Biochemical and Biophysical Research Communications 450 (2014) 160–165

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

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

Kosicek Marko [a], Wunderlich Patrick [b], Walter Jochen [b], Hecimovic Silva [a,*]

[a] Division of Molecular Medicine, Rudjer Boskovic Institute, Bijenicka 54, 10000 Zagreb, Croatia
[b] Department of Neurology, University of Bonn, Sigmund-Freud-Str. 25, 53105 Bonn, Germany

ARTICLE INFO

Article history:
Received 12 May 2014
Available online 24 May 2014

Keywords:
Alzheimer's disease
APP
BACE1
GGA1
Niemann-Pick type C
NPC1

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.

* Corresponding author.
E-mail address: silva.hecimovic@irb.hr (S. Hecimovic).

http://dx.doi.org/10.1016/j.bbrc.2014.05.083
0006-291X/

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

and decreased secretion of sAPPβ in *NPC1*-null cells compared to cells expressing APPwt-6myc alone. A similar result was obtained with CHOwt cells (Fig. 1A and B). Cells overexpressing GGA1DN showed unchanged sAPPα levels but further decreased sAPPβ levels compared to control CHOwt and *NPC1*-null cells. In addition, GGA1wt overexpression decreased APP–CTF levels in *NPC1*-null cells to the levels observed in control CHOwt cells, while the APP–CTFs were undetectable in GGA1DN overexpressing cells (Fig. 1A and B).

In accordance to previous studies, we detected increased intracellular Aβ40 levels (both soluble and insoluble Aβ40, $p < 0.05$), but unchanged secreted Aβ40 levels in *NPC1*-null cells compared to CHOwt (Fig. 1C). In agreement with our data on sAPPβ and APP–CTFs, overexpression of GGA1wt in *NPC1*-null cells decreased the level of intracellular Aβ40 to that detected in CHOwt cells. In parallel, levels of secreted Aβ40 were unaffected by the overexpression of GGA1wt in *NPC1*-null cells, while the overexpression of GGA1DN dramatically reduced secreted Aβ40 in both CHOwt and *NPC1*-null cells. Since GGA1DN overexpression decreased almost all of the secreted APP metabolites, except sAPPα, we further analyzed whether this effect could be due to a general effect of GGA1DN on the secretory pathway and the cellular secretion. To test this, we co-transfected CHOwt and *NPC1*-null cells with the SEAP construct together with GGA1wt, GGA1DN or an empty GFP vector, and analyzed the SEAP activity in the medium. We observed statistically significant reduction of SEAP activity in the medium of CHOwt and *NPC1*-null cells overexpressing GGA1DN, compared to GFP and GGA1wt transfected cells, suggesting that the GGA1DN construct strongly inhibits the total cellular secretory system (not shown). Because of this general effect on secretion, we omitted GGA1DN in our further analyses.

3.2. GGA1wt overexpression does not revert BACE1 accumulation in endolysosomal compartments in NPC1-null cells

To test whether the decreased β-secretase cleavage of APP that was observed upon GGA1wt overexpression in *NPC1*-null cells is due to a GGA1-mediated retrieval of BACE1 out of accumulating endosomes to TGN, we analyzed cellular localization of stably expressed BACE1–GFP in GGA1wt-overexpressing cells by immunocytochemistry (Fig. 2). The controls (nontransfected cells, GGA1wt-negative) are visible on the same image together with transfected cells (GGA1wt-positive). Interestingly, GGA1wt overexpression did not alter the cellular localization of stably expressed BACE1 in both CHOwt and *NPC1*-null cells. In *NPC1*-null cells GGA1wt-overexpression did not reverse BACE1–GFP punctuate staining at the periphery to its perinuclear localization as in CHOwt cells. We also observed perinuclear localization of BACE1–GFP in both GGA1wt-expressing and non-transfected CHOwt cells.

Since the GGA1-mediated retrograde transport of BACE1 is dependent on the phosphorylation state of BACE1, we further tested whether a defect in the retrograde transport of BACE1 in *NPC1*-null cells by GGA1 is dependent on its phosphorylation state. We transiently overexpressed BACE1 phosphorylation mutants BACE1 S498A (which cannot undergo phosphorylation) or BACE1 S498D (which mimics phosphorylated BACE1) and analyzed their cellular localization by immunocytochemistry in CHOwt and *NPC1*-null cells. Both BACE1 variants showed broad distribution

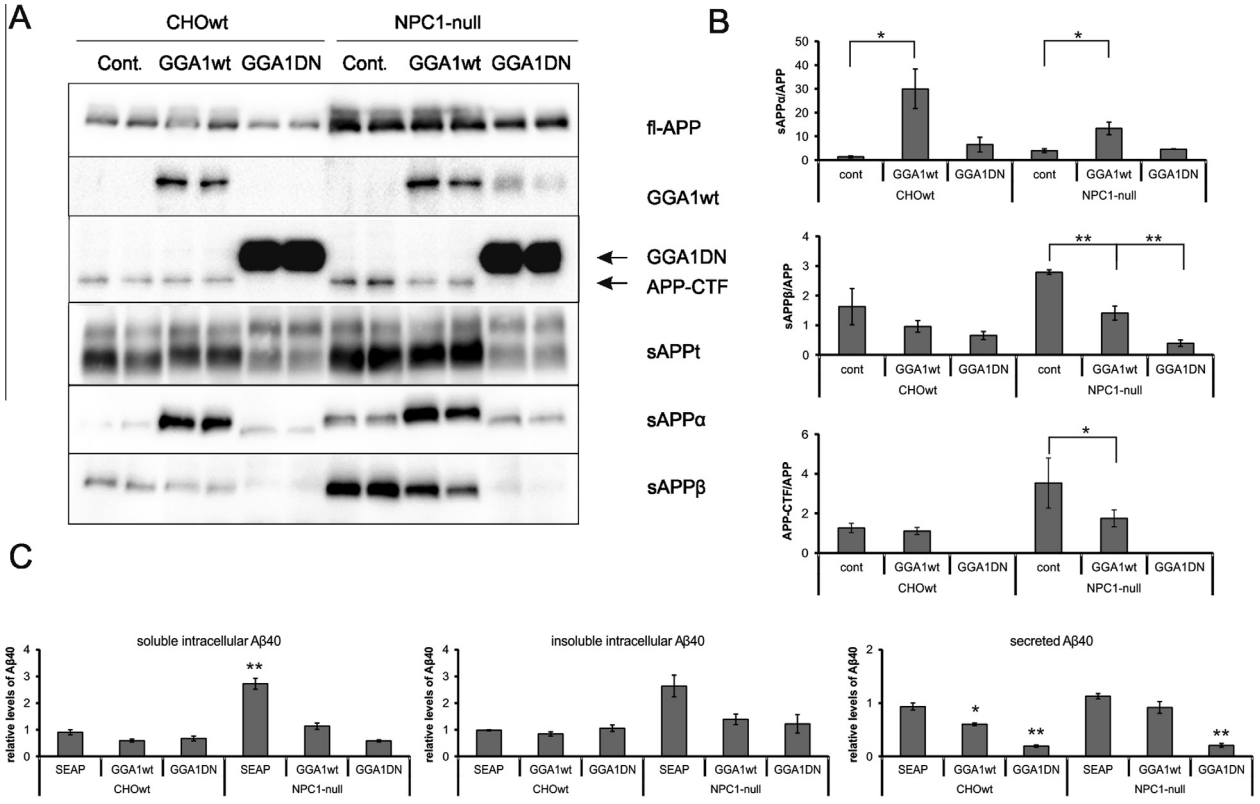

Fig. 1. GGA1wt overexpression increases α- and decreases β-cleavage of APP in *NPC1*-null cells. Cells were transiently co-transfected with APPwt-6myc and GGA1wt/GGA1DN plasmid vectors. In control cells SEAP was co-transfected with APPwt-6myc. (A) fl-APP, APP–CTF, GGA1wt and GGA1DN were analyzed in cell lysates, while secreted sAPP species (total, t; alpha, α; beta, β) were analyzed in cell medium. (B) Western blots were quantified using ImageJ software. Levels of sAPPα, sAPPβ and APP–CTFs were normalized to the levels of their direct precursor fl-APP. (C) Cells were transiently co-transfected with APPwt-6myc and SEAP, GGA1wt or GGA1DN plasmid vector. Three cellular pools of Aβ40 were measured by ELISA: intracellular (soluble and insoluble) and secreted. Statistical analysis was performed using Student *t*-test. Error bars show standard deviation: $^{*}p < 0.05$, $^{**}p < 0.01$.

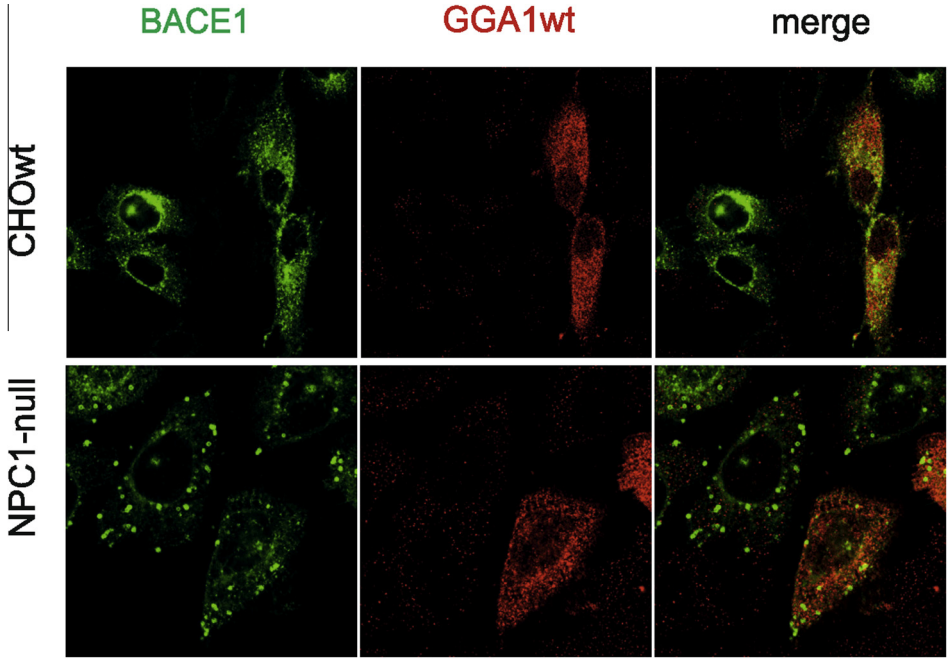

Fig. 2. Cellular localization of BACE1 is not altered by GGA1wt overexpression. CHOwt and *NPC1*-null cells stably expressing BACE1–GFP were transiently transfected with GGA1wt plasmid vector. Cells were visualized under confocal microscope.

of transferrin receptor TfR- and TGN-positive compartments in *NPC1*-null and CHOwt cells and accumulated in enlarged endolysosomal compartments in *NPC1*-null cells (Fig. 3).

3.3. Overexpression of GGA1wt shifts the localization of APP towards the cell surface

The observed accumulation of sAPPα in the medium of GGA1wt overexpressing CHOwt and *NPC1*-null cells indicated that the enhanced non-amyloidogenic processing of APP upon GGA1 expression could be due to a GGA1wt-mediated shift of APP towards the cell surface where α-secretase cleavage mainly takes place. To test this, we labeled endogenous cell surface APP with biotin and compared the cell surface APP levels between control (SEAP transfected) and GGA1wt transfected CHOwt and *NPC1*-null cells. Indeed, we observed a significant increase in the localization of APP at the cell surface in both cell lines upon overexpression of GGA1 (Fig. 4), supporting that the GGA1-dependent enhanced non-amyloidogenic processing of APP involves the accumulation of APP at the cell surface. Due to the low levels of cell surface BACE1 (both on endogenous and exogenous levels) we could not reveal whether GGA1 expression also promotes the localization of BACE1 at the cell surface (not shown). Immunocytochemistry of stably expressed APP in CHOwt and *NPC1*-null cells did not detect any gross changes in the subcellular localization of APP upon overexpression of GGA1wt (not shown), similar to our results on BACE1.

4. Discussion

Endosomes represent a central cellular site of Aβ formation and their dysfunction is an early pathological feature of AD. Intracellular APP trafficking is a main mechanism to control the Aβ generation, and modulating APP trafficking represents a novel therapeutic approach against AD [11]. In this study, we used *NPC1*-null cells as a model to analyze the role of GGA1, a previously described modulator of APP/BACE1 trafficking, on the accumulation of APP/BACE1 within endocytic compartments. Recently, we showed that both APP and BACE1 accumulate within enlarged endosomes in *NPC1*-null cells [12], suggesting that their sequestration within endocytic compartments triggers an enhanced amyloidogenic processing of APP and Aβ production in these cells [13]. In this study, we aimed to analyze whether sequestration of BACE1 in endosomes in *NPC1*-null cells might be caused by its dysfunctional retrograde transport from endosomes to the TGN. It is known that phosphorylation of serine at position 498 in the cytoplasmic domain of BACE1 promotes retrograde transport from endosomes to the TGN by the adaptor protein GGA1 [19]. Indeed, *in vitro* studies have previously shown that overexpression of GGA1wt or GGA1DN mutant proteins modulate the cellular localization of BACE1. In this study, we showed that the GGA1DN variant interferes with the general secretory system in CHO cells and, thus, it was excluded from further studies. In previous work, we analyzed the localization of BACE1 after its uptake from the cell surface in HEK293 cells overexpressing either GGA1wt or GGA1DN. GGA1wt expressing cells revealed predominant localization of BACE1 in juxtanuclear TGN compartments, while expression of GGA1DN led to accumulation of endocytosed BACE1 in endosomal compartments [19]. In CHO cells the steady state distribution of BACE1 was unaltered upon overexpression of GGA1wt in both CHOwt and *NPC1*-null cells. The endosomal accumulation of BACE1 in *NPC1*-null cells could not be recovered by overexpression of GGA1wt, nor by induced overexpression of GGA1DN in CHOwt cells (not shown). This finding suggests that the sequestration of BACE1 within endocytic compartments upon NPC1 dysfunction is independent of the GGA1 expression.

Since phosphorylation of BACE1 promotes the interaction with GGA1, we further analyzed whether the phosphorylation status of BACE1 in *NPC1*-null and CHOwt cells affects its subcellular localization. Interestingly, the mutants mimicking phosphorylated or unphosphorylated BACE1 showed very similar cellular distributions, indicating that neither a dysfunction of GGA1 nor altered phosphorylation of BACE1 is responsible for its accumulation within endocytic compartments in *NPC1*-null cells. Even though we have not observed that GGA1wt overexpression modulates the cellular trafficking of BACE1, we detected that it alters the

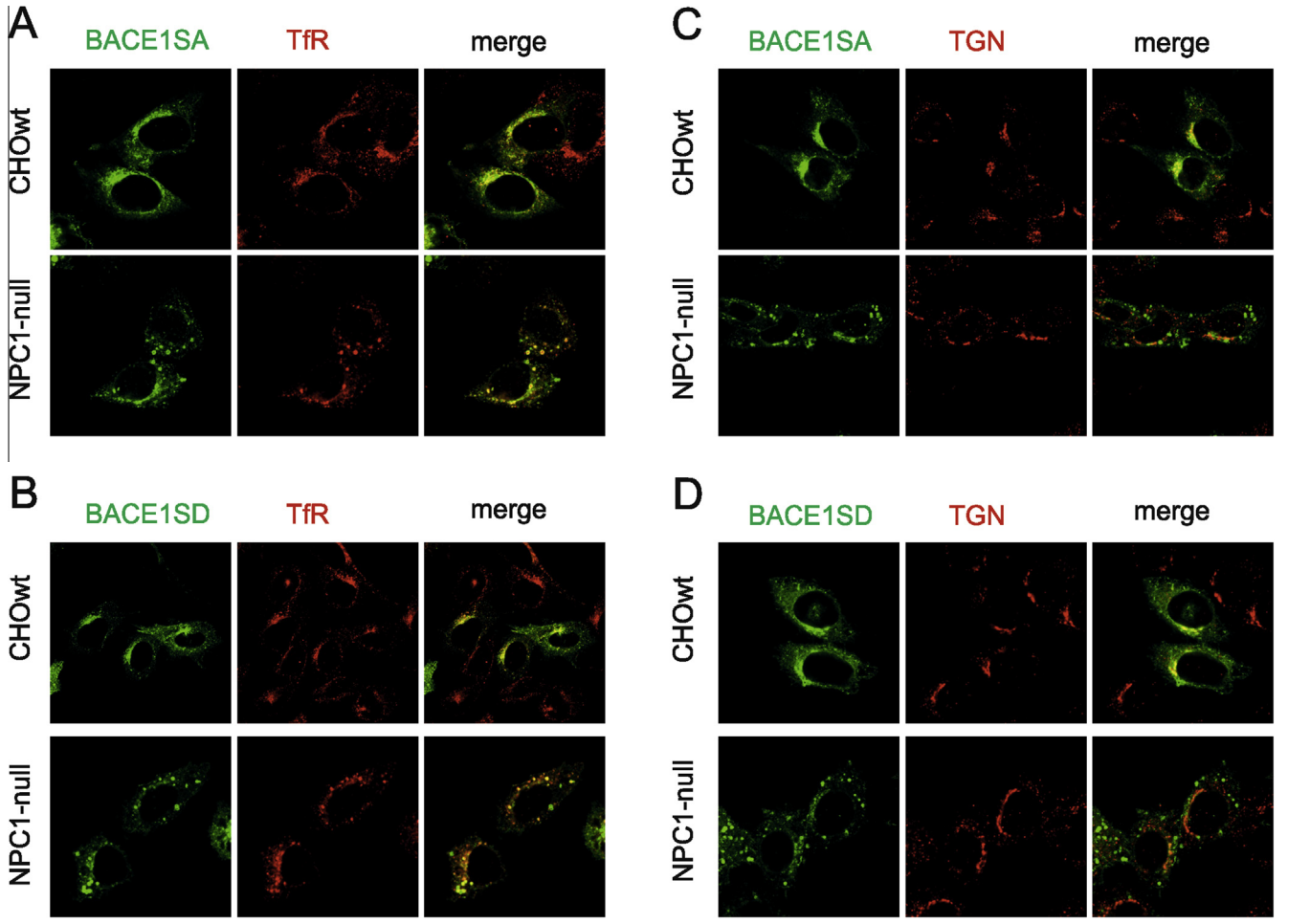

Fig. 3. CHOwt and *NPC1*-null cells show similar localization of BACE1 phosphorylation mutants. CHOwt and *NPC1*-null cells transiently expressing BACE1–GFP S498A (A and C) or S498D (B and D) mutant were stained with anti-Transferrin Receptor antibody (A and B) or anti-Trans Golgi Network antibody (C and D). Cells were visualized under confocal microscope.

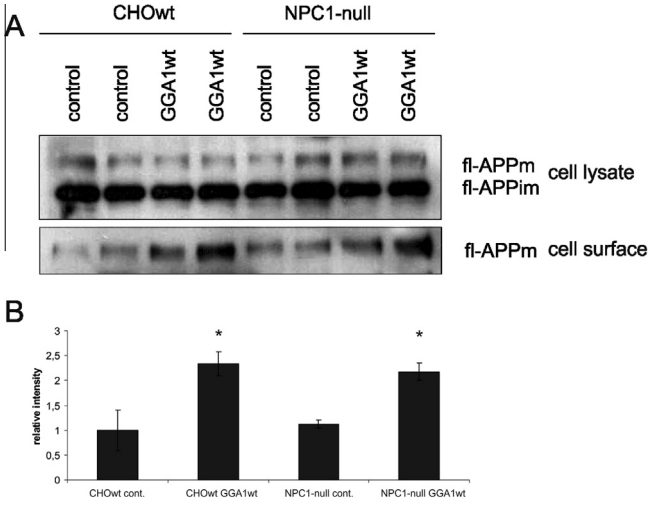

Fig. 4. GGA1wt overexpression increases APP levels at the cell surface. Levels of APP at the cell surface were analyzed by biotinylation assay and western blot using APP N-terminal antibody 22C11 (A). Western blots were quantified using ImageJ software (B). Statistical analysis was performed using Student *t*-test. Error bars show standard deviation: $^{*}p < 0.05$.

expression and processing of APP at the cell surface. Based on these results we proposed that GGA1 may modulate APP cleavage by α- and β-secretase indirectly by regulating the cell surface localization of APP.

The role of GGA1 on APP processing has been addressed previously in HEK293 cells. We found a decreased ratio of CTFβ/CTFα upon overexpression of GGA1, which is consistent with an upregulation of APP cleavage by α-secretase. In addition, the levels of secreted Aβ were decreased [20]. On the other hand, von Arnim and co-workers found a reduction of both sAPPα and sAPPβ levels in HEK293 cells co-expressing APP and GGA1wt [18]. They also showed that GGA1 overexpression reduces APP levels at the cell surface and in the endosomal vesicles, and causes accumulation of APP in TGN. In their experimental system, the observed alterations of APP cellular localization did not lead to changes in intracellular or secreted Aβ levels [18]. The reason of these discrepancies may lie in the used cell line and the fact that we analyzed, and recapitulated our findings in CHOwt cells, in CHO *NPC1*-null cells that have previously shown to favorably cleave APP through the amyloidogenic pathway [13].

In contrast to previous studies, here we used NPC disease cells as a model of an AD-like phenotype to analyze whether GGA1 modulates APP processing and/or APP/BACE1 trafficking. In summary, our findings support a role of GGA1 in APP processing by favoring the non-amyloidogenic *vs.* amyloidogenic pathway. Although the molecular mechanisms underlying the protective effects of GGA1 are still not fully resolved, our studies using the NPC disease model showed that GGA1, cannot retrieve APP and BACE1 from accumulation in endolysosomal compartments.

Instead, GGA1 expression enhanced the localization of APP at the cell surface and thereby could shift APP processing towards non-amyloidogenic pathway further supporting its preventive role against Alzheimer's disease.

Acknowledgments

We would like to thank Dr. Daniel Ory for kindly providing cell lines. In addition, we would like to acknowledge Dr. Christian Haass for his generous gift of antibodies and Dr. Christine von Arnim for sharing her constructs with us. This work was funded by the grant from the Ministry of Science, Education and Sports (MSES) of the Republic of Croatia (to S.H.), a bilateral DAAD-MSES project (to S.H. and J.W.), and a grant from the Deutsche Forschungsgemeinschaft (SFB645, to J.W.).

References

[1] M. Burns, K. Gaynor, V. Olm, M. Mercken, J. LaFrancois, L.L. Wang, P.M. Mathews, W. Noble, Y. Matsuoka, K. Duff, Presenilin redistribution associated with aberrant cholesterol transport enhances beta-amyloid production in vivo, J. Neurosci. 23 (2003) 5645–5649.
[2] S.L. Cole, R. Vassar, The Alzheimer's disease beta-secretase enzyme, BACE1, Mol. Neurodegener. 2 (2007).
[3] D. Edbauer, E. Winkler, J.T. Regula, B. Pesold, H. Steiner, C. Haass, Reconstitution of gamma-secretase activity, Nat. Cell Biol. 5 (2003) 486–488.
[4] R. Fu, N.M. Yanjanin, M.J. Elrick, C. Ware, A.P. Lieberman, F.D. Porter, Apolipoprotein E genotype and neurological disease onset in Niemann-Pick disease, type C1, Am. J. Med. Genet. Part A 158A (2012) 2775–2780.
[5] J. Hardy, D.J. Selkoe, Medicine – The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics, Science 297 (2002) 353–356.
[6] J.T. Huse, D.S. Pijak, G.J. Leslie, V.M. Lee, R.W. Doms, Maturation and endosomal targeting of beta-site amyloid precursor protein-cleaving enzyme. The Alzheimer's disease beta-secretase, J. Biol. Chem. 275 (2000) 33729–33737.
[7] L.W. Jin, I. Maezawa, I. Vincent, T. Bird, Intracellular accumulation of amyloidogenic fragments of amyloid-beta precursor protein in neurons with Niemann-Pick type C defects is associated with endosomal abnormalities, Am. J. Pathol. 164 (2004) 975–985.
[8] W.T. Kimberly, M.J. Lavoie, B.L. Ostaszewski, W.J. Ye, M.S. Wolfe, D.J. Selkoe, Gamma-secretase is a membrane protein complex comprised of presenilin, nicastrin, aph-1, and pen-2, Proc. Natl. Acad. Sci. U.S.A. 100 (2003) 6382–6387.
[9] M. Kosicek, M. Malnar, A. Goate, S. Hecimovic, Cholesterol accumulation in Niemann Pick type C (NPC) model cells causes a shift in APP localization to lipid rafts, Biochem. Biophys. Res. Commun. 393 (2010) 404–409.
[10] S.F. Lichtenthaler, D. Dominguez, G.G. Westmeyer, K. Reiss, C. Haass, P. Saftig, B. De Strooper, B. Seed, The cell adhesion protein P-selectin glycoprotein ligand-1 is a substrate for the aspartyl protease BACE1, J. Biol. Chem. 278 (2003) 48713–48719.
[11] S.F. Lichtenthaler, C. Haass, H. Steiner, Regulated intramembrane proteolysis–lessons from amyloid precursor protein processing, J. Neurochem. 117 (2011) 779–796.
[12] M. Malnar, M. Kosicek, A. Lisica, M. Posavec, A. Krolo, J. Njavro, D. Omerbasic, S. Tahirovic, S. Hecimovic, Cholesterol-depletion corrects APP and BACE1 misstrafficking in NPC1-deficient cells, Biochim. Biophys. Acta-Mol. Basis Dis. 1822 (2012) 1270–1283.
[13] M. Malnar, M. Kosicek, S. Mitterreiter, D. Omerbasic, S.F. Lichtenthaler, A. Goate, S. Hecimovic, Niemann-Pick type C cells show cholesterol dependent decrease of APP expression at the cell surface and its increased processing through the beta-secretase pathway, Biochim. Biophys. Acta-Mol. Basis Dis. 1802 (2010) 682–691.
[14] Y. Saito, K. Suzuki, E. Nanba, T. Yamamoto, K. Ohno, S. Murayama, Niemann-Pick type C disease: accelerated neurofibrillary tangle formation and amyloid beta deposition associated with apolipoprotein E epsilon 4 homozygosity, Ann. Neurol. 52 (2002) 351–355.
[15] N. Takasugi, T. Tomita, I. Hayashi, M. Tsuruoka, M. Niimura, Y. Takahashi, G. Thinakaran, T. Iwatsubo, The role of presenilin cofactors in the gamma-secretase complex, Nature 422 (2003) 438–441.
[16] G. Tesco, Y.H. Koh, E.L. Kang, A.N. Cameron, S. Das, M. Sena-Esteves, M. Hiltunen, S.H. Yang, Z.Y. Zhong, Y. Shen, J.W. Simpkins, R.E. Tanzi, Depletion of GGA3 stabilizes BACE and enhances beta-secretase activity, Neuron 54 (2007) 721–737.
[17] R. Vassar, B.D. Bennett, S. Babu-Khan, S. Kahn, E.A. Mendiaz, P. Denis, D.B. Teplow, S. Ross, P. Amarante, R. Loeloff, Y. Luo, S. Fisher, L. Fuller, S. Edenson, J. Lile, M.A. Jarosinski, A.L. Biere, E. Curran, T. Burgess, J.C. Louis, F. Collins, J. Treanor, G. Rogers, M. Citron, Beta-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE, Science 286 (1999) 735–741.
[18] C.A.F. von Arnim, R. Spoelgen, I.D. Peltan, M. Deng, S. Courchesne, M. Koker, T. Matsui, H. Kowa, S.F. Lichtenthaler, M.C. Irizarry, B.T. Hyman, GGA1 acts as a spatial switch altering amyloid precursor protein trafficking and processing, J. Neurosci. 26 (2006) 9913–9922.
[19] T. Wahle, K. Prager, N. Raffler, C. Haass, M. Famulok, J. Walter, GGA proteins regulate retrograde transport of BACE1 from endosomes to the trans-Golgi network, Mol. Cell. Neurosci. 29 (2005) 453–461.
[20] T. Wahle, D.R. Thal, M. Sastre, A. Rentmeister, N. Bogdanovic, M. Famulok, M.T. Heneka, J. Walter, GGA1 is expressed in the human brain and affects the generation of amyloid beta-peptide, J. Neurosci. 26 (2006) 12838–12846.
[21] T. Yamazaki, T.Y. Chang, C. Haass, Y. Ihara, Accumulation and aggregation of amyloid beta-protein in late endosomes of Niemann-Pick type C cells, J. Biol. Chem. 276 (2001) 4454–4460.