviruses associated with arthropathy (HAAP) [15], and *tax*, the viral transforming gene that causes HAAP and its product, pp40Tax, could transform synovial cells into RSCs in patients and overexpressing mice [16–18]. However, expression of pp40Tax is not observed in human RSCs. Thus, to determine the functionally equivalent endogenous gene products in RSCs, comprehensive immunoscreening was carried out using anti-RSC antibodies, and synoviolin was cloned successfully [19], a human homolog of the yeast ubiquitin ligase (E3) Hrd1p/Del3p [20]. Synoviolin is an endoplasmic reticulum (ER)-resident membrane protein with a RING-H2 motif and is highly expressed in the rheumatoid synovium [19]. Since it is expected that this endogenous molecule might elucidate the cause of RA, the distribution and functional properties of synoviolin was investigated further.

Arthropathy in *synoviolin*-overexpressing mice

To investigate physiologically the role of this molecule, the tissue distribution of synoviolin was first examined by northern blot analysis. The results in mice showed that *synoviolin* was ubiquitously expressed in various tissues. This was an unexpected result, since synoviolin was cloned in RSCs and it was predicted that *synoviolin* was specifically expressed in the rheumatoid synovium.

To gain insight into the function of synoviolin *in vivo*, the *synoviolin*-overexpressing mouse was generated. Essentially, it is desirable to analyze the

Figure 1. RA is a 'hyper-ERAD disease'.



Synoviolin is highly expressed in RSCs, which leads to hyperactivation of the ERAD system. Synoviolin is an E3 ubiquitin ligase and functions in the ERAD system. When the misfolded proteins accumulate in the ER they are eliminated by ubiquitin- and proteasome-dependent degradation processes to avoid cell death caused by dysfunction of ER. The ERAD system is activated by overexpression of synoviolin, which prevents apoptotic death of synovial cells, consequently leading to synovial hyperplasia.
ER: Endoplasmic reticulum; ERAD: ER associated degredation; RA: Rheumatoid arthritis; RSC: Rheumatoid synovial cell.

synoviolin function by using a promoter expressed specifically in synovial cells. However, such a promoter has not yet been identified. Thus, human *synoviolin*-overexpressing mice were established using a β-actin promoter, which drives systemic protein expression, including synovial cells. Strikingly, approximately 30% of *synoviolin*-overexpressing mice developed spontaneous arthropathy with marked joint swelling even in the C57BL/6 strain. Interestingly, no other abnormality was apparent in these mice throughout their life, apart from the spontaneous arthritis. In spite of the ubiquitous expression of *synoviolin*, this phenotype of *synoviolin*-overexpressing mice exhibited pathological features similar to those of patients with RA, indicating that these mice are a suitable animal model of RA.

Collagen-induced arthritis model of *synoviolin*-deficient mice

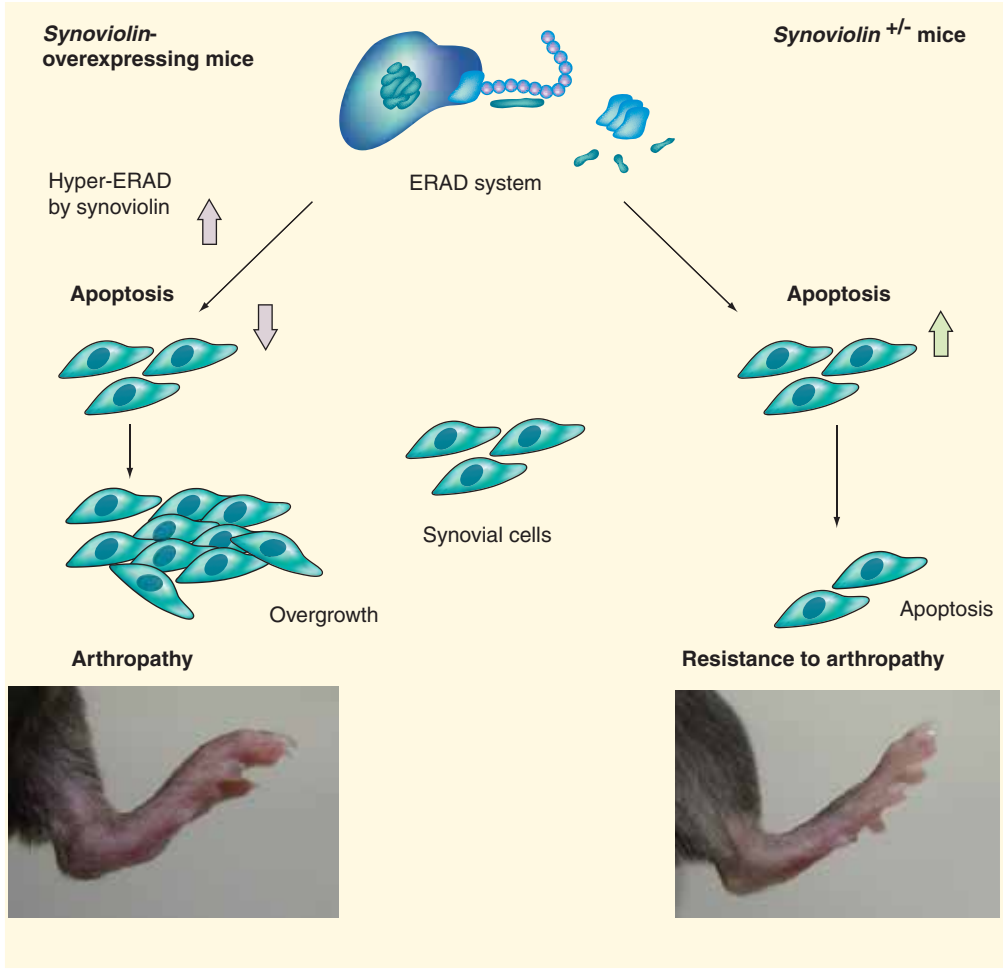
The results of the gain-of-function study suggested that synoviolin acts as an inducer of synovial cell hyperplasia. An attempt to verify this hypothesis was subsequently made in *synoviolin*-deficient mice, that is, loss-of-function. Mice with type II collagen-induced arthritis (CIA) are used commonly as a model of experimental arthritis. This model can also be considered suitable for analyzing the function of synoviolin in RA. Thus, *synoviolin*-deficient (*syno*$^{-/-}$) mice were generated by gene-targeted disruption.

Unfortunately, all fetuses lacking synoviolin died *in utero* at around E13.5 [21], although Hrd1p/Del3p, a yeast ortholog of synoviolin, was described as nonessential for survival [22]. *Syno*$^{-/-}$ were anemic owing to enhancement of apoptosis in the fetal liver [21]. In the next series of studies, the relationship between synoviolin and flares of RA was clarified. The CIA model was applied to *synoviolin*-heterozygous mice (*syno*$^{+/-}$). The results showed that *syno*$^{+/-}$, treated for induction of CIA, were resistant to the development of arthritis. Furthermore, CIA-*syno*$^{+/-}$ mice exhibited intact immunoreactions and demonstrated inflammatory cell infiltration but lacked advanced synovial cell hyperplasia [19]. These results indicate that the synovial cell hyperplasia process is independent of proceeding immunoreactions, and is an indispensable process in the pathogenesis of arthropathy. This conclusion was confirmed *in vitro*; *synoviolin* small interfering RNA (siRNA) suppressed the growth of synovial cells even when these cells were stimulated by cytokines [19]. When combined, these results of loss-of-function indicate that synoviolin is essential for the crisis of arthritis.

New disease concept: hyper-ERAD disease

How does synoviolin, an E3 ubiquitin ligase resident in the endoplasmic reticulum (ER), participate in synovial cell hyperplasia? In the ER of eukaryotic cells, newly synthesized proteins are transported for correct folding. Under normal conditions, the transport and folding processes in the ER match the requirement of the secretory pathway. Alternatively, since various environmental insults can overwhelm the efficacy of intracellular protein folding, cells have a self-protective mechanism for survival following an increased demand for protein folding. ER stress can trigger a cellular response termed the unfolded protein response (UPR) [23], during which the synthesis of new proteins is inhibited globally and genes encoding the ER chaperone proteins are also upregulated to refold the misfolded proteins correctly [24]. However, when the UPR fails to deal with this problem, misfolded proteins are eliminated by ubiquitin- and proteasome-dependent degradation processes, known as the ER-associated degradation (ERAD) system, and thus spare

Figure 2. Drug targets for treatment of RA.



Gain-of-function of synoviolin results in the development of spontaneous arthropathy through the anti-apoptotic effects of synoviolin. On the other hand, *syno*$^{+/-}$ mice are resistant to the CIA model. Therefore, synoviolin could be a novel therapeutic target for RA.
CIA: Collagen-induced arthritis; ERAD: Endoplasmic reticulum associated degredation
RA: Rheumatoid arthritis.

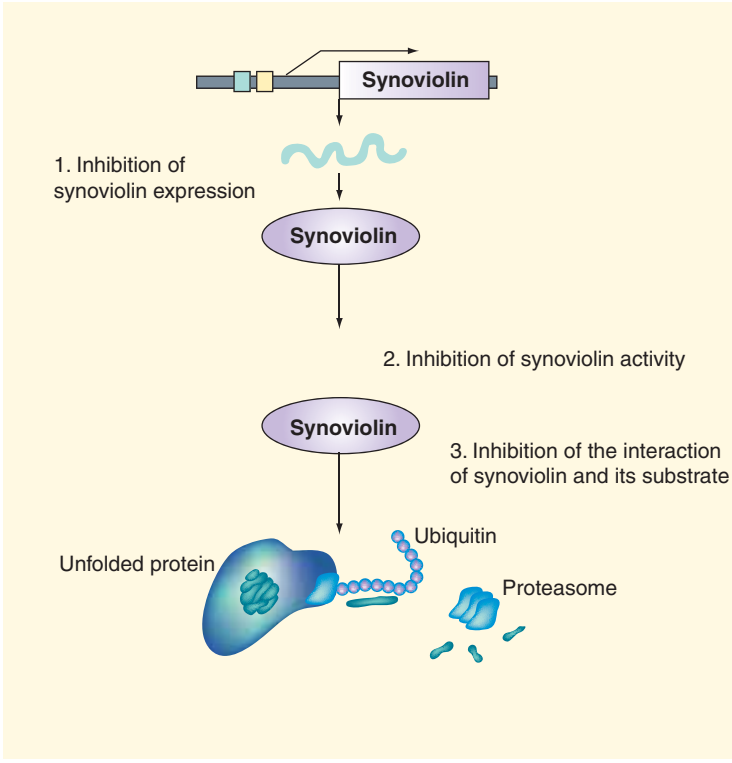
the cells from cell death through ER dysregulation [25–27]. Synoviolin is involved in this ERAD system as an ubiquitin ligase [19,28,29] and takes part in rescuing the cells from cell death. In synovial cells of CIA-*syno*$^{+/-}$, which have impaired the ERAD system owing to the lack of synoviolin, the apoptotic cells are significantly increased [19]. Therefore, in CIA-*syno*$^{+/-}$, synovial cells cannot avoid apoptotic cell death because of lack of synoviolin, thus preventing synovial cell overgrowth. However, in RSCs, hyperactivation of the ERAD system by overexpression of synoviolin could prevent synovial cell apoptotic death, consequently leading to synovial hyperplasia.

Dysfunction of the ERAD system has been implicated in various disorders. For instance, production of expanded polyglutamine causes certain inherited neurodegenerative disorders [30–32]. Furthermore, mutation of the *parkin* gene, a well known ubiquitin ligase protein in the ERAD system, is thought to result in neuronal death of the substantia nigra in patients with autosomal recessive juvenile parkinsonism [33]. The authors first postulated that hyperactivation of the ERAD system could lead to proliferative diseases and then introduced a novel concept, the hyper-ERAD disease (Figure 1).

Possible drug target for the treatment of RA

It was demonstrated that the etiology of RA could be based on hyperactivation of the ERAD system due to overexpression of synoviolin and it was concluded that synoviolin could be considered as

Figure 3. Development of synoviolin-based therapy for rhematoid arthritis.



Possible pathways in which synoviolin could be targeted for the treatment of rheumatoid arthritis.

a novel therapeutic target of RA (Figure 2). In making synoviolin a therapeutic target, three methods can be considered (Figure 3).

First, overexpression of synoviolin in RSCs could lead to the suppression of hyper-ERAD. In order to control the amount of synoviolin, it is important to elucidate the transcriptional regulation of synoviolin. Clarification of the mechanisms of transcriptional regulation of synoviolin should allow the suppression of transcription of synoviolin, thus avoiding a hyper-ERAD state. The authors recently identified a crucial site for *synoviolin* expression in the *synoviolin* proximal promoter, in other words, the Ets binding site, and that the growth-associated binding protein (GABP)-α/β complex is essential for its transcriptional regulation [34]. Thus, it is expected that RA gene therapy will become possible in the future.

Second, synoviolin is an E3 ubiquitin ligase that acts in the ERAD system, which suggests inhibition of its enzymatic activity. Blockade of synoviolin enzymatic activity should be associated with suppression of the hyper-ERAD state. In this regard, human *synoviolin*-overexpressing mice with a lack of enzymatic activity did not show any phenotype, including arthritis [Unpublished Data]. Therefore, there is a need to develop synoviolin inhibitors.

Third, since synoviolin is an E3 ubiquitin ligase, one could intercept synoviolin-substrate interaction. Synoviolin cannot function as an enzyme in the absence of an interaction with the substrate. Moreover, it is conceivable that not only synoviolin but also its substrate are expressed highly or specifically in RSCs. This is supported by the results of studies in *synoviolin*-overexpressing mice only showing arthritis despite the systemic expression. However, a substrate of synoviolin has not yet been identified. Thus, there is a need for further studies to identify a synoviolin substrate. In this regard, the authors are currently conducting such studies using the yeast two-hybrid system [Yamasaki S *et al.*, Zhang L *et al.*, Unpublished Data]. If a specific substrate of synoviolin in RSCs is identified, it is expected that disturbances of the synoviolin–substrate interaction could be used to prevent RA flares. Moreover, it is predicted that this may enhance the development of antibody therapies.

In any case, since CIA was almost completely suppressed in *syno*$^{+/-}$, further studies should be conducted to investigate the impact of approximately 50% inhibition of the amount and/or activity of synoviolin. Moreover, since synovial cell outgrowth is a common event in RA, new drugs designed to block *synoviolin* expression/activity might help find the cure of RA.

The development of a synoviolin-based marker for the diagnosis of RA, together with the development of drugs designed to block synoviolin expression and/or activity could perhaps allow the identification of the disease at an early stage and administration of effective therapy for RA.

Conclusion

Synoviolin is an important causative factor of RA, and our findings could open new avenues of investigation into the pathogenesis of RA.

Future perspective

RA has a negative impact on quality of life. Cytokines released from immune cells cause chronic inflammation and stimulate the proliferation of synovial cells that destroy bone and cartilage of joints. However, nearly 25% of RA patients do not respond to anticytokine or anti-inflammatory therapies. This may be because synovial cells acquire the autonomous proliferation ability that could be controlled by an ERAD-associated E3 ubiquitin ligase synoviolin. Therefore, we believe

that our findings will help design novel therapies for RA and expect development of selective inhibitors of synoviolin in the future.

Moreover, bone and cartilage destruction of joints in RA is one of the most serious terminal symptoms, thus we are considering the possibility of synoviolin involvement in these phenomena. Clarification of this point is our next subject, and we hope our research will help in the establishment of a new therapy.

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Executive summary

Background

- Rheumatoid arthritis (RA) affects approximately 1% of the adult population worldwide.
- RA is a disease associated with generalized symptoms related to the whole body especially to painful joints.
- The burden of musculoskeletal diseases on society has been recognized throughout the world and RA is defined as one of the most important diseases in the Bone and Joint Decade, launched by the World Health Organization in 2000 to reduce the social and financial costs of musculoskeletal disorders to society.

Pathology

- RA includes chronic inflammation of systemic joints associated with overgrowth of synovial cells, which eventually causes cartilage and bone destruction in the joints.

Synoviolin

- Cloning of synoviolin from rheumatoid synovial cells ocurred using immunoscreening.
- Synoviolin is an endoplasmic reticulum (ER)-resident membrane protein.
- Synoviolin is an E3 ubiquitin ligase associated with ER-associated degredation (ERAD).
- Synoviolin is expressed ubiquitously, however it is highly expressed in the rheumatoid synovium.

Animal study

- Mice overexpressing *synoviolin* exhibit spontaneous arthropathy and a progressive synovial hyperplasia characteristic of RA patients.
- Reduced expression of *synoviolin* in mice correlated with protection from arthritis. This resistance is not due to an impaired cytokine response or reduced inflammatory cell infiltration, but to an increase in synovial cell apoptosis.

New disease concept

- RA is an ERAD activated disease caused by by overexpressed *synoviolin* in rheumatoid synovial cells.

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