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RESEARCH ARTICLE

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Regulatory role of Golgi brefeldin A resistance factor-1 in amyloid precursor protein trafficking, cleavage and Aβ formation

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Abstract

β-amyloid peptide (Aβ) deposition derived from sequential cleavage of the amyloid precursor protein (APP) through the amyloidogenic pathway is an important characteristic feature of Alzheimer's disease (AD). During this process, cellular trafficking plays a crucial role. A large Sec7-domain containing ADP-ribosylation factor guanine nucleotide exchange factor (ARF-GEF), Golgi brefeldin A resistance factor 1 (GBF1) has been reported to initiate the ADP-ribosylation factor (Arf) activation cascade at trans-Golgi network, which plays a crucial function at the endoplasmic reticulum-Golgi interface. In this study, we investigated the role of GBF1 in APP transmembrane transport and Aβ formation. Using APP/PS1 (presenilin 1) overexpressing transgenic mice, we demonstrate that GBF1 has upregulated the expression of APP, indicating a role for GBF1 in APP physiological process. Knocking down of GBF1 using small interfering has significantly increased the intracellular but not the surface expression of APP. In contrast, overexpression of wild-type (WT) and guanine nucleotide exchange factor (GEF) in the activated form but not the GEF deficient mutation induced continuous activation of GBF1, which subsequently increased the surface level of APP. Interestingly, inhibition of GBF1 by c(BFA) also impaired APP trafficking and induced endoplasmic reticulum (ER) stress in SH-SY5Y cells. Our results thus for identified the role of GBF1 in APP trafficking and cleavage, and provide evidence for GBF1 as a possible therapeutic target in AD.

KEYWORDS
amyloid precursor protein, β-amyloid peptide, endoplasmic reticulum stress, Golgi brefeldin A resistance factor 1, transmembrane trafficking

Kaifei Liu and Ying Liu contributed equally to this work.

1 | INTRODUCTION

Alzheimer's disease (AD) is one of the most common neurodegenerative disorders in the elderly population all over the world. Although the molecular mechanisms are not fully understood, there are so many hypotheses, including choline, genetics, inflammation, and so on.[1] Amount these, extracellular amyloid plaque formation and intraneuronal neurofibrillary tangles composed of the β-amyloid peptide (Aβ) and hyperphosphorylated tau respectively are well known pathological markers of AD.[2] Recent reports have shown that synaptic activity promotes the secretion of Aβ in slice cultured hippocampal and also in the mouse brain. Aβ peptides are derived from a sequential cleavage of amyloid precursor protein (APP) through the amyloidogenic pathway.[3] In this pathway, APP is first cleaved within the cytoplasmic domain by α-secretase (BACE2), or β-secretase (BACE1) enzyme resulting in the shedding of almost its entire ectodomain and subsequent generation of the membrane-tethered APPα or APPβ carboxy-terminal fragments. The β-cleavage-derived fragment is further cleaved within their transmembrane domains by the γ-secretase complex to release the Aβ peptide to form amyloid plaque, and a cytoplasmic APP intracellular domain (AICD).[4] After the cleavage by γ-secretase complex, APPα will become soluble, which might be used as a nutritious component by neuronal cells.[5]

It is clear that the molecular mechanisms underlying AD pathogenesis involve alterations in APP cleavage, which leads to increased Aβ production or, decreased enzymatic degradation and clearance of Aβ, alternatively.[6] On the basis of the localization of BACE1 and BACE2, it is plausible that the α-secretase cleavage of APP is primarily occurring on the cell surface, whereas β-secretase cleavage mainly takes place in endosomes.[7] BACE1 is located in the Golgi and trans-Golgi network (TGN), whereas BACE2 is mainly present in the plasmalemma. Impairments in APP trafficking between the cell surface and different intracellular organelles or its consequent localization might influence the preference for α- or β-secretase mediated cleavage. If the cleavage happens in Golgi, then the production of Aβ will increase, otherwise sAPPα will be produced preferentially. Indeed, increased APP levels on the cell surface or decreased APP internalization has been shown to reduce Aβ release,[8,9] whereas accelerated endocytosis in neuron enhances β-cleavage. Grbovic et al.[10] Thus, from Golgi to the membrane, vesicle trafficking plays a critical role in APP physiological processes.[11]

The formation and maturation of the vesicle require the Procedural action of two cytosolic coat protein complexes, named COPI and COPII.[12] Current evidence suggests that endoplasmic reticulum (ER) cargo is selected at ER exit sites (ERES) into COPII-coated structures that subsequently bud and fuse to generate the transporting vesicles.[13,14] However brefeldin A (BFA), which blocks COPI but not COPII function, prevents the COPII-driven accumulation of anterograde cargo at ERES structures.[15] Some evidence also indicates that COPII function is tightly linked and dependent on COPI recruitment on ERES structures.[16]

The ADP ribosylation factor (Arf) family of small G-proteins constitutes a critical component of the intracellular membrane trafficking machinery, which plays fundamental roles in the regulation of membrane transporting at multiple sites in all the eukaryotes, including the Golgi, endosomes, ER, ER-Golgi intermediate compartments, as well as the plasma membrane.[17] The activation of Arf depends on guanine nucleotide exchange factor (GEF),[18] especially the Sec7 domain.[19] Although multiple GEFs activate Arfs in a different manner, their functional mechanism still needs more work to clarify. The specific cargo has the capability to regulate the Arf-dependent recruitment of the adaptors,[20] which indicates that a functional complex between the cargo, the cognate Arf, and an Arf GEF exists during membrane trafficking. However, up to now, we have not found any evidence for such a complex.[21]

The BIG/GBF family consists of large Golgi-localized Arf GEFs containing the highly conserved Sec7 domain, which contributes in the nucleotide exchange activity, surrounded by several conserved domains participating in the functional interactions that regulate the activity and membrane connection.[22,23] Golgi brefeldin A resistance factor 1 (GBF1) is one of the high-molecular-weight GEF, which functions within the early Golgi and Golgi and trans-Golgi network (TGN), and its activity is inhibited by BFA.[24] It has also been reported that GBF1 is targeted to Golgi membranes through a special PIP-binding domain,[25] and required for the polarized cell growth in *Arabidopsis*, which plays a crucial role in mediating endosomal recycling.[26] At the TGN, GBF1 initiates an Arf activation cascade through direct interactions with Arf4,[27] and regulates the Arf/COPI-dependent trafficking at the ER-Golgi interface.[28] GBF1, Arf1 and COPI complex is involved in the lipid droplet (LD) metabolism, mediating recruitment of the proteins, Perilipin 2 (Plin2) and Patatin Like phospholipase domain containing 2 (ATGL) to the LD surface. GBF1 and Arf1 have COPI-independent functions of the clathrin-independent endocytosis, mitochondria, and in the recruitment of golgin160 to Golgi membranes.[29]

To date, specific diseases related to GBF1 are not yet identified. However, GBF1 is mainly involved in viral RNA replication, including coxsackie virus B3,[30] hepatitis

C virus,[31] and polio virus.[32] Here, we report a critical role of GBF1 in mediating APP trafficking to cell surface, to increase α-cleavage of APP leading to decrease in Aβ formation during AD pathological processes.

2 | MATERIALS AND METHODS

2.1 | Animals, antibodies, plasmids, and reagents

Specific pathogen-free (SPF) Sprague-Dawley (SD) rats were provided by the Laboratory Animal Center, Sun Yat-Sen University, APP/PS1 double transgenic and wild-type (WT) C57BL/6 J mice were purchased from Shanghai SLAC Laboratory Animal Co. Ltd. (Shanghai, China) SH-SY5Y cell line was obtained from ATCC (Manassas, VA). Purified mouse anti-GBF1 antibody was from BD Biosciences (Sparks, MD). APP Antibody, Presenilin 1 (PS1) antibody, β-amyloid (1-40 Specific) (D8Q7I) rabbit mAb, phospho-eIF2α (Ser51) (D9G8) XP rabbit mAb, ATF-6 (D4Z8V) Rabbit mAb, IRE1α (14C10) Rabbit mAb, and phospho-PERK (Thr980) (16F8) rabbit mAb were products of Cell Signaling Technology, Inc (Boston, MA). GRP78/BIP rabbit polyclonal antibody was purchased from Proteintech (Wuhan, China); mouse monoclonal anti-β-actin antibody was from Abcam (Cambridge, UK). Mouse monoclonal anti-GAPDH antibody was purchased from Beyotime (Jiangsu, China), Alexa Fluor 488, 594-conjugated secondary antibodies and ProLong Gold Anti-fade mount with 4′,6-diamidino-2-phenylindole (DAPI) were from Molecular Probes (Carlsbad, CA); enhanced chemiluminescence (ECL) kit was from GE Healthcare (Fairfield, CT); ethylenediaminetetraacetic acid (EDTA)-free protease inhibitor was from Roche (Basel, Switzerland), BFA was from Sigma Aldrich (St Louis, Mo), Golgicide A from Selleck Chemicals (Shanghai, China). EGFP-GBF1-WT, EGFP-GBF1-E794K, and EGFP-GBF1-M832L plasmids were kind gift from Dr. Martha Vaughan in the Laboratory of Metabolic Regulation, National Heart, Lung, and Blood Institute (Bethesda, MD). EGFP-APP^{swe} was constructed by cleaving the APP sequence from Flag-APP^{swe}, a gift from Prof. Lin Zhong, School of Pharmaceutical Sciences, Sun Yat-Sen University.

2.2 | Protein extraction from mouse brain and Western blot analysis

4-month-old WT and APP/PS1 transgenic mice were humanely euthanized by CO_2 asphyxiation, according to the National Institutes of Health guide for the care and use of laboratory animals, and the brains were separated and homogenized in radioimmunoprecipitation assay (RIPA) buffer (Beyotime) supplemented with EDTA-free protease inhibitor cocktail. Equal amounts of proteins (30 μg) from the soluble fractions of the lysates were separated by 8% SDS-PAGE and transferred to polyvinylidene fluoride membrane (Merck Millipore, Bedford, MA). After blocking with 5% nonfat milk solution in Tris-buffered saline (TBS) containing 0.1% Tween 20 for 1 hour at room temperature (RT), the membranes were incubated with appropriately diluted primary antibodies (1:100-1:1000) at 4°C overnight and then probed with horseradish peroxidase-conjugated secondary antibodies (goat anti-rabbit or mouse IgG) for 1 hour at RT. Immunoreactive bands were detected by ECL and visualized using the ChemiDoc system (Bio-Rad, Hercules, CA). The intensities of the blots were quantified by densitometry using Quantity One software (Bio-Rad, Hercules, CA) according to manufacturer's instructions, as described earlier.[33]

2.3 | Primary cortical neuron culture, small interfering RNA, and BFA treatment

Cortical neurons were dissociated from embryos of E19 from SD rats. Briefly, cortices were aseptically dissected and incubated with 2 mg/mL of papain (Sigma-Aldrich, St. Louis, MO) for 15 minutes at 37°C. Dissociated neurons were plated at a density of 2×10^6 cells per mL into poly-L-lysine-treated coverslips or cell culture plates. Cultures were maintained in 5% fetal bovine serum (FBS) Dulbecco's modified Eagle's medium (DMEM) containing 100 U/ml of penicillin and 100 μg/mL of streptomycin for 8 hours at 37°C. Subsequently, the medium was removed and neurobasal medium containing 1% B27 supplement (Invitrogen, Carlsbad, CA) was added. Experiments were carried out on neurons after 7 to 10 days culture in vitro.

Small interfering RNA (siRNA) targeting GBF1 was designed (E11), and synthesized by GenePharma (Suzhou, China). After the neurons were cultured for 6 to 7 days, they were transfected with E11 or control scrambled siRNA with Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. Seventy-two hours after transfection, cells were fixed and stained for immunofluorescence or lysed for Western blot analysis.

For the functional assays of BFA on GBF1 expression, APP/PS1/Aβ formation, and for analysis of ER stress markers, hippocampus neuronal cells were cultured for 7 days and then treated with 0.5 μg/mL of BFA for 12, 24, 36 hours or at 0.1, 0.5, 1.0 μg/mL concentrations for 24 hours. Dimethyl sulfoxide (DMSO) was used as menstruum control. Total protein was extracted and used for Western blot analysis.

2.4 | Immunofluorescence staining

After the treatment described before, neuronal cells plated on coverslips were fixed with Immunol Staining

Fix Solution (Beyotime) and blocked for 30 minutes with 10% normal goat serum in PBS containing 0.1% Saponin. Cells were then incubated with appropriate diluted primary antibodies for 1 hour at RT, followed by incubation with Alexa Fluor-labelled secondary antibodies (1:1000, in blocking buffer). Subsequently, cells were washed and the coverslips were mounted in Prolong Gold anti-fade reagent with DAPI (Molecular Probes, Waltsham, MA) and inspected using a confocal microscope (Zeiss 710, Oberkochen, Germany).

2.5 | Overexpression of APP and GBF1 plasmid in SH-SY5Y cells

SH-SY5Y cells were cultured in DMEM supplemented with 10% FBS (Gibco, Waltsham, MA) without antibiotics. When the cells were 80% to 90% confluent, empty vector, EGFP-APP^{swe}, EGFP-GBF1-WT, EGFP-GBF1-E794K, or EGFP-GBF1-M832L were transfected with Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. Twenty-four hours after transfection, cells were lysed with RIPA buffer to extract surface or total proteins for Western blot analysis.

2.6 | Labeling and extraction of surface APP

Cell surface proteins were biotinylated using EZ-Link Sulfo-NHS-SS-Biotin Reagent (Pierce, Rockford, IL) for 30 minutes at 4°C. After quenching, cells were washed, lysed and the membrane proteins were isolated by incubating with streptavidin-agarose beads (Pierce) at RT for 1 hour. After recovering the beads, all the bound proteins were eluted using loading buffer and then analyzed by Western blot analysis with anti-APP antibody as described earlier.[34]

2.7 | Ethics statement

I certify that this manuscript is original and has not been published and will not be submitted elsewhere for publication while being considered by Journal of Cellular Biochemistry. And the study is not split up into several parts to increase the number of submissions and submitted to various journals or to one journal over time. No data have been fabricated or manipulated (including images) to support your conclusions. No data, text, or theories by others are presented as if they were our own.

The submission has been received explicitly from all coauthors. And authors whose names appear on the submission have contributed sufficiently to the scientific work and therefore share collective responsibility and accountability for the results.

2.8 | Statistical analysis

All the data are shown as mean ± SEM. Statistical comparisons for Western blot analysis and neurite length were performed using a nondirectional the Student t test by SPSS (version 13.0, IBM Corp, Armonk, NY). Statistical significance is defined as $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. All the data points shown are the average from a minimum of three separate experiments.

3 | RESULTS

3.1 | GBF1 was upregulated along with APP expression

To explore whether GBF1 participates in APP physiology, we first used APP/PS1 transgenic mice and Western blot analysis. We analyzed whether overexpression of APP could increase the protein level of GBF1 in vivo. The results showed that the transgenic mice have a high level of APP expression, suggesting that APP/PS1 mice could be used as AD model to understand the underlying pathological mechanisms. Surprisingly, in these mice, GBF1 was also upregulated significantly (Figure 1A). To confirm our observation, we transfected EGFP-APP^{swe} plasmid into SH-SY5Y cells, then checked the expression of APP, GBF1, PS1, and Aβ. From the results, it is clear that our in vitro results are similar to our in vivo findings (Figure 1B).

3.2 | Knockdown of GBF1 causes APP accumulation

From the previous data, we identified that GBF1 is related to APP expression, but its role in APP pathology is still not clear. Considering the vesicle transporting function, we surmised that if GBF1 is crucial for APP trafficking, then it could have a pathological role as well. Hence, we designed and synthesized a specific siRNA sequence targeting GBF1 to knockdown its expression. As shown in Figure 2A, after transfected with E11 siRNA, the protein level of GBF1 was significantly decreased. However, APP and PS1 expressions were increased in the intracellular compartment. By confocal microscope imaging, GBF1 was found to be localized in the ER-Golgi apparatus. In the presence of GBF1, APP staining was weak, suggesting that the intracellular APP is present in small amounts only. In E11 transfected group, GBF1 staining was obviously reduced and APP has accumulated significantly (Figure 2B). Moreover, colocalization between APP and GBF1 (the yellow part) was observed, which suggests that GBF1 might be involved in transporting APP to the plasma membrane.

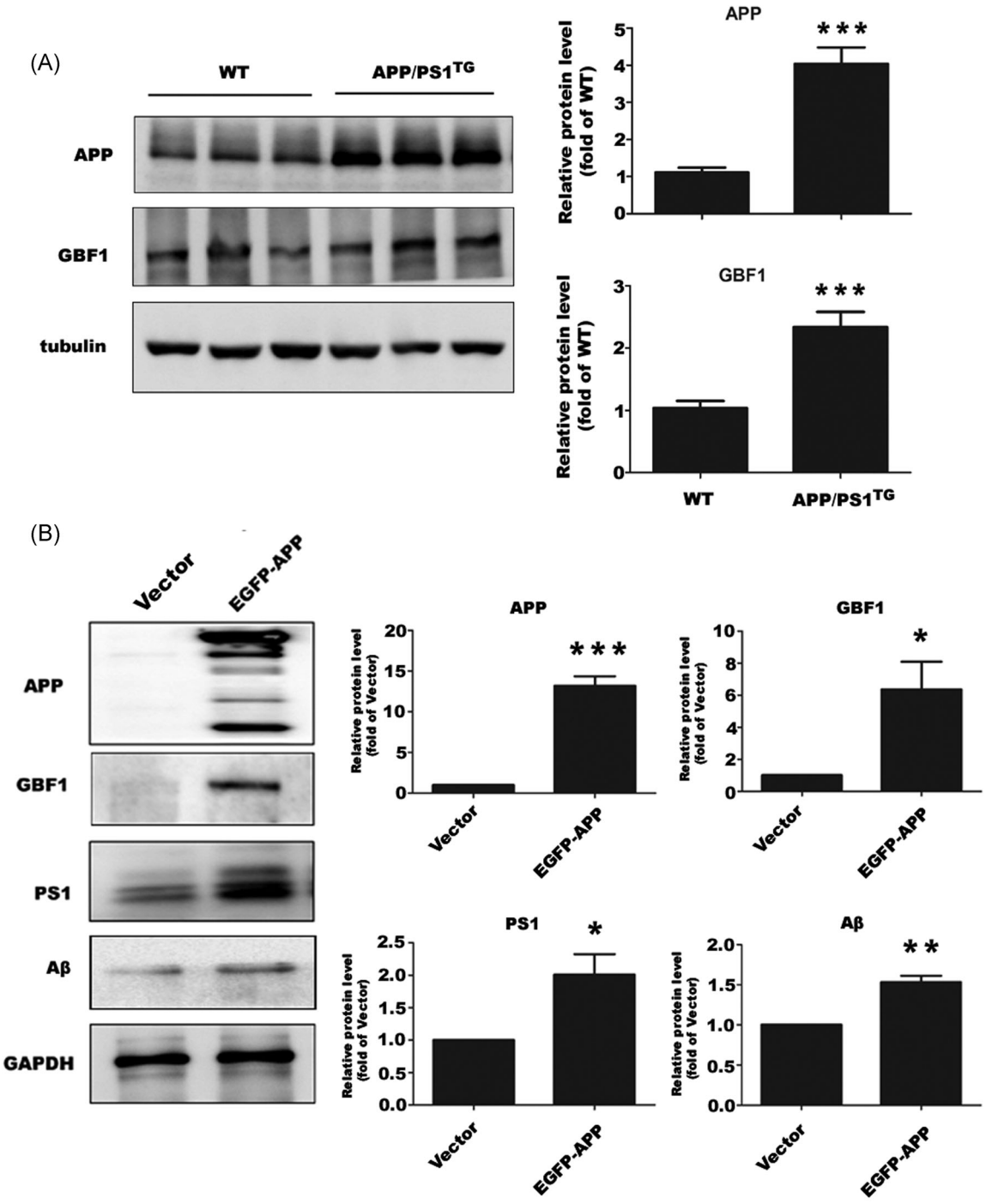

FIGURE 1 Increase in the level of APP upregulates GBF1 expression. Cerebral cortices of WT and APP/PS1 transgenic mice were separated and homogenized to extract total proteins for Western blot analysis. Expression of APP and GBF1 were detected (A). After overexpression of EGFP-APP^{swe} plasmid in SH-SY5Y cells, cell lysate was used to evaluate the protein levels of APP, GBF1, PS1, and Aβ (B). *P< 0.05; **P < 0.01; ***P < 0.001 Transgenic mice were compared with WT mice and plasmid groups were compared with the vector group. Aβ, β-amyloid peptide; APP, amyloid precursor protein; EGFP, enhanced green fluorescent protein; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; PS1, presenilin 1; WT, wild-type

3.3 | Overexpression of GBF1 rescues APP accumulation

As we mentioned earlier, the function of GBF1 is dependent on its GEF activity. Here, we used three GBF1 plasmids, viz, EGFP-GBF1-WT, EGFP-GBF1-E794K, and EGFP-GBF1-M832L. E794K is the loss-of-function mutant, whereas M832L has continuous GEF activity. These plasmids were transfected with EGFP-APP^{swe} and the intracellular amount of APP was investigated. The data

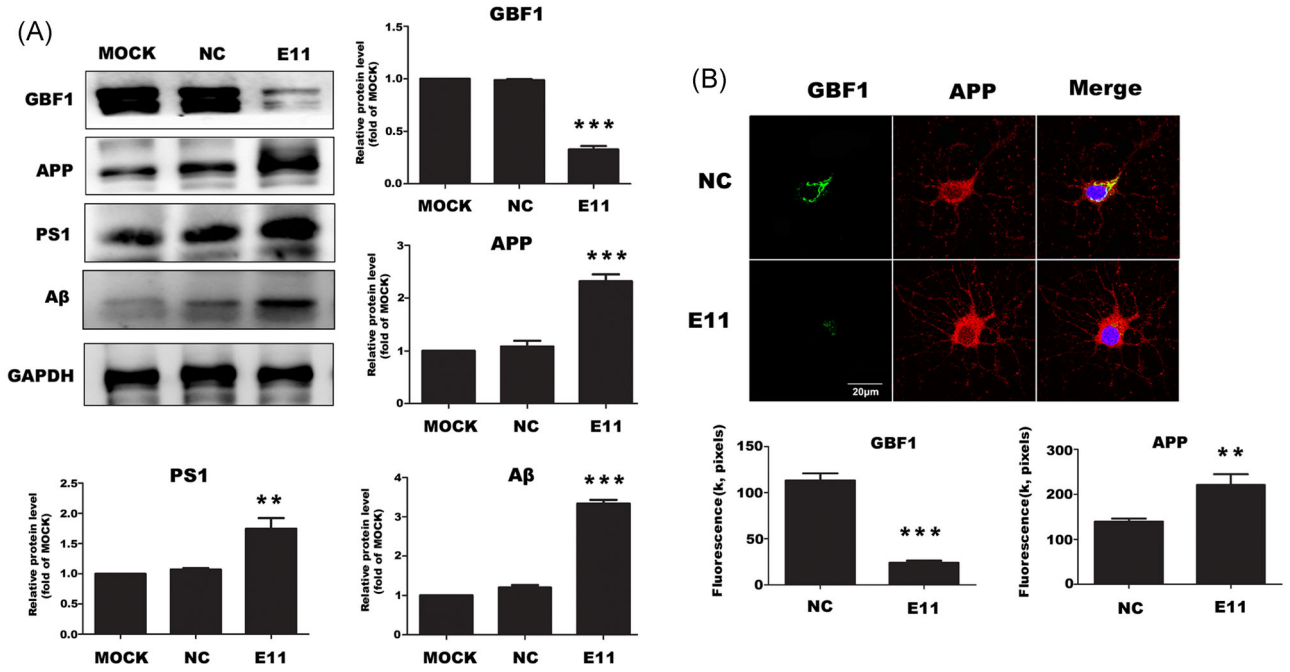

FIGURE 2 GBF1 knockdown leads to intracellular APP accumulation. A, Cultured primary cortical neurons (4 DIV) were transfected without siRNA (mock, MC) or with scrambled siRNA (NC) as controls, or with GBF1 siRNA E11. Three days after the transfection, cells were harvested for Western blot analysis and analyzed with GBF1, APP, PS1 and Aβ—specific antibodies. The intensity of the blots was quantified using densitometry scan and Quantity One software. B, Cortical neurons (4 DIV) were cultured on cover slips and transfected with scrambled siRNA as control (NC), or E11. Three days after the transfection, cells were fixed for immunostaining with an antibody against GBF1 or APP, followed by imaging with confocal microscope. $^{**}P < 0.01$; $^{***}P < 0.001$. E11 transfections were compared with NC for statistical analysis. Aβ, β-amyloid peptide; APP, amyloid precursor protein; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GBF1, Golgi brefeldin A resistance factor 1; NC, negative control; PS1, presenilin 1; WT, wild-type

in Figure 3A-E depict GBF1 dependent trafficking of APP, as well as the formation of PS1 and Aβ , especially the GEF activity. In E794K group, GBF1 showed no effect on APP transportation, which resulted in the accumulation of APP, PS1, and Aβ.

3.4 | BFA inhibited APP trafficking in a dose and time-dependent manner

As a next step, we used siRNA and plasmid transfection to evaluate the critical role of GBF1 in APP transportation and cleavage to form Aβ. It is possible that BFA might act on membrane trafficking by inhibiting Golgi-associated guanine nucleotide exchange protein on ADP-ribosylation factor (ARF), which plays a central role in controlling vesicular traffic from the ER to the Golgi complex and also between Golgi compartments. In our study, when SH-SY5Y cells were treated with BFA in different dosages and time points, APP accumulation and increased the formation of Aβ were observed by Western blot analysis (Figure 4A and 4B) and immunostaining (Figure 4C,D).

3.5 | GBF1 maintains the trafficking of APP to the plasma membrane

Both the knockdown of GBF1 and treatment with BFA led to APP conglomeration, hence we next analysed whether the increased level of APP causes plasmalemma impairment. To explore this idea, we isolated the plasmalemma from conditioned SH-SY5Y cells and detected the membrane expression of APP. Knocking down of GBF1 has significantly reduced the level of APP transport to the cell surface (Figure 5A). In contrast, both the overexpression of WT and M832L but not E794K GBF1 promoted plasmalemma recruitment of APP (Figure 5B). Inhibition of GBF1 by BFA has also impaired APP trafficking in a dose and time-dependent manner (Figure 5C,5D).

3.6 | Inhibition of GBF1 induced ER stress

As observed using confocal microscope, GBF1 is mainly localized in the ER. Hence, we hypothesized that if GBF1 activity is depleted; the architecture of the ER would be impaired, which could lead to ER stress. The results from

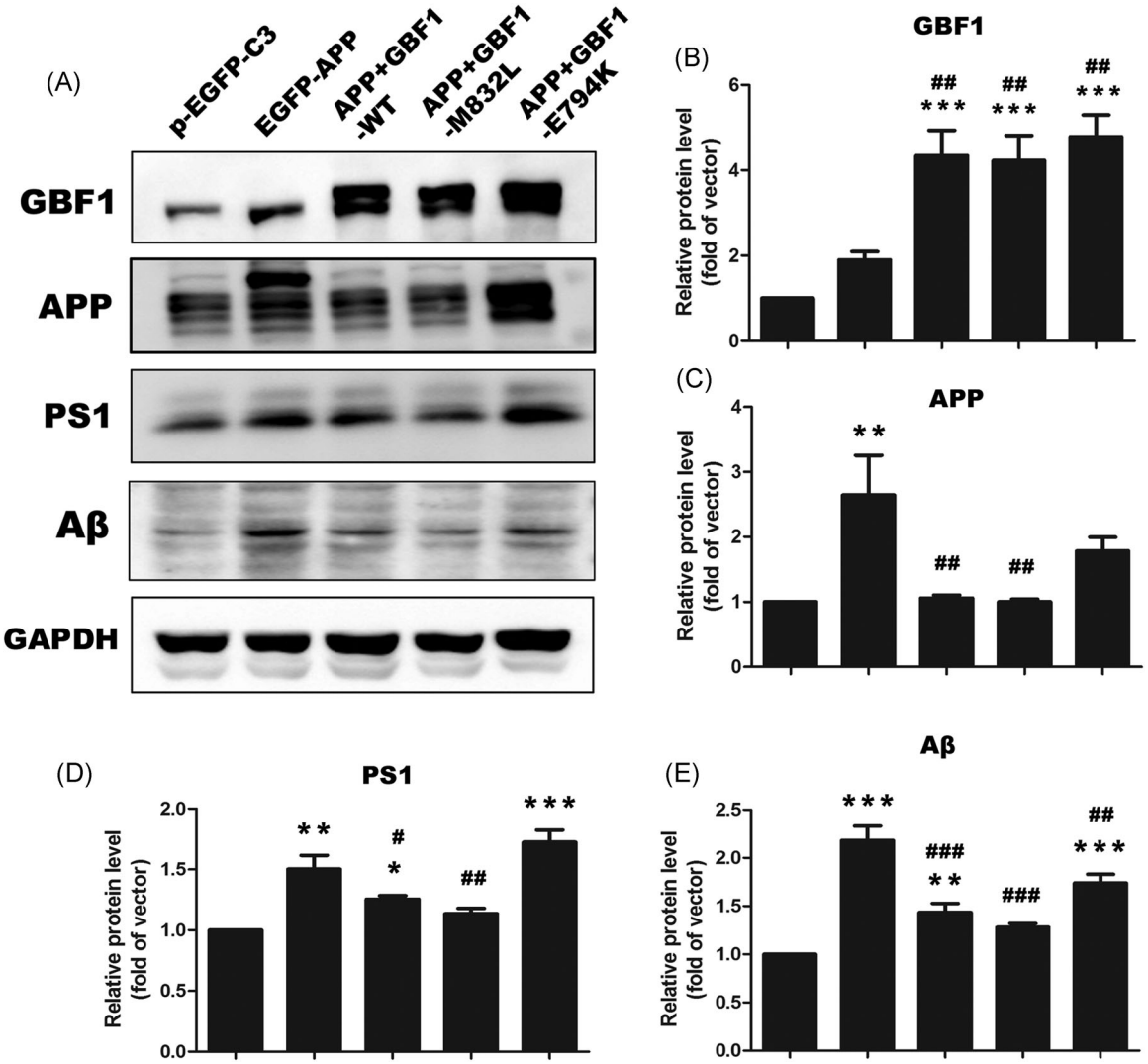

FIGURE 3 Overexpression of GBF1 contributes to APP transmembrane trafficking that is dependent on its GEF activity. SH-SY5Y cells were transfected with vector, APP^{swe}, APP^{swe} plus WT-GBF1, GBF1-M832L, or GBF1-E794K. After 24 hours, cells were harvested for Western blot analysis and the blots were analyzed with GBF1, APP, PS1, and Aβ—specific antibodies (A). The intensity of the blots was quantified using densitometry scan and Quantity One software (B-E). $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ APP^{swe} plus WT-GBF1, GBF1-M832L, or GBF1-E794K groups were compared with the vector group for statistical analysis. $^{\#}P < 0.05$; $^{\#\#}P < 0.01$; $^{\#\#\#}P < 0.001$, when APP^{swe} plus WT-GBF1, GBF1-M832L, or GBF1-E794K were compared with the APP^{swe} group. Aβ, β-amyloid peptide; APP, amyloid precursor protein; EGFP, enhanced green fluorescent protein; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GBF1, Golgi brefeldin A resistance factor 1; PS1, presenilin 1; WT, wild-type

both the knockdown of GBF1 and BFA treatment, inhibition of GBF1 by siRNA(Figure 6A) or BFA (Figure 6B) was found to remarkably upregulate the ER stress markers viz, expression of binding protein (BIP), the phosphorylation level of protein kinase R-like ER kinase (PERK), and eukaryotic translation initiation factor 2α (EIF2α).

4 | DISCUSSION

AD is a heterogeneous multifactorial disease and one of the most common, progressive and aging-related neurodegenerative disorders that severely reduce the patients' quality of life. The main neuropathological characters of AD are extracellular amyloid plaque deposition and intracellular neurofibrillary tangles (NFTs) consisting of aggregated Aβ and hyperphosphorylated microtubule-associated protein τ, respectively. These histopathological lesions are mainly present in the hippocampus and the cerebral cortex, two large forebrain regions that are crucial for memory acquisition and storage, along with other higher-order cognitive functions. These characteristic pathological features ultimately lead to the typical clinical symptoms

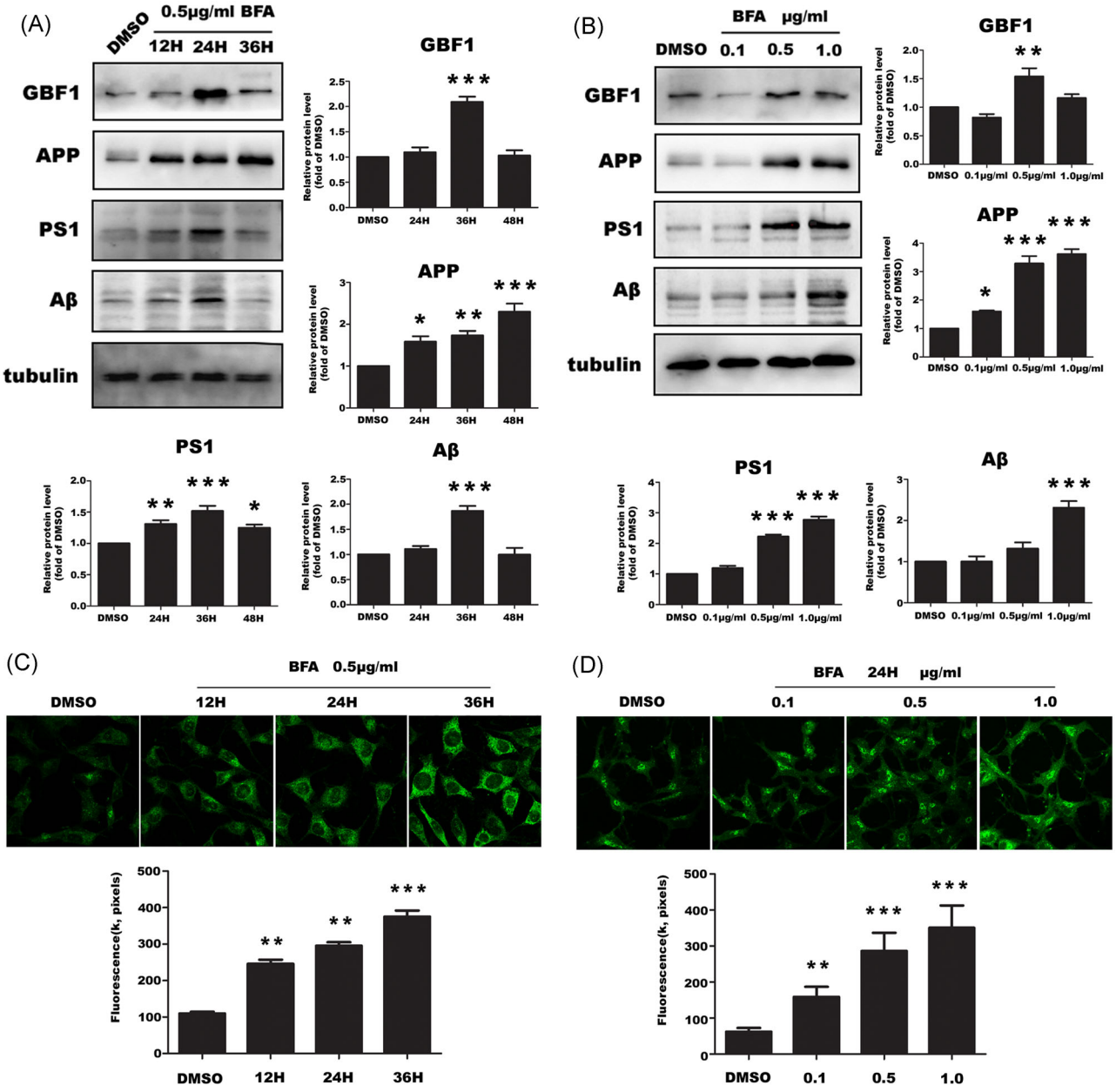

FIGURE 4 Effect of BFA, an uncompetitive inhibitor of the GEF activity of GBF1 on APP accumulation and Aβ formation. Cultured primary cortical neurons (4 DIV) were treated with 0.5 μg/mL of BFA (A) for 12, 24, 36 hours or at 0.1, 0.5, 1.0 μg/mL concentrations of BFA for 24 hours (B). Cells were harvested, lysed and used for Western blot analysis and the blots were analyzed with GBF1, APP, PS1, and Aβ—specific antibodies. The intensity of the blots was quantified using densitometry scan and Quantity One software. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ test groups were compared with DMSO group for statistical analysis (C,D). After BFA treatment, cells cultured on coverslips were fixed for immunostaining with antibodies specific for APP, followed by imaging with confocal microscope. Aβ, β-amyloid peptide; APP, amyloid precursor protein; BFA, brefeldin A; DMSO, dimethyl sulfoxide; GBF1, Golgi brefeldin A resistance factor 1; GEF, guanine nucleotide exchange factor; PS1, presenilin 1

associated with AD, such as general cognitive decline, dramatic memory loss, and severe personality changes.[35,36]

Eukaryotic cells contain numerous membrane-enclosed compartments, which are called vacuole or vesicle, each with a distinct biochemical function and composition. Proteins and lipids are continuously exchanged between these compartments, and this process is largely mediated through vesicular traffic and microtubule. This traffic occurs via transport vesicles that bud from the donor membrane, translocate through the cell and then fuse with a specific acceptor compartment.[22] The formation of a vesicle is a complex process that involves the admission of cargo proteins and vesicle, a new bud was

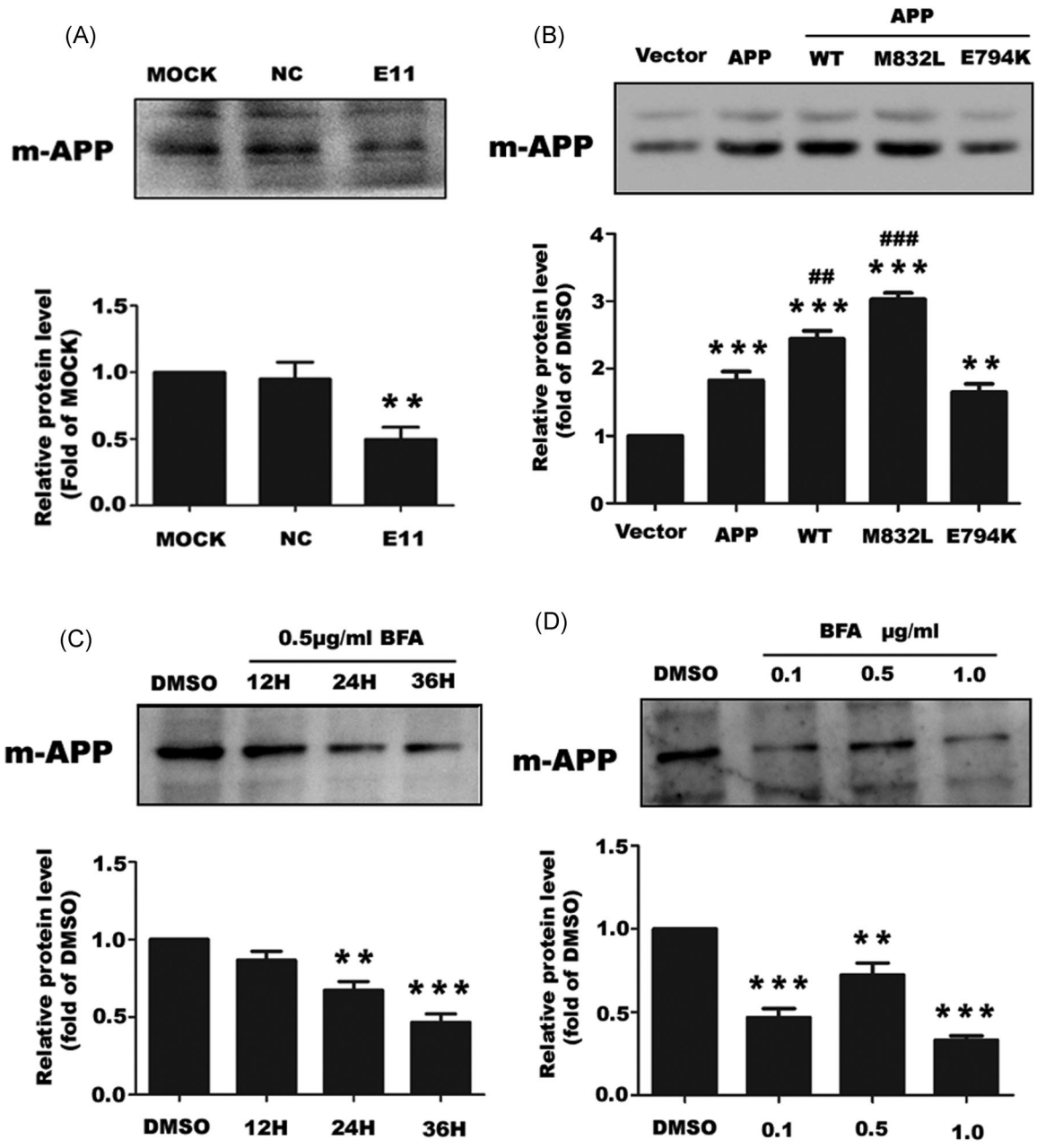

FIGURE 5 GBF1 is critical for APP transfer to the cell surface. Cells were treated as previously described in Figure 4, and the surface proteins were isolated for Western blot analysis to detect the levels of APP. **and $^{\#\#}P < 0.01$; *** and $^{\#\#\#}P < 0.001$. APP, amyloid precursor protein; BFA, brefeldin A; DMSO, dimethyl sulfoxide; WT, wild-type

identified in a process that included the deformation of the planar membrane and the eventual disconnection of the mature bud from the donor membrane.[37] The Golgi apparatus is a highly dynamic organelle, many of its resident proteins circulate continuously in the endoplasmic reticulum or the endosomes, while after treatment with BFA), the mammalian cis^{-}/medial-Golgi merges with the ER and the TGN mixes with the recycling endosomes only to reappear after removal of BFA.[38]

Aβ has been strongly implicated in AD due to its presence in the analysis of early-onset cases, which is genetic in nature. Although many studies have focused on APP trafficking and cleavage, the details of this process are not yet fully understood. All the mutations that increase the risk of AD to affect the production of Aβ, which may play a key role in sporadic forms of the disease. Cortes-Canteli et al.[39] Here we focus on the member of ARF-GEF, GBF1, by finding that GBF1's vesicle trafficking function is upregulated in APP/PS1 transgenic mice, as well as under APP overexpression conditions. Although it has been published earlier that GBF1 contributes to the membrane transporting,[29] but

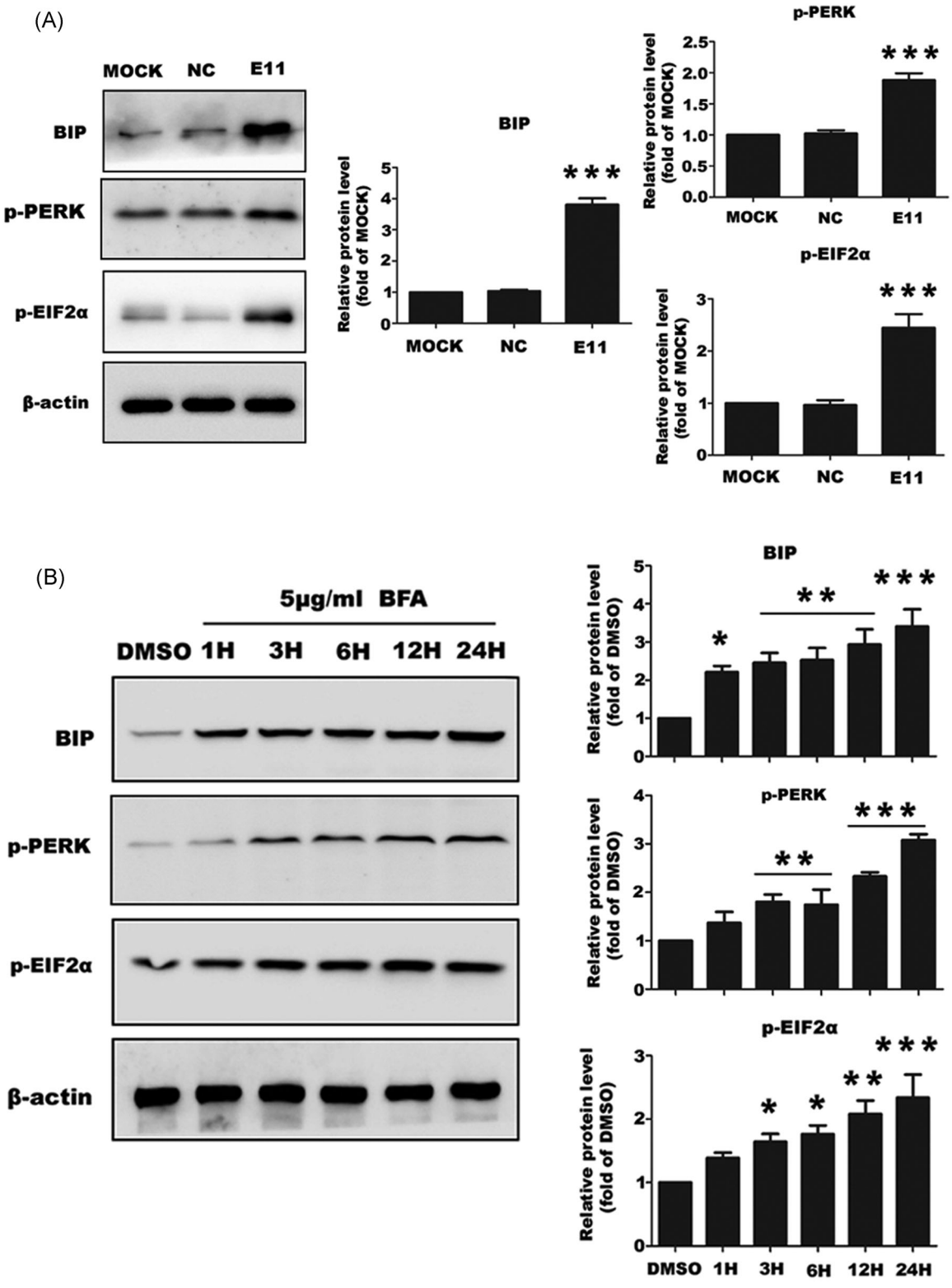

FIGURE 6 Inhibition of GBF1 induced ER stress. A, Cortical neurons (4 DIV) were transfected without siRNA (mock, MC) or with scrambled siRNA (NC) as controls, or GBF1 siRNA E11. Three days after the transfection, cells were harvested for Western blot analysis. The blots were analysed with BIP, Phospho-PERK, or Phospho-EIF2α—specific antibodies to verify the status of ER stress. B, Cortical neurons (4 DIV) were treated with 0.5 μg/mL of BFA for 1, 3, 6, 12, and 24 hours and then harvested to detect ER stress markers. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. GBF1 siRNA E11group was compared with control groups for statistical analysis. BFA, brefeldin A; BIP, binding protein; DMSO, dimethyl sulfoxide; EIF2α, eukaryotic translation initiation factor 2α; ER, endoplasmic reticulum; GBF1, Golgi brefeldin A resistance factor 1; PERK, protein kinase R-like ER kinase; siRNA, small interfering RNA

this information is limited only to virus replication. Considering the importance of axonal transport of APP and, the spatial regulation of APP cleavage and function in neuronal cells, we for the first time, explored the role of GBF1 in APP transport and Aβ formation.

Interestingly, like other vesicle trafficking proteins, when ER-Golgi localized GEF was impaired in its expression or activity, intracellular APP aggregation and increased Aβ formation were observed. As noted earlier, β-cleavage is the dominant cleavage type involved in the Aβ production, when APP is located at the Golgi and trans-Golgi membrane. Otherwise, α-cleavage is involved in the production of sAPPα in the cytosolic membrane. Our data demonstrate that inhibition of GBF1 could reduce the cell surface localization of APP, indicating an increased rate of β-cleavage and Aβ production. We also found that the vesicle trafficking inhibition either by knocking down of GBF1 or BFA treatment could induce stress in the ER compartment. Unresolved ER stress causes neuronal cell death and has been implicated in neurodegenerative conditions, especially in AD.[40] Due to ER and Golgi stress, ER-Golgi SNARE Syntaxin5 expression was found to be increased leading to the induction of βAPP processing in cultured hippocampal neurons.[41] However, another study showed that APP is rapidly degraded by the ubiquitin-proteasome system (UPS) in the CHO cell line in response to ER stress, caused by calcium ionophore, a compound A23187 that induced calcium influx. Increased levels of intracellular calcium by A23187 induces poly-ubiquitination of APP, causing its cleavage process.[42] Our data is in accordance with the former work, that is, ER stress increases β-cleavage and leads to Aβ formation.

In conclusion, our findings suggest that the involvement of GBF1 is critical for APP transmembrane transport and also demonstrate that GBF1 regulates Aβ production by influencing the trafficking of APP to the cell surface for α-cleavage, implicating GBF1 as a putative target for AD treatment.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

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