Role of synoviolin in rheumatoid arthritis: possible clinical relevance

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Rheumatoid arthritis (RA) is a disease associated not only with painful joints, but also general symptoms related to the whole body such as fever, 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 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.

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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 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
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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 2. Drug targets for treatment of RA.
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
RA is an ERAD-activated disease caused by overexpressed 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.

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 occurred using immunoscreening.
- Synoviolin is an endoplasmic reticulum (ER)-resident membrane protein.
- Synoviolin is an E3 ubiquitin ligase associated with ER-associated degradation (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 overexpressed synoviolin in rheumatoid synovial cells.

Bibliography
Papers of special note have been highlighted as either of interest (●) or of considerable interest (●●) to readers.


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