

Commentary

Rheumatoid arthritis as a hyper-endoplasmic reticulum-associated degradation diseaseSatoshi Yamasaki¹, Naoko Yagishita¹, Kaneyuki Tsuchimochi¹, Kusuki Nishioka²
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Arthritis Research & Therapy 2005, **7**:181-186 (DOI 10.1186/ar1808)**Abstract**

We introduce Synoviolin as a novel pathogenic factor in rheumatoid arthritis (RA). Experimental studies indicate that this endoplasmic reticulum (ER)-resident E3 ubiquitin ligase has important functions in the ER-associated degradation (ERAD) system, an essential system for ER homeostasis. Overexpression of Synoviolin in mice causes arthropathy with synovial hyperplasia, whereas heterozygous knockdown results in increased apoptosis of synovial cells and resistance to collagen-induced arthritis in mice. On the basis of these experimental data, we propose that excess elimination of unfolded proteins (that is, 'hyper-ERAD') by overexpression of Synoviolin triggers synovial cell overgrowth and hence a worsening of RA. Further analysis of the hyper-ERAD system may permit the complex pathomechanisms of RA to be uncovered.

Introduction

There is a general agreement that synovial cells have a crucial function in rheumatoid arthritis (RA) by forming a mass of synovial tissue, which promotes the production of matrix-degrading proteases and osteoclast activation that lead to joint destruction [1-6]. In a series of experiments that focused on synovial cells, we determined that human T cell leukemia virus type I (HTLV-I) causes arthropathy [7], and that *tax*, the viral transforming gene of HTLV-1, and its product, pp40Tax, could transform synovial cells of patients as well as those of *tax*-overexpressing mice [8-10]. These results suggest that synoviocytes can acquire the ability to overgrow autonomously in RA.

Here we discuss the role of a novel pathogenic factor for RA named 'Synoviolin' (GenBank accession no. AB024690) [11]. This novel molecule is an endoplasmic reticulum (ER)-resident ubiquitin ligase and is involved in the ER-associated

degradation (ERAD) system [12-17]. ERAD is an important processing system for ER homeostasis, and its disruption is known to result in cellular apoptosis [18]. Surprisingly, both the amount and enzymatic activity of Synoviolin regulate synovial cell proliferation and apoptosis, at least in mice [11].

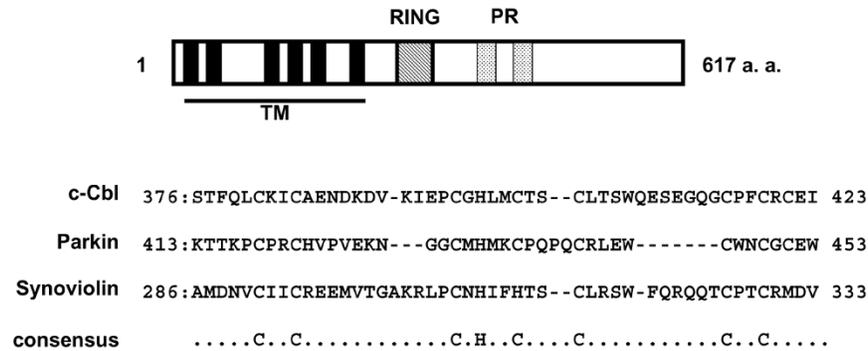
Cloning of Synoviolin

We identified Synoviolin from a human cDNA library of rheumatoid synovial cells (RSC) by immunoscreening with anti-RSC antibodies to isolate a molecule promoting the autonomous proliferation and activation of synovial cells in RA [11]. Structurally, Synoviolin has a putative six-transmembrane domain and a RING-H2 motif (Fig. 1). As reported previously, proteins with a RING finger domain act as E3 ubiquitin ligases [19], Synoviolin also exhibits a clear auto-ubiquitination activity [11]. By using immunostaining, we also determined that Synoviolin is located in the ER of synovial cells. We therefore concluded that Synoviolin is an ER-resident E3 ubiquitin ligase [11].

Previous studies in yeast and human cells concluded that ER-resident E3 ubiquitin ligases are important for ER homeostasis [20]. Because it is estimated that 30 to 40% of the newly synthesized proteins fail to fold properly in the ER [21], these unfolded proteins eventually induce severe damage of the ER (so-called ER stress) or even apoptosis of the cell (ER stress-induced apoptosis) unless two biological processes, unfolded protein response (UPR) and ERAD, work properly [20,22,23]. In brief, UPR contains two systems involved in the attenuation of global translation to stop the influx of proteins into the ER and increasing the transcription of chaperones to refold the unfolded proteins in the ER again. In contrast, the ERAD system eliminates unfolded proteins that

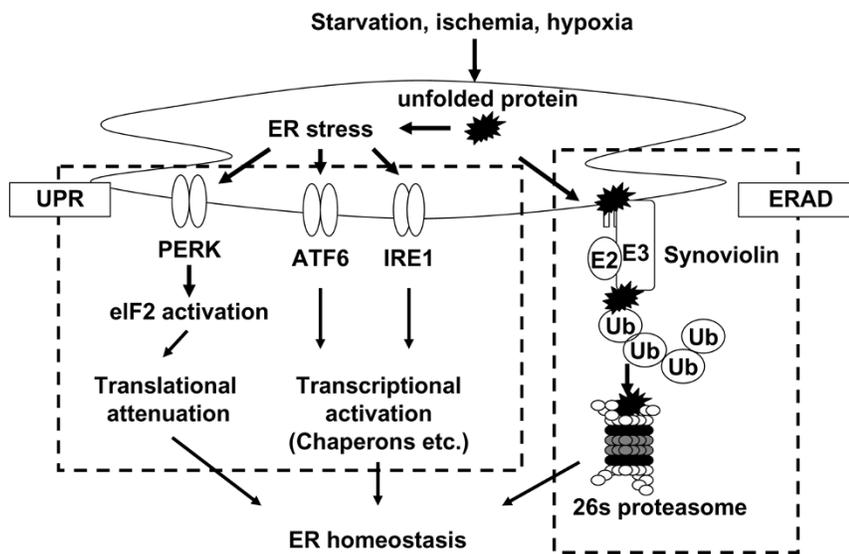
CIA = collagen-induced arthritis; ER = endoplasmic reticulum; ERAD = endoplasmic reticulum-associated degradation; RA = rheumatoid arthritis; RSC = rheumatoid synovial cells; siRNA = small interfering RNA; TNF = tumor necrosis factor; TUNEL = TdT-mediated dUTP nick end labelling; UPR = unfolded protein response.

Figure 1



Synoviolin is a RING type E3 ubiquitin ligase. Top, schematic diagram of the primary structure of Synoviolin. a.a., amino acid residues; PR, proline-rich domain; RING, RING finger domain; TM, transmembrane domain. Bottom, consensus sequence of the RING finger domain. Mutations in consensus cysteine or histidine abolish the enzymatic activity of Synoviolin.

Figure 2



Schematic representation of the proposed biological functions of Synoviolin. Synoviolin has important functions in the endoplasmic reticulum (ER)-associated degradation (ERAD) system. Synoviolin maintains ER function by eliminating unfolded proteins. ATF6, activating transcription factor 6; IRE1, inositol-requiring enzyme 1; PERK, PKR-like ER kinase; Ub, ubiquitin; UPR, unfolded protein response.

accumulate in the ER through the ubiquitin-proteasome system (Fig. 2) [18,20].

Synoviolin transgenic mice and arthropathy

To study the role of Synoviolin in RA, we reported previously the establishment of Synoviolin-overexpressing and Synoviolin knockout mice [11]. Analyses of these mice demonstrated both the induction of arthritis by overexpression and the inhibition of arthritis by knockout of a single gene, that encoding Synoviolin.

First, we established human Synoviolin-overexpressing mice by using a β -actin promoter to drive systemic expression of

the gene, because a northern blot analysis demonstrated that the tissue distribution of Synoviolin in the mouse is ubiquitous. Surprisingly, 10 of 33 Synoviolin-overexpressing mice developed spontaneous arthropathy after 20 weeks of age [11], and a histological analysis of joints of these mice demonstrated synovial cell hyperplasia and bone destruction, which resembled typical pathological features of RA joints. It should be noted that no other abnormalities are apparent in these mice throughout their life.

Next, we attempted to verify the hypothesis that Synoviolin is important for the development of arthritis using Synoviolin-knockout mice; that is, a 'loss-of-function' study. Collagen

injection can induce arthritis in experimental mice, a model known as collagen-induced arthritis (CIA). Because Synoviolin homozygous knockout (*syno*^{-/-}) mice die *in utero* [24], the 'loss-of-function' experiments were conducted in Synoviolin heterozygous knockout (*syno*^{+/-}) mice. The incidence of arthritis in *syno*^{+/-} mice (7%) was significantly lower than that in wild-type counterparts (*syno*^{+/+}) (65%). Examination of the joints by soft X-ray revealed that bone destruction in *syno*^{+/-} mice was much milder than that in *syno*^{+/+} mice. Immunological responses, including the production of type II collagen antibody, inflammatory cell infiltration, and elevation of inflammatory cytokine levels, were not impaired in *syno*^{+/-} mice. Histological analysis of synovial tissues showed marked differences between *syno*^{+/+} and *syno*^{+/-} mice. No advanced synovial cell hyperplasia was detected in CIA-*syno*^{+/-} mice, even though inflammatory cell infiltration was clearly observed in them. Detailed analysis of synovial tissues showed that the number of proliferating-cell nuclear antigen (PCNA)-positive cells in CIA-*syno*^{+/-} mice was not different from that in *syno*^{+/+} mice, but TdT-mediated dUTP nick end labelling (TUNEL) analysis demonstrated a significant increment of apoptotic cells in CIA-*syno*^{+/-} mice. Consistent with these results, *synoviolin* homozygous knockout was associated with aberrantly increased apoptosis of liver and severe impairment of erythropoiesis, and embryonic death [24]. These data suggested the importance of Synoviolin in inhibiting apoptosis.

Synoviolin in human synovial cells

Because Synoviolin-overexpressing mice show synovial cell hyperplasia, and *syno*^{+/-} mice are resistant to CIA because of increased apoptosis of synovial cells, we expected that Synoviolin has both cell-proliferating and anti-apoptotic effects. In a small-scale study we showed that suppression of Synoviolin by small interfering RNA (siRNA) inhibited the growth of RSC, even under mitogenic stimulation by tumor necrosis factor (TNF)- α and interleukin-1 β [11]. These results suggested the possible role of Synoviolin in cell proliferation. We also examined the effect of tunicamycin (a glycosylation inhibitor that inhibits proper protein folding in ER) on RSC treated with siRNA to test whether the downregulation of Synoviolin increases their susceptibility to apoptosis caused by disruption of ER function. TUNEL staining of RSC revealed enhanced susceptibility to tunicamycin-induced apoptosis, similar to Synoviolin knock-down [11], implicating the anti-apoptotic effect of Synoviolin in ER stress. Further, larger, studies are needed to confirm the relevance of Synoviolin to human RA. It is also important to explain the molecular basis of these Synoviolin-induced cellular regulatory processes to determine the underlying pathomechanism of synovial cell overgrowth in RA.

Results of a preliminary study from our laboratories suggest that RSC are basically refractory to ER stress-induced apoptosis: the concentration of tunicamycin necessary to induce apoptosis of RSC was about tenfold that required by

other human cell lines such as HEK-293 or HeLa cells. In addition, among synovial cells, RA synovial cells ($n=5$) were more refractory to ER stress-induced apoptosis than OA synovial cells ($n=5$) (Yamasaki S, Yagishita N, Tsuchimochi K, Kato Y, Sasaki T, Amano T, Beppu M, Nakamura H, Nishioka K, Nakajima T, unpublished data). These results suggest that RA synovial cells are refractory to ER stress-induced apoptosis. Accordingly, our working hypothesis in human RA is that Synoviolin promotes synovial cell proliferation and inhibits ER stress-induced apoptosis, leading to RA progression.

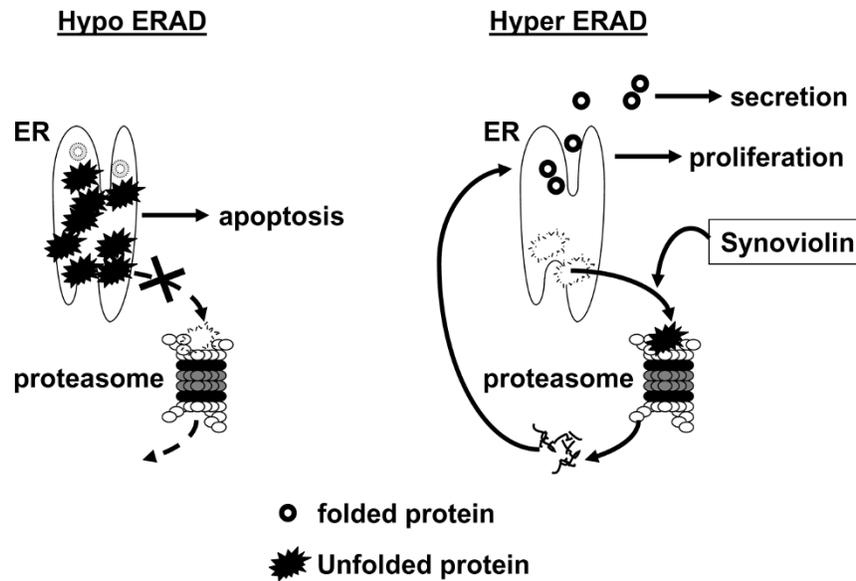
Hyper-ERAD in RA

It is generally accepted that ER-resident E3 ubiquitin ligases including Synoviolin are inherently crucial in the ERAD system, a process indispensable for elimination of unfolded proteins in the ER [11-17]. Furthermore, other studies showed that disruption of the ERAD system (a hypo-ERAD system) causes cell apoptosis and can induce various human diseases such as neurodegenerative diseases [25-27]. What are the consequences of an aberrantly upregulated ERAD (that is, a hyper-ERAD system), induced by Synoviolin overexpression in synovial cells, on the pathological process of RA?

First, a hyper-ERAD state could promote the excess secretion of cytokines and proteases. That is, acceleration of the ERAD system could more efficiently eliminate its client protein in ER (Fig. 3), which could result in the indirect suppression of UPR activation, because unfolded proteins that trigger UPR do not exist any more in such cells, as reported previously in detail [28,29]. The concept of a hyper-ERAD system in RA synovial cells is conceivable because such cells have to keep producing large amounts of proteins for the progression of joint destruction. In other words, RA synovial cells require an extremely efficient ERAD system to maintain ER functions for disease progression. In addition, because UPR includes cell cycle arrest in addition to global attenuation of translation [30], UPR suppression indirectly induced by hyper-ERAD might ultimately promote synovial cell proliferation (Fig. 3). Taking these results together, a hyper-ERAD status could provide favorable cellular conditions for synovial cell overgrowth by escaping negative regulation by UPR.

Second, a hyper-ERAD status could keep synovial cell functioning even in the hostile milieu of inflamed RA synovia [31]. Elevated temperature, starvation, and hypoxia increase the amount of unfolded proteins in organelles [32-35], which has occasionally been observed in the RA joint. In fact, the existence of ER stress in arthritic joints has been demonstrated by the activation of activating transcription factor 6 (ATF6), an ER-resident transcriptional factor, in the nuclei of synoviocytes, because ATF6 is cleaved from ER membrane after the induction of ER stress and is translocated into the nucleus [11,36]. It is therefore possible that hyper-ERAD could keep the ER of synovial cells

Figure 3



The concept of hyper-endoplasmic reticulum (ER)-associated degradation (hyper-ERAD). In cells with functional disturbance of the ERAD system (hypo-ERAD), unfolded proteins accumulate in the ER (left). Conversely, in the hyper-ERAD status, unfolded proteins are promptly eliminated from cells, thus enhancing the ability of the ER to synthesize new proteins (right).

functioning in inflamed joints by overcoming the environmental challenges that cause ER stress.

Third, a hyper-ERAD system could work as an anti-apoptosis system in RA synovial cells. Our previous experimental studies conducted in mice with CIA demonstrated that the downregulation of the *synoviolin* gene promoted the apoptosis of synovial cells in the arthritic joints [11]. Studies by other researchers also confirmed that several E3 ubiquitin ligases (such as Parkin) exhibit a protective function against ER stress-induced apoptosis in neuronal cells [26]. It is possible that Synoviolin also acts as an anti-apoptotic factor, and thus hyper-ERAD could prevent ER stress-induced apoptosis. Support for this conclusion is also provided by Synoviolin knockout; mouse embryonic fibroblasts lacking Synoviolin showed increased susceptibility to ER stress-induced apoptosis as observed in Synoviolin-ablated synovial cells [11,24].

In all, there seems to be sufficient experimental evidence for the following consequences of a hyper-ERAD status: first, enhanced protein production and cell overgrowth; second, maintenance of ER function of synovial cells despite ER stress in the milieu of inflamed joints; and third, prevention of apoptosis induced by ER stress. Consequently, these processes could worsen the pathological process of RA.

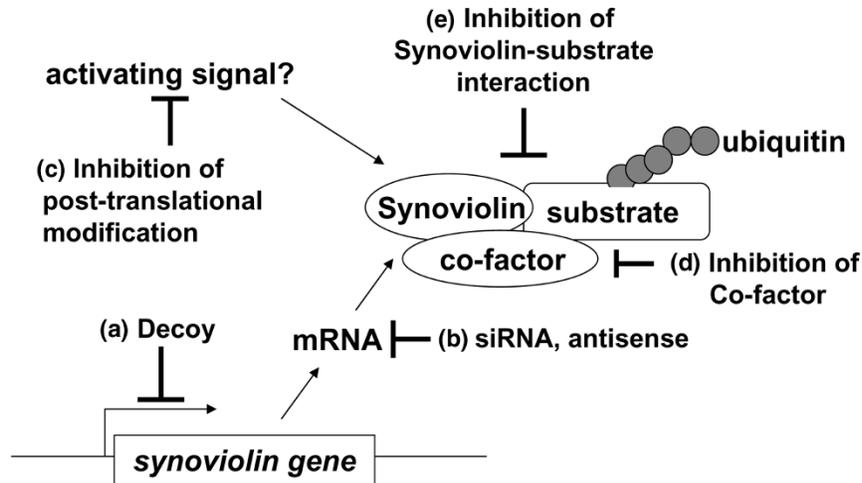
Conclusion

The immunological aspects of RA have been studied extensively over the past several years. However,

understanding these processes and their implementation in the design of new therapies for RA have not been completely successful [37-40]. Here we propose a novel hypothesis for RA pathogenesis: 'hyper-ERAD', which may alter the characteristics of synovial cells in RA. Because Synoviolin knock-down does not affect the immunological pathway [11], this novel concept might explain the underlying pathogenic processes in RA, especially in patients with RA refractory to anti-TNF- α therapies. It is therefore important to investigate the expression of Synoviolin or the status of the ERAD system especially in these patients.

Two questions have to be answered before the design of any new therapies that target Synoviolin and the suppression of the hyper-ERAD. First, what are the mechanism(s) that activate Synoviolin? Second, by what mechanism(s) does Synoviolin regulate cell proliferation and apoptosis? Because the amount of Synoviolin is critical for arthritis [11], a detailed analysis of Synoviolin production and/or activation is also important for its quantity control (Fig. 4). With this in mind, we recently identified the transcriptional regulation of *synoviolin*, which could help in identifying the regulatory pathway that leads to the activation of *synoviolin* in RA synovial cells [41]. Furthermore, these studies could allow the development of decoy nucleic acid-based or siRNA-based therapies (Fig. 4a,b). Thus, in the next step, we need to define the molecular mechanism(s) that activate Synoviolin in RA synoviocytes. The current thinking is that the enzymatic activity of Synoviolin could be regulated by auto-ubiquitination or other forms of post-translational modification, such as

Figure 4



Possible therapeutic approaches against Synoviolin. Five approaches for Synoviolin regulation are described. **(a,b)** Regulation of the amount of Synoviolin by decoy oligonucleotides (a), anti-sense or RNA-mediated interference (b). **(c)** Inhibition of the signal(s) that activate Synoviolin enzyme activity. **(d)** Inhibition of the interaction between Synoviolin and its cofactors. **(e)** Inhibition of the interaction between Synoviolin and its substrates. siRNA, small interfering RNA.

phosphorylation (Fig. 4c). In such processes, cofactors required for Synoviolin activation (Fig. 4d) or interaction with substrates could be crucial for the biological effects of Synoviolin (Fig. 4e). A search for Synoviolin substrates is also indispensable for the discovery of any as yet unknown crosstalk between signaling pathways involved in the regulation of the cell cycle and/or apoptosis and Synoviolin, which could help to uncover the complex pathogenic mechanism of RA.

In this review we have presented a new concept of the hyper-ERAD system in the pathogenic process of RA. Although this concept was formulated through several years of research involving laboratory animals and a limited number of patients with RA, the relevance to human disease remains somewhat speculative at present. More time and efforts are needed to understand the role of the ERAD system in human RA and to define other as yet unknown aspects of RA before the design of any ERAD-based therapy for the disease.

Competing interests

The author(s) declare that they have no competing interests.

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