Supplementary material

Method

Western Blot Analysis

The ARPE-19 cells were seeded at $7.5 \times 10^4$ cells per well in 24-well plates and incubated for 4 days and the medium was replaced with fresh medium. TUDCA was added, followed by an endoplasmic reticulum stress inducer, tunicamycin (Wako), treatment 1 h later, at a final concentration of 2 μg/mL. The ARPE-19 cells were supplemented with a 1% protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO, USA), 1% phosphatase inhibitor cocktails 2 and 3 (Sigma), and sample buffer (Wako). The cells were washed with PBS, harvested, and lysed in RIPA buffer (Sigma) supplemented with a protease inhibitor cocktail and phosphatase inhibitor cocktails 2 and 3. Lysates were centrifuged at 12,000 × g for 15 min at 4°C. Protein concentrations were measured by comparing with known concentrations of bovine serum albumin (BSA), using a BCA Protein Assay Kit (Pierce Biotechnology, Rockford, IL, USA). Subsequently, the samples were boiled in the sample buffer for 5 min. The samples were subjected to a 5–20% gradient SDS polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene difluoride (PVDF) membranes (Immobilon-P; Millipore). The following primary antibodies were used for immunoblotting: Purified Mouse Anti-Bip/GRP78 (1:1,000, BD Biosciences, San Diego, CA, USA) and anti-β-actin mouse monoclonal antibody (1: 5000; Sigma). An HRP-conjugated goat anti-rabbit antibody (1: 2000;
Thermo Fisher Scientific K.K.) and goat anti-mouse antibody (1: 2000; Thermo Fisher Scientific K.K.) were used as secondary antibodies. Immunoreactive bands were visualized using Immunostar-LD (Wako) and LAS-4000 luminescent image analyzer (Fuji Film Co., Ltd., Tokyo, Japan).

**Figure legend**

**The effect of TUDCA on ER stress-induced GRP-78 expression in ARPE-19 cells** Pretreatment with TUDCA (1 mM) (lane 3) was followed by 6 h of additional incubation with 2 μg/mL tunicamycin (lane 2 and 3) in ARPE-19 cells. Representative band images show GRP-78 expression. GRP-78 expression was quantified by densitometry and corrected by reference to β-actin. Data are shown as mean ± SEM (n = 4). **P < 0.01 vs. control (Student’s t-test).**