We recently reported the importance of Synoviolin in quality control of proteins through the endoplasmic reticulum (ER)-associated degradation (ERAD) system and its involvement in the pathogenesis of arthropathy through its anti-apoptotic effect. For further understanding of the role of Synoviolin in vivo, we generated in this study synoviolin-deficient (syno<sup>−/−</sup>) mice by gene-targeted disruption. Strikingly, all fetuses lacking syno died in utero around embryonic day 13.5, although Hrd1p, a yeast orthologue of Synoviolin, is non-essential for survival. Histologically, hypocellularity and aberrant apoptosis were noted in the syno<sup>−/−</sup> fetal liver. Moreover, definitive erythropoiesis was affected in noncell autonomous manner in syno<sup>−/−</sup> embryos, causing death in utero. Cultured embryonic fibroblasts derived from syno<sup>−/−</sup> mice were more susceptible to endoplasmic reticulum stress-induced apoptosis than those from syno<sup>+/+</sup> mice, but the susceptibility was recovered by overexpression of synoviolin. Our findings emphasized the indispensable role of the Synoviolin in embryogenesis.

Quality control of proteins in the endoplasmic reticulum (ER)<sup>1</sup> and transcriptional control of the amount of proteins in the nucleus are important processes that maintain cellular homeostasis (1, 2). In eukaryotic cells, newly synthesized proteins are transported into the ER where they are correctly (4). When the amount of misfolded proteins exceeds the protein folding capacity, despite UPN, misfolded proteins are eliminated by ubiquitin- and proteasome-dependent degradation processes, known as the ER-associated degradation (ERAD) system (1, 5). Misfolded proteins in the ER are translocated into the cytosol, where they are targeted for the 26 S proteasome by the ubiquitin ligase enzymes. Various ubiquitin ligases are reported in the ERAD system of mammalian cells, including C terminus of Hsc70-interacting protein (6–8), PARKIN (9), gp78/AMFR (10, 11), and Fbx2/FBG1/NFB42 (12), and extensive research is currently being conducted to determine the precise mechanisms that regulate the ERAD system in the ER.

It is reported that the ERAD system constitutively functions to eliminate the amount of misfolded proteins produced during cell growth (12). Recent studies have shown that functional disorders of the UPN and/or ERAD system can augment caspase-dependent apoptosis of cells treated with some ER stress-inducible chemical agents (13) that are also known to disturb proper protein folding in the ER (14). These results can explain the molecular pathogenesis of certain human diseases that arise from the ERAD system dysfunction. For instance, production of expanded polyglutamine causes certain inherited neurodegenerative disorders (15–17). Furthermore, mutation of the parkin gene, the product of which is a well known ubiquitin ligase protein in the ERAD system, is thought to result in folded protein response; ERAD, ER-associated degradation; E3, ubiquitin-protein isopeptide ligase; XBP1, X-box-binding protein-1; E, embryonic day; CIA, collagen-induced arthritis; TUNEL, terminal-deoxynucleotidyl transferase-mediated d-UTP nick end labeling; CFU-E, colony-forming unit-erythroid; BFU-E, burst-forming unit-erythroid; MEF, mouse embryonic fibroblast; CFU-M, colony forming unit-macrophage; CFU-GM, colony forming unit-granulocyte/macrophage.
neuronal death of the substantia nigra in patients with autosomal recessive juvenile parkinsonism (18). These findings emphasize the importance of the ERAD system in cell survival in both physiological and pathological conditions and that dysfunction of the ERAD system causes various disorders.

Recently, we used immunoscreening with anti-synovial cell antibodies and cloned Synoviolin, a human homologue of the yeast ubiquitin ligase (E3) Hrd1p/Del3 (19), which is an ER-resident membrane protein with an RING-H2 motif. This molecule is overexpressed in the rheumatoid synovium, and 10 out of 33 littersmates of synoviolin-overexpressing mice developed spontaneous arthropathy. Moreover, in a collagen-induced arthritis (CIA) model, only 7% of synoviolin−/− mice developed arthritis compared with 65% of wild-type littersmates. In addition, the proportion of cells positive for terminal-deoxynucleotidyl transferase-mediated d-UTP nick end labeling (TUNEL) analysis was significantly increased in synovial tissues of synoviolin−/− mice with CIA (20). Indeed, three separate research groups (21–23) reported that this protein has an anti-apoptotic effect against ER stress-induced apoptosis. These findings demonstrated that rheumatoid arthritis is oppositely caused by hyperactivation of the ERAD system through the anti-apoptotic effect of Synoviolin. The present study was designed to determine the function of Synoviolin in vivo. Strikingly, dysfunction of Synoviolin was associated with embryonic lethality, though Hrd1p, a yeast orthologue of Synoviolin, was non-essential for survival (24). Our results clearly indicate that Synoviolin plays an indispensable role in maintenance of life.

**EXPERIMENTAL PROCEDURES**

**Generation of synoviolin-deficient Mice—**Generation of synoviolin-deficient mice was described previously (20). The following primers were used for genotyping of embryos: 5′-ACACAGTCACCCGGTCTTCGTTTCTTCCG-3′ (P1) and 5′-CTCAGTAACAGCGTACCAGGACCGTTCCAG-3′ (P2). PCR analysis using these primers in 1 cycle at 9°C for 1 min followed by 35 cycles at 98°C for 20 s, 68°C for 10 min, with an extension step of 10 min at 72°C at the end of last cycle, produced 6.9- and 2.6-kb fragments from the mutant and wild alleles, respectively.

**Isolation and Histological Analysis of the Embryo—**Mouse embryos were removed from the uterus, and the yolk sac was harvested for genotyping. The embryos were then fixed overnight in 4% paraformaldehyde in phosphate-buffered saline and embedded in paraffin. Nissl sections were cut and stained with hematoxylin and eosin, whereas some sections were used for the TUNEL analysis and immunohistochemistry (wild-type (syno−/−): n = 15, homozygous mutants (syno−/−): n = 10). Staining of peripheral blood and fetal liver cells was performed with the indicated reagents.

**Immunohistochemistry—**Immunohistochemistry was performed as described previously (25). Sections were incubated overnight with anti-glycogen antibody, anti-β-major-glycogen antibody, or anti-GATA-1 antibody (28–29). The specific reaction was visualized using a diamobenidine substrate chromogen system as explained in the manual supplied by the manufacturer (Vector Laboratories, Burlingame, CA). In three different areas, 1000 cells were counted, and the proportion of abnormal erythroblastls was calculated. Furthermore, 100 macrophages were counted, and the rate of erythropagocytosis was calculated.

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**Colon Forming Assays—**Cells were prepared from livers of each genotyoe of E12.5 embryos in Dulbecco's modified Eagle's medium containing 2% fetal calf serum and then counted (syno−/−: n = 5; heterozygous mutants (syno−/−): n = 4; syno−/−: n = 4). Cell suspensions and recombinant cytokines specific for each assay were mixed with MethoCult M3234 (StemCell Technologies, Tokyo) as described previously (29). Cells were plated in 35-mm dishes and cultured at 37°C and 5% CO2. For colony forming unit-erythroid (CFU-E) assay, 2 x 107 cells were cultured in 1 unit/ml recombinant murine erythropoietin (R&D Systems Inc., Minneapolis, MN), and benzidine-positive CFU-E colonies were scored at day 3. For the burst-forming unit-erythroid (BFU-E) assay, 1 x 107 cells were cultured in 2 units/ml erythropoietin and 100 ng/ml stem cell factor (R&D Systems), and benzidine-positive BFU-E colonies were scored at day 7. Colony forming unit-macrophage (CFU-M) and colony forming unit-granulocyte/macrophage (CFU-GM) assay, 5 x 105 cells were cultured in 10 ng/ml macrophage-colony stimulating factor or granulocyte/macrophage-colony stimulating factor (R&D Systems), and CFU-M or CFU-GM colonies were scored at day 7.

**Transplantation of Fetal Liver Cells—**Single cell suspensions prepared from E12.5 fetal liver of the wild-type or syno−/− embryos were pooled (n = 10). 3-month-old female DBA1 mice (Japan SLC, Inc.) were lethally irradiated (900 cGy) and intravenously injected with 1 x 106 fetal liver cells. The mice were maintained on aequous antibiotics (1000 units/ml polymixin B sulfate and 1.1 mg/ml neomycin sulfate). At 1, 2, and 3 weeks after transplantation, 0.4-μl samples of the peripheral blood were lysed and analyzed by electrophoresis on a polyacrylamide gel (30). Proteins were stained with Coomasie Brilliant Blue.

**TUNEL Analysis—**Tissues were prepared as described above. Conditioned culture cells were fixed in 10% formalin for 15 min and attached to aminopropyl triethoxysilane-coated slide glasses. These specimens were subjected to TUNEL analysis. The latter was performed according to the protocol provided by the manufacturer (Clontech Laboratories, Inc.). The viral preparations were titrated with the end-point dilution assay on HEK293 cells. The number of virus particles (measured in plaque-forming units) per cell was expressed in multiplicity of infection values. The infected MEFs were allowed to express targeted genes for 48 h and were then treated with the indicated reagents.

**Western Blotting—**MEFs were collected and lysed in 1% Nonidet P-40, 25 mm Tris-HCl, pH 6.8, 0.25% SDS, 0.05% 2-mercaptoethanol, and 0.1% glycerol. Aliquots of clear cell lysates were separated on SDS-polyacrylamide gels, transferred onto polyvinylidene difluoride or nitrocellulose membrane, and immunoblotted with anti-Synoviolin monoclonal antibody (20), anti-CHOP/ADD153 (Santa Cruz Biotechnol- ogy, Santa Cruz, CA), anti-Big/Ggp7 (Stressgen Biotechnolo- gies, British Columbia, Canada), and anti-β-actin (Sigma). The antibody bands were detected by the peroxidase-conjugated goat anti-mouse immunoglobulins and ECL detection system (Amersham Biosciences).

**Statistical Analysis—**All data are expressed as mean ± S.E. Differences between groups were examined for statistical significance using the Student's t-test. A p value of <0.05 denoted the presence of a statistically significant difference.

**Ethical Considerations—**All experimental protocols described in this study were approved by the Ethics Review Committee of St. Marianna University School of Medicine.

**RESULTS**

**Targeted Disruption of the synoviolin Gene Results in Embryonic Lethality—**To disrupt the mouse synoviolin gene, we constructed the targeting vector (20). Two independently targeted Tg2 embryonic clones were injected into ICR 8-cell embryos and gave germ line transmission (20). F1 mice heterozygous (syno−/+) for the mutation were viable, fertile, and showed no apparent phenotypic abnormalities (data not shown). These heterozygous mice were interbred to generate homozygous mutants (syno−/−), and newborn offspring were genotyped, but no syno−/− mice were identified, indicating that loss of Synoviolin is incompatible with normal embryogenesis (Table I).

**syno−/− Embryos Die in Utero around E13.5—**To identify the stage of embryonic development at which the synoviolin
mutation is lethal, we analyzed E10.5–E18.5 embryos. At E11.5, the majority (88.9%) of synoviolin-deficient embryos were viable; however, at E13.5, only a few (28.6%, Table I) syno−/− embryos were found alive. Morphological analysis showed that E13.5 syno−/− embryos were not different from the wild-type littermates (see Fig. 2A). However, histological examination of syno−/− embryos showed that the cellular density was decreased especially in the liver, although there was no difference with other organs (Fig. 1B–D). Indeed, the livers of syno−/− embryos were smaller in size than those of wild-type embryos (data not shown), and the number of hepatocytes was significantly less than the wild-type littermates (syno−/−: 1.82 ± 0.81 × 10⁵ cells/liver; syno+/+: 10.36 ± 0.72 × 10⁵ cells/liver, p < 0.01).

**Aberrant Hematopoiesis of syno−/− Embryos**—The above results showed that the livers of syno−/− embryos were hypocellular. Next, we examined whether such hypocellularity influenced the hematopoietic status, because the liver becomes the main hematopoietic organ by E12.5 (26–28, 31). First, to investigate the status of hematopoiesis in syno−/− embryos, cytocentrifuge preparations of peripheral blood from E12.5, and the livers of E12.5 embryos were examined. Staining of peripheral blood obtained from E12.5 showed reduced number of erythroblasts in syno−/− embryos (11.58 ± 0.85 × 10⁵ cells/embryo) compared with that of wild-type (25.10 ± 0.66 × 10⁵ cells/embryo). Erythroblasts in syno−/− embryos, the proportion of abnormal erythroblasts showing Howell-Jolly bodies and/or nuclear fragmentation (10.7 ± 0.2%) was markedly higher than that of wild-type littermates (1.8 ± 0.34%, see Fig. 3A, arrowhead). In addition, although there was almost no difference in the number of macrophages, the percentage of macrophages exhibiting erythropagocytosis was significantly higher in the livers of syno−/− embryos (22.0 ± 0.86%) compared with the wild-type (1.7 ± 0.35%, Fig. 2B, arrowhead). At that stage, there was no difference in other lineages (data not shown). These results indicate reduced erythroblast formation and abnormal differentiation in syno−/− embryos.

To confirm the presence of defective hematopoiesis in syno−/− embryos in vivo, the expression levels of globin and GATA-1 were analyzed by immunohistochemistry, because these are marker and master genes in erythropoiesis (26–28, 32). The level of β-globin, which is expressed predominantly during embryonic (primitive) erythropoiesis, was similar in syno−/− and wild-type embryos (syno+/+: 6.25 ± 0.62%; syno−/−: 7.20 ± 0.23%, Fig. 2C). On the other hand, the level of β-major-globin, which first occurs in fetal livers during definitive erythropoiesis, was markedly reduced in syno−/− embryos (syno+/+: 4.60 ± 0.92%; syno−/−: 0.90 ± 0.22%, Fig. 2D, arrowhead). Moreover, in wild-type embryos, the expression of GATA-1, which plays a key role in the gene regulation during erythropoiesis (26–28), was similar in both erythrocytes and megakaryocytes, whereas in syno−/− embryos, GATA-1 was mostly expressed in megakaryocytes (Fig. 2E). These results indicate that the absence of synoviolin results in blockade of definitive erythropoiesis.

Next, we examined erythropoiesis in syno−/− in vitro. Specifically, we examined the ability of cells derived from E12.5 fetal livers to form CFU-M, CFU-GM, CFU-E, and BFU-E. The colony-forming abilities of CFU-M and CFU-GM were not different in wild-type and syno−/− fetal livers (data not shown). Unexpectedly, the proportions of BFU-E and CFU-E were similar in wild-type and syno−/− fetal liver cells (Fig. 2F). These results demonstrate that there was no difference between syno−/− and wild-type with regard to the number of macrophages, granulocytes, and erythroid progenitors. Moreover, erythrocyte progenitors of syno−/− could differentiate in vitro to produce hemoglobin, which was identified by staining for ben-
zytine (data not shown). These findings contradict the results of \textit{in vivo} studies such as reduced number of erythroblasts in peripheral blood of \textit{syno$^{-/-}$}, and the decreased number of $\beta$-globin-positive cells in the fetal liver of \textit{syno$^{-/-}$}. These results suggest abnormality of environment of hematopoiesis in \textit{syno$^{-/-}$}. To determine if the defect in erythropoiesis was cell autonomous, we transplanted fetal liver cells from F8 embryos into wild-type DBA1 mice that had been lethally irradiated (33). The control-irradiated mice did not survive beyond 3 weeks, whereas mice that received the wild-type or \textit{syno$^{-/-}$} fetal liver cells survived for at least 4 weeks. The recipient DBA1 strain is homozygous for the Hbb$^{s}$ haplotype, whereas the liver cells from F8 embryos are homozygous for the Hbb$^{a}$ haplotype (34). As shown Fig. 3G, 30–50% of $\beta$-globin in mice transplanted with either wild-type or \textit{syno$^{-/-}$} fetal liver cells had the Hbb$^{a}$ haplotype at 3 weeks after transplantation. Considered together, these findings indicate that the \textit{syno$^{-/-}$} erythroid cells can develop into mature cells, and that the failure of definitive erythropoiesis in the \textit{syno$^{-/-}$} mice is due to a non-cell-autonomous effect. Therefore, it is expected that abnormal morphology in \textit{syno$^{-/-}$} erythroid is secondary to change in the local environment; i.e. liver.

**Apoptotic Cell Death in \textit{syno$^{-/-}$} Embryos**—The abnormalities of definitive erythropoiesis in \textit{syno$^{-/-}$} were induced by impairment of fetal liver. Moreover, the \textit{syno$^{-/-}$} fetal liver was hypocellular. Thus, we assumed that the low cell density was
due to augmented apoptotic cell death in syno−/− fetal liver since Synoviolin participates in the ERAD system. To determine the extent of apoptotic cell death induced by loss of Synoviolin, TUNEL analysis was performed at each stage of embryogenesis. At E12.5, the number of apoptotic cells in the liver was markedly higher in syno−/− embryos (25.0 ± 0.1%)
than in wild-type counterparts (1.3 ± 0.4%, Fig. 3A), although there was almost no difference in other tissues (e.g. somite, syno−/−: 1.5 ± 0.3%; syno−/−: 1.0 ± 0.2%, data not shown). Moreover, apoptotic cells were hardly detected in E10.5 syno−/− embryos (data not shown). At this stage of embryonic development, Synoviolin was ubiquitously expressed (data not shown). syno−/− MEFs Are Selectively Susceptible to the ER Stress—

The above data indicate enhanced apoptosis in the livers of syno−/− embryos, and such process causes failure of definitive erythropoiesis in non-cell autonomous manner. Apoptosis can be induced through several pathways. To identify the apoptotic pathway that describes Synoviolin involvement, MEFs isolated from syno−/− and wild-type mice were treated in vitro with the following four apoptotic stimuli, monoclonal anti-Fas antibodies, γ-irradiation, tunicamycin (N-glycosylation inhibitor), and thapsigargin (Ca2+-ATPase inhibitor). Under control conditions, the proportion of syno−/− MEF apoptotic cells was higher (16 ± 4%) than the wild-type (6 ± 2%, Fig. 3C). Fas stimulation or exposure to γ-irradiation did not alter the number of apoptotic syno−/− MEFs and wild-type MEFs (Fas: syno−/−, 45 ± 2%; wild-type, 43 ± 4%; γ-irradiation: syno−/−, 34 ± 6%; wild-type, 31 ± 2%; Fig. 3C). In contrast, the ER stress-inducing agents, tunicamycin and thapsigargin, resulted in 1.7- and 2.4-fold increase in the number of TUNEL-positive syno−/− MEFs, respectively, compared with wild-type MEFs (tunicamycin: syno−/−, 56 ± 3%; wild-type, 33 ± 7%; thapsigargin: syno−/−, 90 ± 1%; wild-type, 38 ± 7%; Fig. 3C). Moreover, the sensitivity to the ER stress-induced agents was increased in a dose-dependent manner (Fig. 3D). Furthermore, the ER stress-induced apoptosis of syno−/− MEFs was rescued by infection with synoviolin-adenovirus (Fig. 4E). Finally, we analyzed the expression of the ER stress-inducible proteins, such as Bip/Grp78, CHOP/Gadd153 by Western blotting to rule out the possibility of insufficiency of the UPR in syno−/− MEFs. The results showed that Bip/Grp78 and CHOP/Gadd153 were induced by the ER stress inducers both in syno−/− and syno−/− MEFs (Fig. 3F). These results indicate that breakdown of the ERAD system caused by defect of Synoviolin, but not the UPR dysfunction, contributes to the high sensitivity to the ER stress-induced apoptosis.

**DISCUSSION**

The formation of a proper three-dimensional structure is indispensable for protein function. The “quality control of proteins” by the UPR and ERAD systems plays an important role in maintenance of cellular function. Extensive research in recent years focused on quality control of proteins and its roles in wide variety of cellular functions in vitro (1). However, there is only little information on the quality control system(s) in vivo. With regard to UPR, mice deficient in Pfk2, a eukaryotic initiation factor 2α kinase responsible for UPR-induced repression of protein synthesis, are morphologically normal at birth, but subsequently show progressive degeneration of the ilets of Langerhans, resulting in loss of insulin-secreting β cells and development of diabetes mellitus (35). With regard to the ERAD system in vivo, previous studies reported that breakdown of the ERAD system is associated with the development of various neurodegenerative diseases, such as polyglutamine disease and autosomal recessive juvenile parkinsonism. Thus, there is a need for more in vivo studies to elucidate the physiological function of the ERAD system. To gain insight into the in vivo functions of Synoviolin, a human homologue of the yeast Hrd1p/Del3 (19), which is considered to play a central role among ubiquitin ligases (E3) in the ERAD system, we generated in the present study a synoviolin-deficient mouse using embryonic stem cells.

Our results showed that syno−/− mice died in utero at E12.5–E13.5 (Table 1), and these embryos showed low cellular density in the fetal liver due to extensive apoptosis (Figs. 1 and 3). This phenomenon could explain the defective definitive erythropoiesis in a non-cell autonomous manner (Fig. 2). The results suggested that the impaired differentiation is limited to the erythrocyte lineage, because we observed no abnormality in other lineages from cytocentrifuge preparations (data not shown), and we recognized differentiated macrophages in phagocytosis (Fig. 2B), and multinucleated megakaryocytes that expressed GATA-1 (Fig. 2E) in the syno−/− fetal liver. The impaired hematopoietic microenvironment in syno−/− fetal liver should be examined carefully on each hematopoietic lineage in future investigations. In any case, syno−/− fetal liver cells were found to be selectively impaired, because the total number of fetal liver cells was decreased in syno−/−, although there was no change in the number of α-globin-expressing cells. What is the underlying cause of the limited and selective defect in the fetal liver, despite the ubiquitous expression of Synoviolin in vivo (data not shown)? Two mechanisms may explain these phenomena.

First, because fetal hepatocytes are the major sources of various secretory proteins such as albumin and lipoproteins, homeostasis of the ER may be threatened by increases of these proteins in the ER. In this regard, it was reported that Hrd1p promotes ubiquitination of a misfolded albumin (19). If Synoviolin makes such secretory proteins substrates, the fetal liver might be specifically affected. The ERAD system is important even if it does not only depend on the temporal/spatial expression of Synoviolin but also on the temporal/spatial distribution of its substrate(s). In a related issue, we reported recently that Synoviolin is highly expressed in the rheumatoid synovial tissue, and synoviolin-overexpressing mice induced by using β-ac-
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tin promoter, only develop arthropathy (Fig. 4) (2). In this regard, mutation of Parkin, which is broadly expressed in vivo, causes autosomal recessive juvenile parkinsonism, but such pathology is prescribed by the expression of its substrate, Pael receptor, which is specifically expressed in neuronal cells (9, 36). Thus, the relation of Parkin with its substrate is important to exhibit its unique functions for ubiquitin E3 ligase. The physiological function of Synoviolin in the fetal liver during embryogenesis and its pathological role in arthropathy could be explained by the substrate(s) for Synoviolin. Moreover, Mdm2, a ubiquitin ligase (E3), controls the on/off switch of the apoptosis signal by establishing p53 ubiquitination and this system is generally implicated in the regulation of cell fate (7, 37–39). Therefore, it is conceivable that Synoviolin could also control the ER stress-induced apoptosis by ubiquitinating certain target protein(s). Future studies should identify the mechanisms that control the ERAD system and the effect of the balance of Synoviolin and its substrate(s) on such system. We are currently engaged in several research projects aimed at identifying the substrate(s) of Synoviolin such as using yeast two-hybrid system, proteomics, and DNA microarray.2

Second, various factors are known to cause the ER stress. It is possible that such stress selectively affects the fetal liver and rheumatoid synovial tissues, causing limited abnormalities. Considering the environment prevailing in both physiological and pathological processes, hypoxia could be a common source of stress (40–42). In embryogenesis, the environment that surrounds an embryo is hypoxic, and the type of hemoglobin that delivers oxygen changes from ε-globin to β-globin by E12.5. Therefore, the oxygen partial pressure changes markedly at this stage, and the syno−/− fetal liver would be selectively affected. Interestingly, the joint space is well known for being an exceptionally hypoxic environment (43). Taken together, we speculate that the selective impairment of the fetal liver could be caused by an intrinsic factor; enhancement of substrate production in the fetal liver, and an environmental factor; systemic hypoxia particularly at E12.5.

When the relationship between “quality control of proteins” and “maintenance of life” is discussed, deficiency of synoviolin in mice embryos was lethal. In addition, MEFs derived from the syno−/− embryos exhibited high and selective susceptibility to the ER stress in vitro (Fig. 3, C and D), and expressions of the ER stress-inducible proteins, including Bip/Grp78 and Hsc70-interacting protein, gp78/AMFR, Parkin, development. Several ubiquitin ligases (E3), such as the C terminus of Hsc70-interacting protein, gp78/AMFR, Parkin, and Fbx2/FBG1/NFB42, have been reported to be involved in the ERAD system. However, the loss-of-function of Synoviolin causes autosomal lethality during embryonic development without any redundancy. Our study indicates that Synoviolin could play an important role in embryogenesis. In this regard, the X-box-binding protein-1 (XBP1) is a key regulator of the response to accumulation of unfolded protein in the ER (44). XBP1-deficient mice (XBP1−/−) are embryonically lethal at day E12.5 (45). The XBP1−/− fetuses have severe liver hypoplasia and contain large numbers of apoptotic cells in the liver. This phenotype overlaps with the syno−/− embryos. That loss of only Synoviolin reproduces the phenotypes of XBP1−/− was a surprising finding, even in several downstream factors of XBP1. These results indicate that Synoviolin seems to be the target of XBP1 and that the phenotypes of XBP1−/− would appear by inhibition of Synoviolin function. This speculation was fully confirmed by the study of cultured cell system (21). This is the first report that shows the significance of Synoviolin in embryogenesis in vivo.

In conclusion, we have demonstrated in the present study that the syno−/− embryo exhibits abnormalities of hematopoiesis due to extensive apoptosis in the liver, which ultimately causes death in utero. Our results also demonstrated that the fetal liver is susceptible to the ER stress. In other words, the results suggested a close link between embryogenesis and the quality control of proteins. Further studies should be performed to examine whether the offspring of crosses between liver-specific synoviolin-overexpressing mice derived from albumin promoter and syno−/− mice can survive. Our findings clearly indicate that Synoviolin is essential for the maintenance of life.

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