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Role of the E3 ubiquitin ligase HRD1 in the regulation of serotonin transporter function

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ABSTRACT

To elucidate the regulation of serotonin transporter (SERT) function via its membrane trafficking, we investigated the involvement of the ubiquitin E3 ligase HRD1 (HMG-CoA reductase degradation protein), which participates in endoplasmic reticulum (ER)-associated degradation (ERAD), in the functional regulation of SERT. Cells transiently expressing wild-type SERT or a SERT C-terminal deletion mutant (SERTΔCT), a SERT protein predicted to be misfolded, were used for experiments. Studies using HRD1-overexpressing or HRD1-knockdown cells demonstrated that HRD1 is involved in SERT proteolysis. Overexpression of HRD1 promoted SERT ubiquitination, the effect of which was augmented by treatment with the proteasome inhibitor MG132. Immunoprecipitation studies revealed that HRD1 interacts with SERT in the presence of MG132. In addition, HRD1 was intracellularly colocalized with SERT, especially with aggregates of SERTΔCT in the ER. HRD1 also affected SERT uptake activity in accordance with the expression levels of the SERT protein. These results suggest that HRD1 contributes to the membrane trafficking and functional regulation of SERT through its involvement in ERAD-mediated SERT degradation.

1. Introduction

The serotonin transporter (SERT) is responsible for the termination of serotonergic neural transmission via reuptake of serotonin (5-HT) at nerve terminals [1,2]. SERT is implicated in mood disorders, anxiety disorders, and autism [3–5]. SSRI antidepressants are thought to exert an antidepressive effect by inhibiting SERT function and increasing synaptic 5-HT levels [3].

Membrane proteins, including SERT, are first translated from mRNAs in the endoplasmic reticulum (ER). They are then trafficked to the plasma membrane via the Golgi apparatus. SERT first undergoes incomplete and high-mannose-type glycosylation and is folded into the membrane of the ER. SERT is then transported to the Golgi apparatus, where it is fully and maturely glycosylated before being expressed and becoming functional in the plasma membrane [6,7].

The ER is an important organelle for the membrane trafficking of SERT. Various pathologies, such as hypoxia, impair ER functions, resulting in the accumulation of misfolded proteins in the ER. This condition is defined as ER stress [8]. When ER stress is sustained, apoptosis signals are transmitted from the ER, leading to cell death. The cell activates a defense mechanism called the unfolded protein response (UPR) to prevent ER stress [9,10]. The UPR involves a process by which misfolded proteins are secreted from the ER and degraded. This process is called ER-associated degradation (ERAD) [11,12]. Misfolded membrane proteins that accumulate in the ER undergo retrograde transport into the cytoplasm by ERAD, are ubiquitinated by ubiquitin ligases, and are degraded by the proteasome [8,11,12].

SERT is predominantly localized in the plasma membrane and ER when expressed in cultured cells [13]. Inhibition of ERAD with proteasome inhibitors increases the accumulation of SERT in the ER [13], suggesting that SERT expressed in the ER is misfolded to some extent. Our previous report demonstrated that a SERT C-terminal

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deletion mutant (SERTΔCT) was poorly expressed in the plasma membrane and was retained in the ER as aggregates, inducing ER stress [13–15]. Therefore, SERTΔCT was thought to be a misfolded protein.

HRD1 (HMG-CoA reductase degradation protein) is an E3 ubiquitin ligase that plays a central role in ERAD [16,17]. HRD1 is ubiquitously expressed in many organs [16], including neurons [18]. It has been reported that neurodegenerative disease-related proteins, including amyloid precursor protein and huntingtin, are substrates for HRD1 [19,20], suggesting that HRD1 may be closely implicated in neurodegenerative diseases.

It is important to understand the mechanisms of SERT membrane trafficking in view of SERT degradation. Most misfolded SERT proteins are expected to be degraded by ERAD. However, the detailed mechanism of SERT degradation remains unclear. In this study, we focused on the ubiquitin ligase HRD1 to clarify whether HRD1 influences the regulation of SERT function.

2. Materials and methods

2.1. Materials

An anti-DYKDDDDK tag (anti-FLAG) mouse monoclonal antibody was purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). An anti-Ubiquitin rabbit polyclonal antibody was purchased from Proteintech (Tokyo, Japan). Alexa Fluor 488- or Alexa Fluor 568-conjugated anti-mouse or anti-rabbit IgG antibodies were purchased from Molecular Probes (Eugene, OR, USA). An anti-SYVN1 (HRD1) rabbit monoclonal antibody was purchased from Cell Signaling Technology (Danvers, MA, USA). MG132 was purchased from Sigma-Aldrich. (St. Louis, MO, USA). All other chemicals were of analytical grade.

2.2. Cell culture and transfection

COS-7 cells were cultured as previously described [13,14,21]. HEK293 cells stably expressing WT-HRD1 (designated HEK293 HRD1) and control HEK293 cells transfected with mock vector (designated HEK293 Mock) were generated and cultured as previously reported [22]. Expression plasmids for FLAG-tagged rat or human SERT were constructed as previously described [23]. These plasmids are designated herein as pFLAG-rSERT and pFLAG-hSERT, respectively. An expression plasmid for FLAG-SERTΔCT was constructed as described previously [13] and is designated pFLAG-SERTΔCT. To produce cells transiently expressing FLAG-SERT or FLAG-SERTΔCT, plasmids were transfected into the desired cells by electroporation using an NEPA21 electroporator (NEPA GENE, Chiba, Japan).

2.3. Cycloheximide (CHX) chase assay

Forty-eight hours after transfection, cells were treated with 50 μg/ml cycloheximide (CHX). After starting CHX treatment, cells were harvested at the time points indicated in the figures, and prepared samples were subjected to Western blot analysis.

2.4. Generation of HRD1 knockdown cells

To generate HRD1 knockdown cells, double-stranded siRNAs corresponding to the human HRD1 cDNA sequence were purchased from Dharmacon (Lafayette, CO, USA). The target siRNA sequence was 5′-UCAUCAAGGUUCUGCUGUA-3'. HRD1-siRNA or control siRNA (100 pmol for both) was simultaneously transfected into COS-7 cells with pFLAG-rSERT or pFLAG-SERTΔCT by electroporation.

2.5. Measurement of SERT uptake activity

A Neurotransmitter Transporter Uptake Assay Kit (Molecular Devices, San Jose, CA) was used as previously described [15]. In brief, the fluorescence of the substrate incorporated into cells was measured using an Opera Phenix system (PerkinElmer, Waltham, MA, USA). The fluorescence intensity per cell 45 min after the application of substrates was determined to be the SERT uptake activity.

2.6. Western blot analysis

Western blot was carried out as previously described [13,14,21]. In brief, cell lysate samples were separated on 9% SDS-PAGE gels and transferred onto polyvinylidene difluoride (PVDF) membranes. Membranes were incubated first with the anti-DYKDDDDK-tag antibody (diluted 1:1000), anti-SYVN1 antibody (diluted 1:2000) or anti-ubiquitin antibody (diluted 1:600) for 16 h at 4 °C, and then with a horseradish peroxidase-conjugated anti-mouse or rabbit IgG antibody (diluted 1:10,000) for >1 h at room temperature. Immunoreactive bands were visualized with a chemiluminescence detection kit (Chemi-Lumi One, Nacalai Tesque). The band densities were measured with a chemiluminescence image analyzer (EZ-Capture MG, ATTO, Tokyo, Japan).

2.7. Immunocytochemistry

Immunocytochemistry was carried out as previously described [13,14,21]. The primary antibodies used were the anti-DYKDDDDK-tag antibody (diluted 1:200) and anti-SYVN1 antibody (diluted 1:200). The secondary antibodies used were Alexa Fluor 488- or Alexa Fluor 568-conjugated anti-mouse or rabbit IgG (diluted 1:500). Fluorescence signals were observed by confocal laser scanning fluorescence microscopy (LSM 780, Carl Zeiss, Jena, Germany).

2.8. Immunoprecipitation and detection of SERT ubiquitination

To assess the ubiquitination of SERT, pFLAG-hSERT and pHA-ubiquitin were transfected into HEK293 Mock or HEK293 HRD1 cells. MG132 (20 μg/ml), a proteasome inhibitor, was added to the culture medium 24 h after transfection. Forty-eight hours after transfection, an immunoprecipitation assay was performed as previously described [23]. The supernatants of cell lysates were incubated with anti-DDDDK-tag mAb-conjugated magnetic agarose beads (MBL, Nagoya, Japan) with rotation at 4 °C overnight. Cells transfected with pHA-ubiquitin alone were used as negative controls. To examine the interaction between HRD1 and SERT, pFLAG-hSERT alone was transfected into HEK293 HRD1 cells. Non-FLAG-SERT-expressing cells were used as negative controls.

2.9. Statistical analysis

Statistical analysis was performed using Prism 4 software (GraphPad Software, San Diego, CA). Statistical significance was determined by Student's t-test. If the p value was less than 0.05 ($P < 0.05$), the difference was considered significant.

3. Results

3.1. Effects of HRD1 overexpression on degradation of the SERT protein

The expressed FLAG-SERT and FLAG SERTΔCT were visualized as multiple bands in COS-7 cells, as previously reported [13] (Fig. 1A).

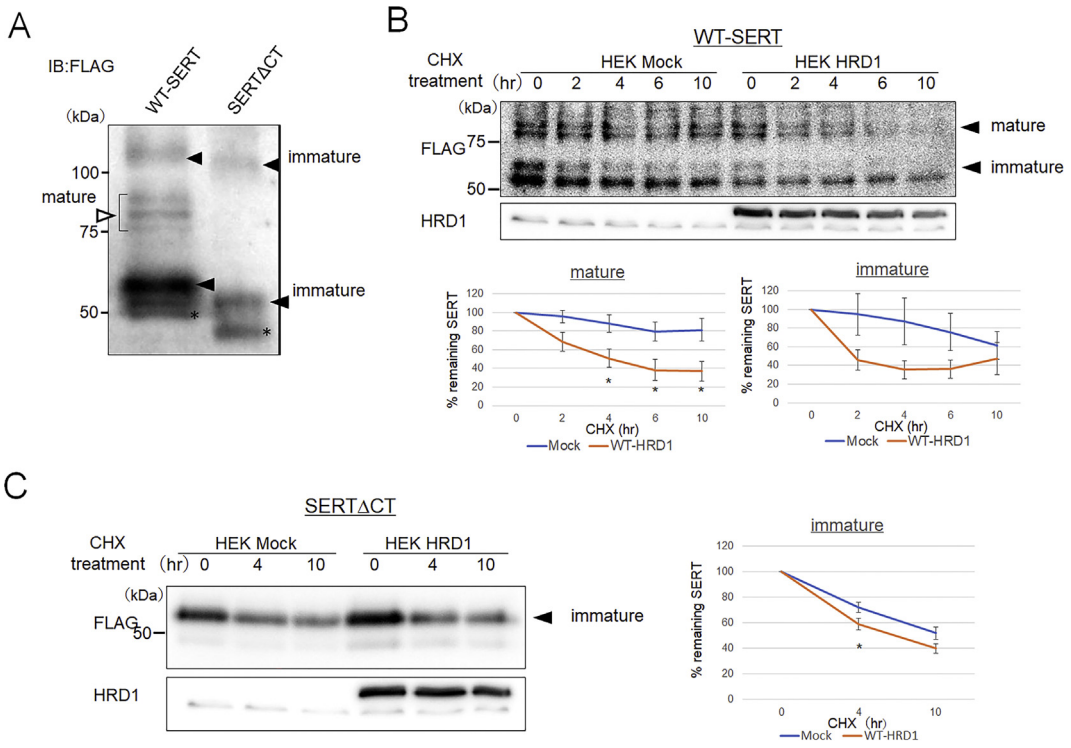

Fig. 1. Effects of HRD1 overexpression on the degradation of WT-SERT and SERTΔCT. A: Immunoblotting of WT-SERT and SERTΔCT in COS-7 cells expressing FLAG-WT-SERT or FLAG-SERTΔCT. The hollow arrowhead, solid arrowheads and asterisks indicate the maturely glycosylated, immaturely glycosylated and unglycosylated forms of SERT, respectively. B: Effects of HRD1 overexpression on the degradation of WT-SERT. HEK 293 HRD1 and Mock cells were transfected with pFLAG-hSERT. Overexpression of HRD1 significantly enhanced the degradation of maturely glycosylated SERT at 4, 6, and 10 hours after CHX treatment (*$p < 0.05$, Student's t-test, n=5). For mature SERT, the p value was 0.053 at 2 hours after CHX treatment. For immature SERT, the p values were 0.083, 0.087, 0.116, and 0.546 at 2, 4, 6, and 10 hours after CHX treatment, respectively. The data are presented as the mean ± SEM.C: Effects of HRD1 overexpression on the degradation of SERTΔCT. HEK 293 HRD1 and Mock cells were transfected with pFLAG-SERTΔCT. Overexpression of HRD1 significantly enhanced the degradation of SERTΔCT 4 hours after CHX treatment (*$p < 0.05$, Student's t-test, n=11). The p value was 0.053 at 10 hours after CHX treatment. The data are presented as the mean ± SEM.

The bands at approximately 80 kDa were considered to be maturely glycosylated SERT (mature), and the bands at approximately 63 kDa were considered to be immaturely glycosylated SERT (immature). Bands larger than 100 kDa were considered to be dimers of immaturely glycosylated SERT. A prominent decrease in maturely glycosylated SERT was observed in SERTΔCT-transfected cells, indicating that SERTΔCT is trafficked at very low levels to the Golgi apparatus and plasma membrane.

Initially, we examined the effect of HRD1 overexpression on degradation of the SERT proteins using a cycloheximide (CHX) chase assay. HEK293 HRD1 or HEK293 Mock cells expressing FLAG-WT-SERT were treated with CHX and harvested at the indicated times after the start of CHX treatment (Fig. 1B). Overexpression of HRD1 significantly enhanced the degradation of maturely glycosylated WT-SERT (Fig. 1B). In addition, there was a trend toward enhanced degradation of immaturely glycosylated WT-SERT (Fig. 1B). In addition, overexpression of HRD1 significantly enhanced the degradation of immaturely glycosylated SERTΔCT (Fig. 1C).

3.2. Effects of HRD1 knockdown on degradation of the SERT protein

COS7 cells were transfected with pFLAG-WT-rSERT and siRNAs and were treated with CHX 48 h after transfection. Cells were harvested at the indicated times after CHX treatment (Fig. 2A). Transfection of HRD1 siRNA sufficiently suppressed the expression of HRD1 (Fig. 2A, 2B and 4A). HRD1 knockdown significantly inhibited the degradation of the immaturely glycosylated WT-SERT (Fig. 2A) 2 h after CHX treatment. In addition, HRD1 siRNA-treated cells showed a trend toward reduced degradation of maturely glycosylated WT-SERT (Fig. 2A). HRD1 knockdown significantly suppressed the degradation of SERTΔCT 2 h after CHX treatment. (Fig. 2B).

3.3. Effects of HRD1 overexpression and treatment with the proteasome inhibitor MG132 on the ubiquitination of SERT

The effects of HRD1 overexpression and treatment with the proteasome inhibitor MG132 on the ubiquitination of SERT were examined. HEK293 HRD1 cells expressing FLAG-WT-SERT and HA-ubiquitin were treated with the proteasome inhibitor MG 132 (20 μg/ml) for 24 h.

In FLAG-SERT-expressing cells, immunoblotting with the anti-FLAG antibody showed that SERT was sufficiently recovered in immunoprecipitates with anti-FLAG antibodies. (Fig. 3A, upper, right). In contrast, no specific SERT band was identified in non-FLAG-SERT-expressing control cells (Fig. 3A, upper, left). When immunoprecipitates were immunoblotted with anti-ubiquitin antibodies, possibly ubiquitinated SERT was observed in the region corresponding to a molecular mass of more than 95 kDa, and this band appeared to be more prominent in HRD1-overexpressing cells (Fig. 3A, lower, right). SERT ubiquitination was further enhanced by MG132 treatment (Fig. 3A, lower, right). These results suggest that HRD1 promotes the ubiquitination of SERT, which was more prominent in MG132-treated cells.

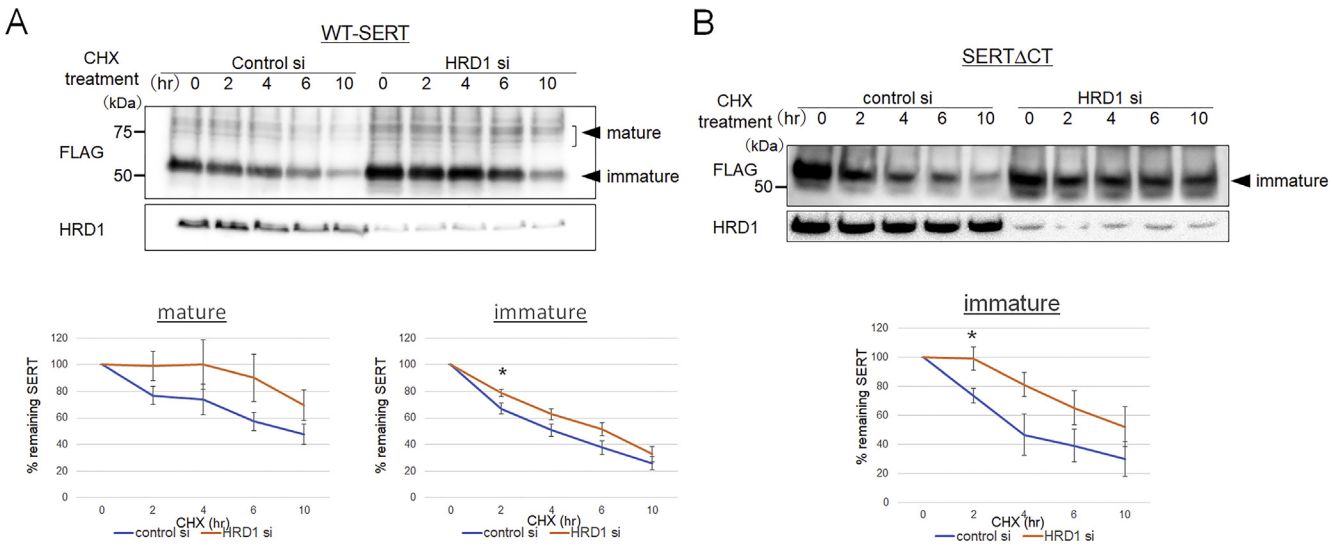

Fig. 2. Effects of HRD1 knockdown on the degradation of WT-SERT and SERTΔCT proteins. A: Effects of HRD1 knockdown on the degradation of WT-SERT. COS-7 cells were transfected with pFLAG-rSERT and siRNAs. HRD1 knockdown significantly reduced the degradation of the immaturely glycosylated WT-SERT 2 h after CHX treatment ($p < 0.05$, Student's t-test, $n = 13$). For mature SERT, the p values were 0.096, 0.234, 0.099 and 0.124 at 2, 4, 6 and 10 h after CHX treatment, respectively. For immature SERT, the p values were 0.064, 0.067 and 0.373 at 4, 6 and 10 h after CHX treatment, respectively. The data are presented as the mean ± SEM. B: Effects of HRD1 knockdown on the degradation of SERTΔCT.Knockdown of HRD1 significantly suppressed the degradation of SERTΔCT at 2 hours after CHX treatment (*$p < 0.05$, Student's t-test, $n=5$). The p values were 0.068, 0.148 and 0.261 at 4, 6 and 10 hours after CHX treatment, respectively. The data are presented as the mean ± SEM.

3.4. Interaction and localization of HRD1 and SERT

The interaction and localization of HRD1 and SERT were examined. HEK293 HRD1 cells expressing WT-FLAG-SERT were treated with the proteasome inhibitor MG132 for 24 h. Cell lysates were immunoprecipitated with the anti-FLAG antibody.

In FLAG-SERT-expressing cells, immunoblotting with the anti-FLAG antibody demonstrated that SERT was recovered in immunoprecipitates with the anti-FLAG antibody (Fig. 3B, lower, right). In contrast, SERT bands were not identified in non-FLAG-SERT-expressing control cells (Fig. 3B, upper, right). When cells were treated with the proteasome inhibitor MG132, coimmunoprecipitation of FLAG-SERT and HRD1 was confirmed in HRD1-overexpressing cells (Fig. 3B, lower, right, asterisk). Prolonged exposure of this immunoblot revealed that FLAG-SERT and HRD1 were coimmunoprecipitated also in non-MG132-treated cells (data not shown). Thus, these results suggest that HRD1 interacts with SERT and that the interaction is more pronounced with MG132 treatment.

Next, the localization of HRD1 and SERT was examined by immunostaining. HEK293 HRD1 cells expressing FLAG-SERT or FLAG-SERTΔCT were immunostained with anti-FLAG and anti-HRD1 antibodies. WT-SERT was predominantly expressed in the plasma membrane (Fig. 3C, upper). Interestingly, cells with relatively high HRD1 expression tended to express relatively low levels of SERT (Fig. 3C, upper, triangles). Conversely, cells with higher SERT expression tended to express lower levels of HRD1 (Fig. 3C, upper, asterisks). The localization of SERT and HRD1 was further investigated in magnified images. HRD1 was partially colocalized with WT-SERT, which was expressed intracellularly (Fig. 3C, lower, arrowheads). HRD1 was colocalized with SERTΔCT intracellularly and accumulated in the aggregates of SERTΔCT (Fig. 3D, arrowheads). These results suggest that HRD1 colocalizes and interacts with SERT at intracellular sites, probably in the ER.

3.5. Effect of HRD1 on the protein expression and function of SERT

Finally, the effect of HRD1 on the protein expression level and uptake activity of SERT was examined using HRD1-knockdown COS-7 cells. One and two days after transfection, the protein level and uptake activity of SERT were analyzed.

It was confirmed that HRD1 siRNA treatment significantly decreased the expression of HRD1 both 1 and 2 days after transfection (Fig. 4A, Day 1 and Day 2, respectively). HRD1 knockdown significantly increased the protein levels of both maturely and immaturely glycosylated SERT on Day 1 and Day 2 (Fig. 4B and C). In addition, HRD1 knockdown significantly increased SERT uptake activity on Day 1, in accordance with the increased SERT protein levels, but not on Day 2. These results suggest that HRD1 regulates SERT functions by altering the protein level of SERT.

4. Discussion

The aim of this study was to clarify a regulatory mechanism of SERT function via the ERAD-related E3 ubiquitin ligase HRD1. In this study, we used two types of SERT, WT-SERT and SERTΔCT, a C-terminal deletion mutant of SERT. Previous studies have shown that both maturely glycosylated and immaturely glycosylated SERT are recognized by immunoblotting of WT-SERT. On the other hand, SERTΔCT was almost recognized as immaturely glycosylated SERT [13,14], as reconfirmed in this study (Fig. 1A). In addition, since SERTΔCT forms aggregates in the ER and induces ER stress [14], SERTΔCT is believed to be a misfolded protein.

First, we investigated the effect of HRD1 overexpression on SERT degradation by a CHX chase assay. Overexpression of HRD1 significantly promoted the degradation of maturely glycosylated WT-SERT (Fig. 1B). A tendency to accelerate the degradation of immaturely glycosylated WT-SERT was also shown (Fig. 1B). In addition, overexpression of HRD1 significantly accelerated the degradation of SERTΔCT (Fig. 1C).

Next, the effect of a decrease in endogenous HRD1 on SERT degradation was examined using siRNA-treated COS-7 cells, which were most suitable for transient transfection in our experiments. In HRD1 knockdown cells, the degradation of immaturely glycosylated WT-SERT and SERTΔCT was significantly suppressed. These results of experiments using HRD1-overexpressing and HRD1-

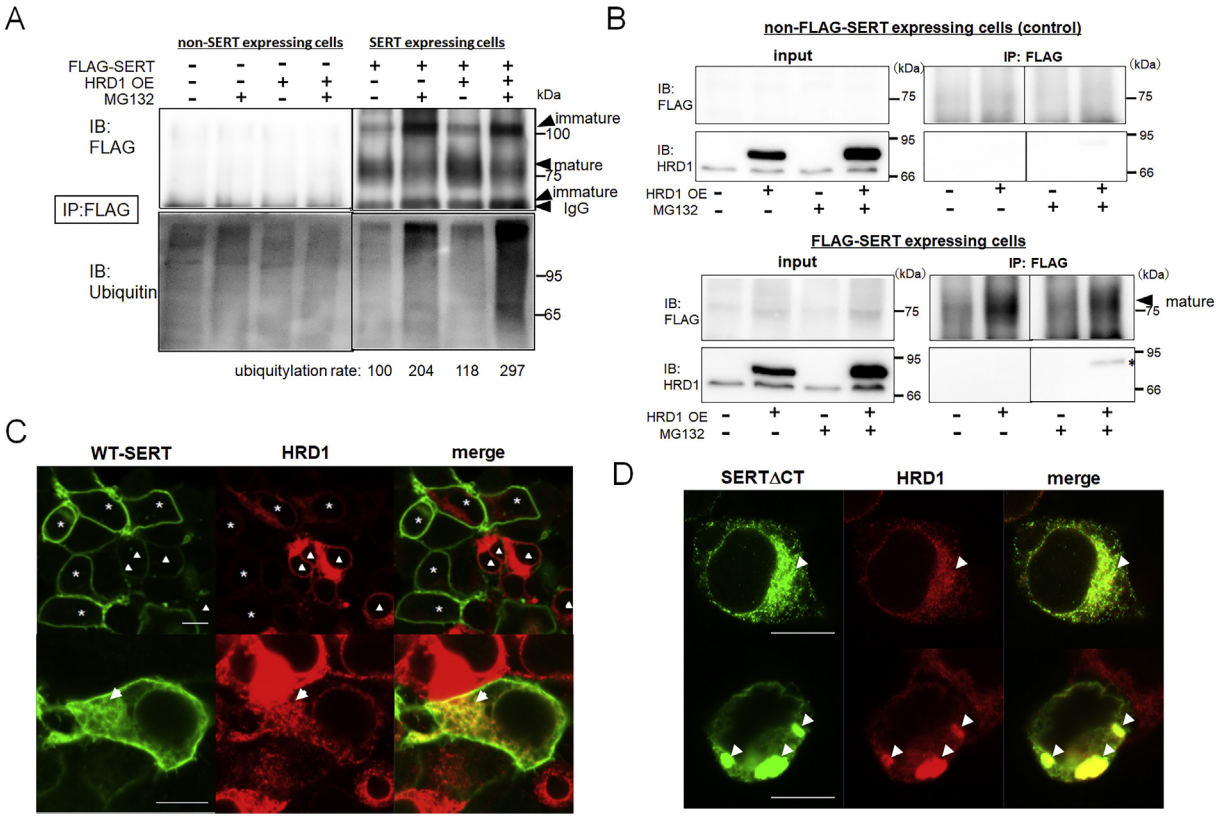

Fig. 3. A: Effects of HRD1 overexpression and treatment with the proteasome inhibitor MG 132 on the ubiquitination of SERT. HEK293 HRD1 and HEK293 Mock cells were transfected with pFLAG-hSERT and pHA-ubiquitin. Twenty-four hours after transfection, cells were treated with 20 mg/ml MG132 for 24 hours. Cell lysates were subjected to immunoprecipitation (IP) with an anti-FLAG antibody and immunoblotted (IB) with an anti-FLAG or anti-ubiquitin antibody. For the negative control study, non-FLAG-SERT-expressing cells were used. Representative data from 3 independent experiments are shown. For ubiquitin immunoblotting, the densities of bands in regions corresponding to greater than 95 kDa were measured to calculate the ubiquitination rate of each sample. The band densities of samples prepared from non-HRD1-overexpressing, non-MG132-treated and FLAG-SERT-expressing cells were set as 100%, and each experimental band density was normalized to these band densities. B: Interaction and localization of HRD1 and SERT Immunoprecipitation assays were performed using HEK293 HRD1 and HEK293 Mock cells overexpressing FLAG-SERT. Twenty-four hours after transfection, cells were treated with MG132 for 24 hours. Cell lysates were subjected to IP with anti-FLAG antibody and analyzed by IB with an anti-FLAG antibody or anti-HRD1 antibody. For the negative control study, a similar experiment was carried out using non-FLAG-SERT-expressing cells. Representative data from 3 independent experiments are shown. C: Immunocytochemical analysis of HEK293 HRD1 cells expressing WT-SERT. Forty-eight hours after transfection, immunocytochemical assays using anti-HRD1 and anti-FLAG antibodies were performed. The triangles and asterisks indicate cells with high and low HRD1 expression levels, respectively (lower panel). WT-SERT was partially colocalized with HRD1 in the intracellular region (arrowheads, lower panel). Bar: 10 μm. D: Immunocytochemical analysis of HEK293 HRD1 cells expressing SERTDCT. HRD1 was colocalized with SERTDCT and accumulated in the aggregation-like form of SERTDCT (Fig. 3D, arrowheads). Bar: 10 μm

knockdown cells suggest that HRD1 is involved in SERT degradation.

ERAD appears to be primarily involved in the degradation of the misfolded immaturely glycosylated SERT protein. However, this study showed that the degradation of maturely glycosylated SERT was also affected by HRD1. Mechanistically, normally folded SERT may also be incorrectly degraded by ERAD. Alternatively, there may be a defense mechanism against ER stress that converts misfolded immaturely glycosylated SERT into normally folded SERT through the action of a molecular chaperone. Since HRD1 promotes the degradation of misfolded SERT, its conversion to normally folded SERT may be reduced, resulting in a decrease in maturely glycosylated SERT.

We next examined the role of HRD1 in the ubiquitination of SERT. The level of ubiquitinated SERT was higher in cells overexpressing HRD1, and SERT ubiquitination was further enhanced by treatment with the proteasome inhibitor MG132. These results indicate that HRD1 is involved in the ubiquitination of SERT (Fig. 3A).

In addition, the interaction and localization of HRD1 and SERT were investigated. MG132 treatment increased HRD1 expression, suggesting that HRD1 itself is also regulated by the ubiquitin-proteasome system (Fig. 3B). In HRD1-overexpressing cells treated with MG132, FLAG-SERT and HRD1 were coimmunoprecipitated. This finding indicates that SERT and HRD1 interact at the protein level and that this interaction is enhanced under MG132 treatment.

The expression levels of HRD1 were not uniform in HRD1-overexpressing cells (Fig. 3C, upper). Interestingly, SERT expression tended to be lower in cells with higher HRD1 expression (Fig. 3C, upper), suggesting that HRD1 is involved in the regulation of SERT protein levels. The magnified image shows that intracellularly-expressed WT-SERT, and SERTΔCT were consistent with its localization with HRD1 (Fig. 3C, lower, 3D). In addition, HRD1 apparently colocalized with aggregates of SERTΔCT (Fig. 3D, lower). Collectively, these results suggest that SERT likely colocalizes and interacts with HRD1 in the ER.

Finally, HRD1-mediated regulation of the SERT protein level and uptake activity was investigated in HRD1 knockdown cells. HRD1 knockdown increased the expression level of both immaturely glycosylated and maturely glycosylated SERT on both Day 1 and Day 2. (Fig. 4B and C). Consistent with the increase in the protein level, the uptake ability of SERT was also increased on Day 1, suggesting that HRD1 can regulate the function of SERT by affecting its

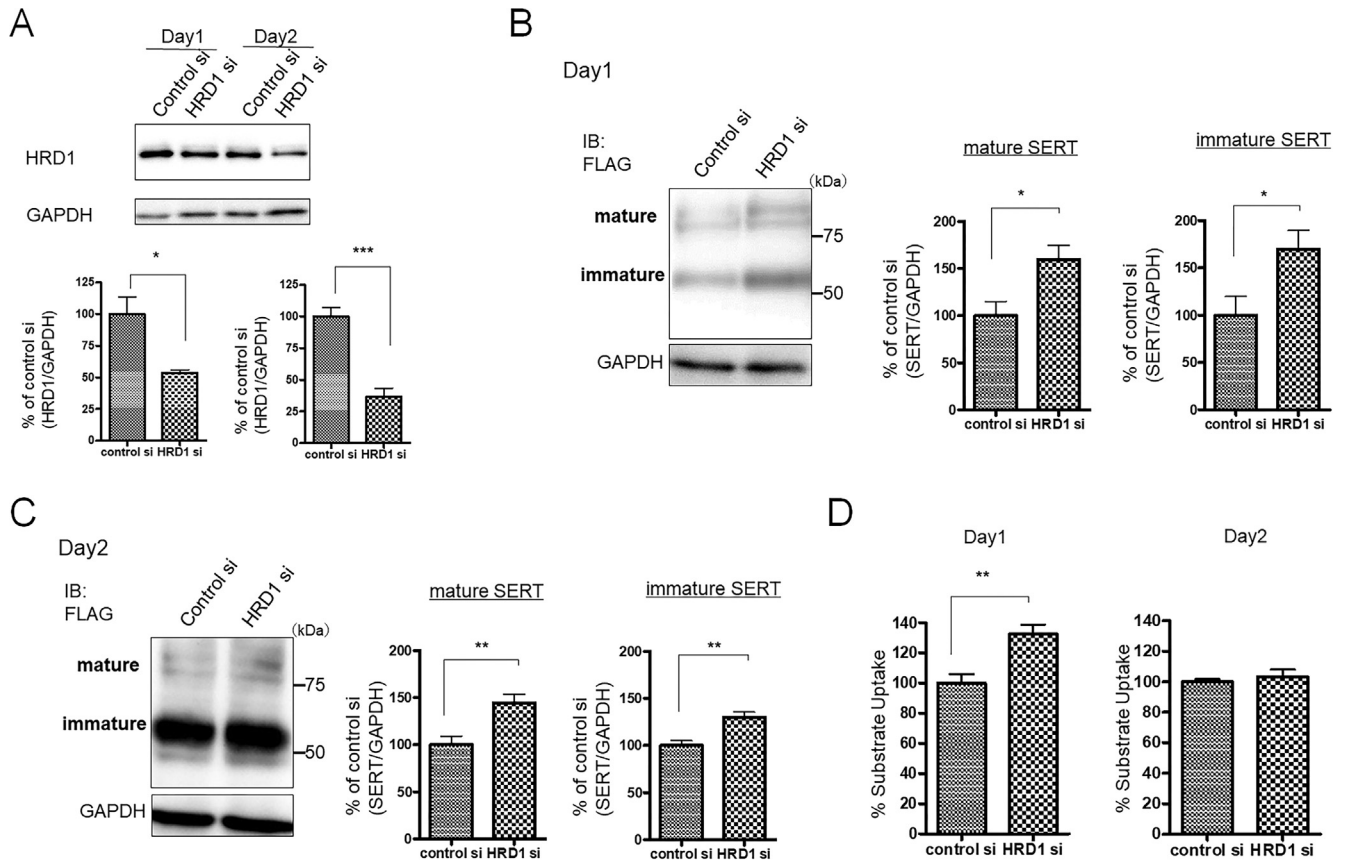

Fig. 4. Effect of HRD1 knockdown on SERT protein expression and function. HRD1 siRNA or control siRNA was cotransfected into COS-7 cells with pFLAG-rSERT. A: Confirmation of HRD1 knockdown. HRD1 levels were significantly reduced by HRD1 siRNA on both Day 1 and Day 2 (*p<0.05, ***p<0.001 Student's t-test, n=4 on Day 1, n=6 on Day 2). The data are presented as the mean ± SEM. B: Effect of HRD1 knockdown on the protein level of FLAG-SERT on Day 1. HRD1 knockdown significantly increased the protein levels of both maturely and immaturely glycosylated SERT (*p<0.05, Student's t-test, n=6). The data are presented as the mean ± SEM. C: Effect of HRD1 knockdown on the protein level of SERT on Day 2. HRD1 knockdown significantly increased the protein levels of both maturely and immaturely glycosylated SERT (**p<0.01, Student's t-test, n=6). The data are presented as the mean ± SEM. D: Effect of HRD1 knockdown on SERT uptake activity. The uptake of fluorescent SERT substrates per cell was measured and considered the 5-HT uptake activity. HRD1 knockdown significantly increased SERT uptake activity on day 1 (**p<0.01, Student's t-test, n=9, 3 independent experiments). HRD1 knockdown had no significant effect on SERT uptake activity on Day 2 (ns, Student's t-test, n=21, 7 independent experiments). The data are presented as the mean ± SEM.

degradation. However, no increase in uptake consistent with an increase in the protein level was observed on Day 2. SERT uptake activity is determined by the amount of SERT expressed in the plasma membrane, whereas the density of mature SERT is determined by the total amount of SERT expressed in the plasma membrane and Golgi apparatus. The plasma membrane became saturated with SERT on day 2, which may have caused the discrepancy between the uptake activity and the level of mature SERT.

In conclusion, our results suggest that HRD1 contributes to the membrane trafficking and functional regulation of SERT by participating in ERAD-mediated SERT degradation. HRD1 may be involved in the pathogenesis of a variety of SERT-related diseases.

Declaration of competing interest

The authors have no conflicts of interest regarding this study.

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