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GGA1 overexpression attenuates amyloidogenic processing of the amyloid precursor protein in Niemann-Pick type C cells

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ABSTRACT

Alzheimer's disease (AD) and a rare inherited disorder of cholesterol transport, Niemann-Pick type C (NPC) share several similarities including aberrant APP processing and increased Aβ production. Previously, we have shown that the AD-like phenotype in NPC model cells involves cholesterol-dependent enhanced APP cleavage by β-secretase and accumulation of both APP and BACE1 within endocytic compartments. Since retrograde transport of BACE1 from endocytic compartments to the trans-Golgi network (TGN) is regulated by the Golgi-localized γ-ear containing ADP ribosylation factor-binding protein 1 (GGA1), we analyzed in this work a potential role of GGA1 in the AD-like phenotype of *NPC1*-null cells. Overexpression of GGA1 caused a shift in APP processing towards the non-amyloidogenic pathway by increasing the localization of APP at the cell surface. However, the observed effect appear to be independent on the subcellular localization and phosphorylation state of BACE1. These findings show that the AD-like phenotype of NPC model cells can be partly reverted by promoting a non-amyloidogenic processing of APP through the upregulation of GGA1 supporting its preventive role against AD.

1. Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disorder among older population. According to the amyloid cascade hypothesis, accumulation of amyloid-β (Aβ) in the brain by impaired balance of Aβ production and clearance, is the primary cause of AD. Aggregated forms of Aβ trigger a cascade of disease processes, including formation of neurofibrillary tangles, synaptic dysfunction and neurodegeneration [5]. Aβ is produced by proteolytic processing of the amyloid precursor protein (APP), which is sequentially cleaved by two proteases termed β- and γ-secretase. The β-secretase is the aspartic protease BACE1 [17], while the γ-secretase is an intramembranous proteolytic complex, consisting of four components with presenilins constituting the active site [3,8,15]. In an alternative, the so-called non-amyloidogenic pathway, APP can be cleaved within the Aβ domain by the α-secretase, a metalloprotease of the ADAM family, thereby precluding the formation of Aβ.

BACE1 has maximal activity around pH 4–6. Accordingly, it is mainly localized in acidic compartments such as endosomes, lysosomes and the TGN, although some BACE1 is transiently associated with the plasma membrane [6]. Phosphorylation of BACE1 at serine 498 in the cytoplasmic domain does not directly affect its enzymatic activity, but can modulate its intracellular transport. Phosphorylation of BACE1 promotes the retrograde transport from endosomes to the TGN or lysosomes, while non-phosphorylated BACE1 stays in endosomes [2]. This process is regulated by the Golgi-localized γ-ear containing ADP ribosylation factor-binding proteins (GGA). GGA1 facilitates retrograde transport of phosphorylated BACE1 from endosomes to the TGN [19] and may modulate APP processing, most likely indirectly by altering the trafficking of BACE1 [18]. Depletion of GGA proteins or inhibition of BACE1 phosphorylation increases accumulation of BACE1 in endosomes, which favors β-secretase cleavage of APP and thereby the production of

Abbreviations: Aβ, amyloid beta peptide; AD, Alzheimer's disease; ADAM, A disintegrin and metalloproteinase; APOE, apolipoprotein E; APP, amyloid precursor protein; BACE1, beta-secretase; CHO, Chinese hamster ovary; CTF, C-terminal fragment; DN, dominant negative; FA, formic acid; GFP, green fluorescent protein; GGA1, Golgi-localized γ-ear containing ADP ribosylation factor-binding protein 1; HEK, human embryonic kidney; HRP, horseradish peroxidase; NPC, Niemann-Pick type C; PBS, phosphate buffer saline; sAPP, soluble N-terminal fragment of amyloid precursor protein; PAGE, polyacrylamide gel electrophoresis; PVDF, polyvinylidene difluoride; RIPA, radio-immunoprecipitation assay buffer; SDS, sodium dodecyl sulfate; SEAP, secreted alkaline phosphatase; TfR, transferrin receptor; TGN, trans-Golgi network; wt, wild type.

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Aβ [16,19]. The connection between GGA1 and BACE1 localization, APP processing and Aβ production has been analyzed in several cellular models [20].

It has been recently shown that a rare inherited lipid storage disorder Niemann-Pick type C (NPC), caused by dysfunction of the cholesterol transporters NPC1/NPC2, shares several similarities with AD, including neurodegeneration, accumulation of Aβ/APP–CTFs, endosomal/lysosomal dysfunction and APOE ε4-mediated increased progression of the disease [1,4,7,14,21]. NPC disease has also been referred to as childhood Alzheimer's, thereby representing an interesting model to analyze the mechanism of an AD-like pathogenesis. Our recent studies have shown that upon loss-of-function of NPC1, APP is favorably cleaved by BACE1 [13] most likely due to enhanced accumulation of both APP and BACE1 within endocytic compartments [12] and within cholesterol-rich lipid rafts [9]. In this study we analyzed the involvement of GGA1 in defective transport of BACE1 and/or APP and enhanced amyloidogenic processing of APP in NPC model cells.

2. Methods

2.1. Cell lines

Chinese hamster ovary wild type cells (CHOwt) and CHO cells in which the *NPC1* gene has been deleted (*NPC1*-null) were kindly provided by Dr. Daniel S. Ory, Washington University School of Medicine, USA. Cells were grown in DMEM/F12 medium (1:1) supplemented with 10% Fetal Bovine Serum, 2 mM L-glutamine and antibiotic/antimycotic solution, all from Sigma–Aldrich.

2.2. Plasmid vectors

SEAP (secreted alkaline phosphatase, R. Kopan, Washington University, USA); APPwt-6myc; BACE1–GFP (C. von Arnim, University of Ulm, Germany); GGA1wt-myc and GGA1DN-myc [20]; BACE1 S498A-GFP and BACE1 S498D-GFP (C. von Arnim, University of Ulm, Germany).

2.3. Primary antibodies

For Western blot we used: C-myc antibody 9E10 (Sigma–Aldrich); APP N-terminal antibody 22C11 (Chemicon); sAPPα specific antibody 14D6; sAPPβ specific antibody 8C10 (all kindly provided by C. Haass, Adolf Butenendt Institute, Germany).

For immunocytochemistry we used: C-myc antibody 9E10 (Sigma–Aldrich); anti-transferrin receptor antibody (Zymed laboratories); anti-TGN46 antibody (Abcam).

2.4. Transient transfection

For transfection we used Lipofectamine LTX (Invitrogen), according to the supplier's instructions. In order to compare APP processing products between CHOwt and *NPC1*-null cells we co-transfected APPwt-6myc with GGA1wt-myc, GGA1DN-myc or SEAP construct in control cells. SEAP activity measured in cell medium was an indicator of transfection efficiency between CHOwt and *NPC1*-null cells [10].

2.5. Analysis of APP processing

Soluble, insoluble and secreted Aβ40 was measured according to the previously optimized protocol [13]. Briefly, the cells were grown in Ø10 cm-plate. 48 h after transfection and 24 h after changing the medium, the cells were washed and collected. Cells were centrifuged and the pellet was resuspended and lysed in either RIPA buffer containing a protease inhibitor cocktail (Roche) for analysis of soluble, intracellular Aβ40 or were resuspended and lysed in formic acid (FA) for analysis of insoluble, intracellular Aβ40. The medium was collected and used for analysis of secreted Aβ40. The levels of secreted and intracellular Aβ40 were determined by ELISA Aβ40 kit (Invitrogen) according to the manufacturer's protocol. Aβ40 levels were normalized to the concentration of total protein (measured by Bio-Rad DC protein assay in RIPA lysates) in the sample and corrected for transfection efficiency between CHOwt and *NPC1*-null cells using SEAP activity measurement. Statistical validation of the data was achieved by Student *t*-test.

sAPP was analyzed in collected medium, while fl-APP, APP–CTFs, GGA1wt-myc and GGA1DN-myc levels were analyzed in RIPA lysates. RIPA lysates and medium were mixed with sample buffer and subjected to Tris–Glycine SDS–PAGE.

2.6. Biotin labeling of cell surface proteins

Biotin labeling of cell surface proteins was performed according to the previously optimized protocol [13]. Cells were grown in Ø10 cm-plates. Forty-eight hours after transfection and 24 h after changing the medium cells were washed with PBS (pH 8.5) and then processed for biotin labeling as described. For the analysis of the cell surface APP the samples were subjected to Tris–Glycine SDS–PAGE.

2.7. Western blot

After SDS–PAGE proteins were transferred to PVDF membrane (Roche), and subjected to blotting using HRP conjugated secondary antibodies (Bio-Rad). Proteins were visualized by chemiluminescence using POD chemiluminescence blotting substrate (Roche Applied Science). Western blots were quantified using ImageJ software (National Institutes of Health, USA). Statistical validation of the data was achieved by Student *t*-test.

2.8. Immunocytochemistry

Cells were grown on Ø12 mm-coverslips. 48 h after transfection, cells were fixed in 4% paraformaldehyde, permeabilized with 0.2% saponin and blocked in 5% goat serum. Immunostaining was performed with primary antibodies at 4 °C overnight in a dark wet chamber, following incubation with secondary Alexa488 or Alexa594 conjugated antibody (Invitrogen). The cover slips were mounted and analyzed by a Leica inverted fluorescent confocal microscope.

3. Results

3.1. Expression of GGA1wt increases the α- and decreases the β-cleavage of APP in NPC1-null cells

We previously showed that in *NPC1*-null cells both APP and BACE1 accumulate within endocytic compartments causing enhanced APP cleavage by β-secretase (BACE1) and increased production of sAPPβ, APP–CTFs and intracellular Aβ [12,13]. Since GGA1 controls the retrograde transport of BACE1 and probably APP from endocytic compartments to the TGN, we hypothesized that sequestration of these proteins within endosomes upon cholesterol accumulation in *NPC1*-null cells could be due to inefficient GGA1 function. To test this, we transiently co-transfected GGA1wt or a GGA1 dominant negative (DN) mutant or the SEAP plasmid vector together with a APPwt-6myc construct in CHOwt and CHO *NPC1*-null cells and analyzed APP processing. Upon overexpression of GGA1wt we observed significantly increased secretion of sAPPα

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