EXPERIMENTAL CELL RESEARCH 332 (2015) 11–23

Available online at www.sciencedirect.com

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journal homepage: www.elsevier.com/locate/yexcr

Research Article

ER stress response in NG108-15 cells involves upregulation of syntaxin 5 expression and reduced amyloid β peptide secretion

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ARTICLE INFORMATION

Article Chronology:
Received 20 July 2014
Received in revised form
5 January 2015
Accepted 7 January 2015
Available online 14 January 2015

Keywords:
Amyloid precursor protein (APP)
Amyloid-beta (Aβ)
Endoplasmic reticulum stress
SNARE proteins
Stress response
Syntaxin 5

ABSTRACT.

Endoplasmic reticulum (ER) stress plays a role in the pathogenesis of neurodegenerative diseases such as Alzheimer's disease (AD). We previously showed that manipulation of the ER–Golgi-soluble *N*-ethylmaleimide-sensitive factor-attachment protein receptors (ER–Golgi SNARE) syntaxin 5 (Syx5) causes changes in Golgi morphology and the processing of AD-related proteins. To understand the pathophysiologic significance of these phenomena, we examined whether the expression of Syx5 is altered by ER stress. De novo synthesis of ER–Golgi SNARE Syx5 and Bet1 was induced by various ER stressors. Elevated expression of Syx5 and Bet1 was associated with increased levels of these proteins in vesicular components, including ER–Golgi-intermediate-compartment/vesicular tubular clusters. In addition, ER stress diminished amyloid β (Aβ) peptide secretion. Knockdown of Syx5 expression enhanced the secretion of Aβ peptides under condition without ER stress. Moreover, diminished Aβ peptide secretion resulting from ER stress was significantly reversed by Syx5 knockdown. These findings suggest that Syx5 plays important roles in β-amyloid precursor protein processing and in the ER stress response that precedes apoptotic cell death and may be involved in the crosstalk between these two pathways.

Abbreviations: Aβ peptide, amyloid-β peptide; AD, Alzheimer's disease; APP-CTF, C-terminal fragment of β-amyloid precursor protein; BFA, brefeldin A; βAPP, β-amyloid precursor protein; COP, coatmer protein; ER, endoplasmic reticulum; CHX, cyloheximide; CPA, cyclopiazonic acid; ERGIC/VTC, ER–Golgi-intermediate-compartment/vesicular tubular clusters; HA, hemagglutinin; PS, presenilin; RNAi, RNA interference; SDG, sucrose density gradient; SERCA, sarco/endoplasmic reticulum Ca^{2+}-ATPase; siRNA, small interfering RNA; SNARE, soluble N-ethylmaleimide-sensitive factor-attachment protein receptor; STS, staurosporine; Syx5, syntaxin 5; Tg, thapsigargin; Tm, tunicamycin

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http://dx.doi.org/10.1016/j.yexcr.2015.01.001
0014-4827/

Introduction

Biogenesis of nascent secretory and membrane proteins in the endoplasmic reticulum (ER) is carefully controlled, both qualitatively and quantitatively [1]. These proteins are subsequently packaged into transport vesicles for exiting the ER to the Golgi apparatus along the secretory pathway. This transport system is governed by various proteins, including Rab and Arf GTPases, soluble *N*-ethylmaleimide-sensitive factor-attachment protein receptors (SNAREs), and Sec1/Munc 18 [2–4]. SNAREs are highly conserved proteins that act as the major players in the final docking and subsequent fusion stages in a diverse array of vesicle-mediated transport events [5,6]. ER–Golgi SNAREs are vesicle delivery proteins that regulate the proper targeting and sorting of membrane proteins in the early secretory compartments. The syntaxin (Syx) family of proteins is mammalian SNAREs that localize to specific membrane compartments along the secretory and endocytic pathways [2,7]. Syx5 resides in the ER and throughout the Golgi compartments are involved in the early secretory pathways and act as a target-SNARE (t-SNARE) in vesicular transport [8–10]. Syx5 regulates the targeting and fusion of carrier vesicles at multiple membrane fusion interfaces along the early secretory pathways by constructing selective combinations of SNARE complexes with other proteins [2,8,9,11–13].

We previously showed that downregulation of Syx5 expression by small interfering RNA (siRNA) results in Golgi fragmentation [14], which is one of the phenomena seen in the neurons of Alzheimer's disease (AD)-affected brain [15,16]. Furthermore, we found that Syx5 isoforms specifically interact with the presenilin (PS) holoprotein, which is a key player in the pathogenesis of AD [17,18]. PS is a component of the γ-secretase complex that cleaves β-amyloid precursor protein (βAPP) to produce amyloid-β (Aβ) peptide [19–21]. In addition, we showed that Syx5 overexpression causes accumulation of βAPP holoprotein in the ER and suppresses its further processing into the βCTF (C-terminal fragment of βAPP) and Aβ peptide [22]. Recently, we also reported that treatment with the ER stress inducer, brefeldin A (BFA), which inhibits trafficking of proteins from the ER to the Golgi complex[23], results in the retention of βAPP holoprotein in the ER [22,23]. It has been reported that various stimuli lead to altered distribution of βAPP to the ER and affect the processing of βAPP in some cells [24,25]. Disturbances in the balancing of protein synthesis, folding, and secretion in the ER lead to ER stress. Prolonged ER stress can induce apoptosis [26] and has been implicated in neurodegenerative disorders such as AD, Parkinson's disease, and Huntington's disease (for review, see [27]). ER stress is also associated with the familial type of AD [28,29]. However, as there are few reports concerning ER stress and the sporadic type of AD [30], whether there is a correlation between ER stress-associated changes in vesicle transport regulators and AD is unclear.

In the present study, we examined the effects of ER stress and apoptosis induction on the expression of Syx5 and other ER–Golgi SNAREs. We also examined the pathophysiologic significance of Syx5 expression on βAPP processing during ER stress. We found that whereas ER stress led to upregulated expression of Syx5 isoforms and Bet1, induction of apoptosis led to downregulated Syx5 expression. Suppression of Syx5 expression increased the production of Aβ peptides without ER stress. In addition, suppression of Syx5 expression attenuated the ER stress-induced reduction in Aβ peptide secretion. Thus, we suggest that Syx5 plays a role in the ER stress response and in βAPP metabolism.

Material and methods

Materials

Tunicamycin (Tm), Hoechst 33342, and brefeldin A (BFA) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). BFA was stored as a 5 mg/mL solution in methanol. Staurosporine (STS), thapsigargin (Tg), and cyclopiazonic acid (CPA) were purchased from Merck (Darmstadt, Germany) and dissolved in dimethyl sulfoxide (DMSO). Cyloheximide (CHX) and cell permeable Caspase3 inhibitor (II: Z-D(OMe)E(OMe)VD(OMe)-FMK) were obtained from Merck. Protease inhibitor cocktail was purchased from Wako Chemicals (Osaka, Japan). All other reagents were of the highest grade available, unless otherwise noted.

Antibodies

Mouse anti-Syx5 monoclonal (clone 1C5) and anti-HPC-1/Syx1A (14D8) antibodies were prepared as described previously [14,31]. Mouse monoclonal antibodies against α-tubulin, β-actin, and Sec22b, and Cy3-conjugated rabbit polyclonal antibody against ERGIC53/p58 were obtained from Sigma. Mouse monoclonal antibodies against caspase 3/CPP32, GM130, BiP/GRP78, Syx6, and γ-adaptin were obtained from BD Transduction Laboratories (San Diego, CA, USA). Antibodies against GS28 and Bet1 were obtained from Stressgen (Victoria, BC, Canada). Rabbit anti-β-coatmer protein (COP) polyclonal antibody was obtained from Thermo Scientific (Rockford, IL, USA), and anti-calnexin and -membrin antibodies were obtained from Stressgen. Rabbit polyclonal anti-βAPP (APPC) antibody was obtained from IBL (Gunma, Japan). Rat monoclonal anti-hemagglutinin (HA) antibody 3F10 was purchased from Roche Diagnostics (Indianapolis, IN, USA). Alexa fluor 488-conjugated anti-mouse and rabbit IgG antibodies were obtained from Molecular Probes (Eugene, OR, USA). Cy3-labeled anti-rat and anti-mouse IgGs were obtained from Jackson Immunoresearch Laboratories (West Grove, PA, USA).

Cell culture and transfection

Mouse neuroblastoma and rat glioma hybrid NG108-15 cells were cultured in Dulbecco's modified Eagle's medium containing 4 mM L-glutamine, 100 U/mL of penicillin, 100 μg/mL of streptomycin, 2.5 mM hypoxanthine, 10 μM aminopterin, 0.4 mM thymidine, and 10% fetal bovine serum in a humidified incubator with 5% CO_2 at 37 °C, as described previously [22]. The cells were inoculated into type IV collagen-coated 6- or 24-well plates or 100-mm dishes (BD Bioscience, Bedford, MA, USA) for extract preparation, into poly-L-lysine-coated 96-well plates (Greiner Bio-One, Frickenhausen, Germany) for cell viability assays, or 35-mm dishes (BD Bioscience) with glass bottoms for immunocytochemical and time lapse imaging analyses. The cells were transfected with annealed duplex siRNA (final concentration of 10 nM) using LipofectAMINE RNAiMax transfection reagent (Life Technologies, Rockland, MD, USA).