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Modulation of Cholesterol, Farnesylpyrophosphate, and Geranylgeranylpyrophosphate in Neuroblastoma SH-SY5Y-APP695 Cells: Impact on Amyloid Beta-Protein Production

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Abstract There is keen interest in the role of the isoprenoids farnesylpyrophosphate (FPP) and geranylgeranylpyrophosphate (GGPP) in protein prenylation and cell function in Alzheimer's disease (AD). We recently reported elevated FPP and GGPP brain levels and increased gene expression of FPP synthase (FPPS) and GGPP synthase (GGPPS) in the frontal cortex of AD patients. Cholesterol levels and gene expression of 3-hydroxy-3-methylglutaryl-coenzyme A reductase were similar in AD and control samples, suggesting that homeostasis of FPP and GGPP but not cholesterol is specifically targeted in brain tissue of AD patients (Neurobiol Dis 2009 35:251–257). In the present study, it was determined if cellular levels of FPP, GGPP, and cholesterol affect beta-amyloid (Aβ) abundance in SH-SY5Y cells, expressing human APP695. Cells were treated with different inhibitors of the mevalonate/isoprenoid/cholesterol pathway. FPP, GGPP, cholesterol, and $A\beta_{1\text{-}40}$ levels were determined, and activities of farnesyltransferase and geranylgeranyltransferase I were measured. Inhibitors of different branches of the mevalonate/isoprenoid/cholesterol pathway as expected reduced cholesterol and isoprenoid levels in neuroblastoma cells. $A\beta_{1\text{–}40}$ levels were selectively reduced by cholesterol synthesis inhibitors but not by inhibitors of protein isoprenylation, indicating that changes in cholesterol levels per se and not isoprenoid levels account for the observed modifications in Aβ production.

Keyword Cholesterol · Farnesylpyrophosphate · Geranylgeranylpyrophosphate · Beta amyloid · Alzheimer · Isoprenoid · Rho protein · Statin · Cell · Neuroblastoma · Farnesyltransferase · Geranylgeranyltransferase

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Introduction

The mevalonate (MVA) pathway is a crucial metabolic pathway in almost all eukaryotic cells, which converts MVA into cholesterol (Fig. 1) [1]. It is most recognized for the biosynthesis of cholesterol, as shown in Fig. 1, but it also provides the cell with indispensable lipids such as squalene and the isoprenoids farnesylpyrophosphate (FPP) and geranylgeranylpyrophosphate (GGPP). Biosynthesis mainly occurs in the endoplasmic reticulum (ER) but also in peroxisomes [2]. Initial steps of the MVA-isoprenoid pathway involve the synthesis of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) from acetyl-CoA via acetoacetyl-CoA (Fig. 1). Subsequently, activity of HMG-CoA reductase, the rate limiting step in this pathway leads to production of MVA [1]. MVA kinase (MK) then catalyzes the phosphorylation of mevalonic acid to phosphormevalonate. Downstream products including geranylpyrophosphate (GPP) and FPP regulate MK activity by transcriptional and posttranslational mechanisms [3]. The phosphomevalonate kinase catalyzes the reaction of MVA 5-phosphate and adenosine

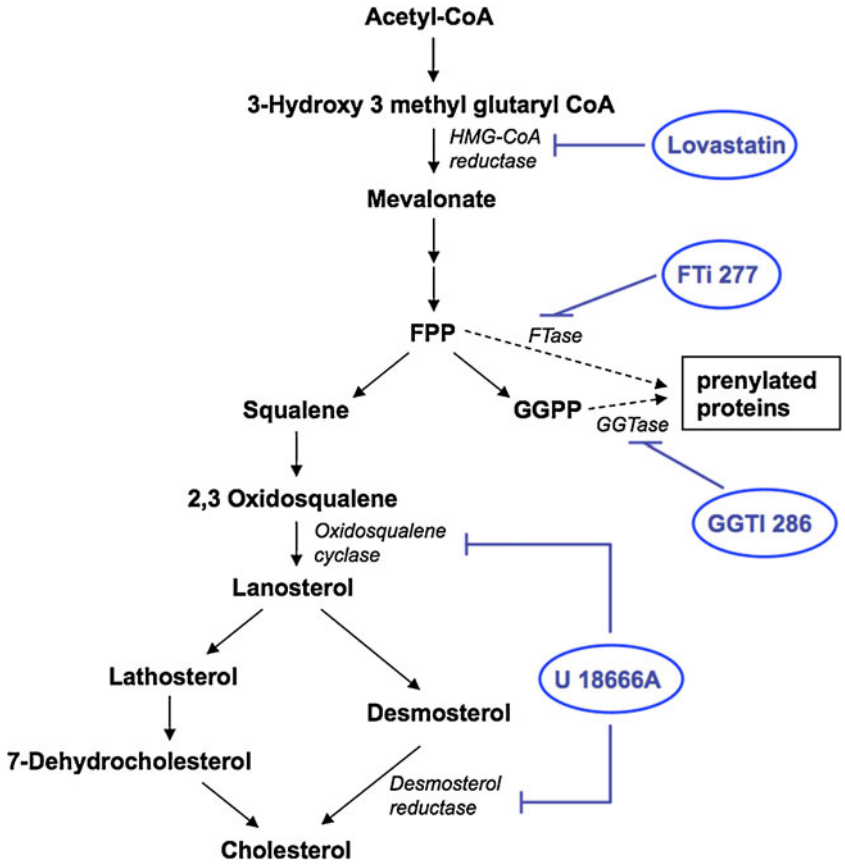

Fig. 1 Abbreviated mevalonate/isoprenoid/cholesterol pathway showing the main intermediates, the employed inhibitors (*in blue*), and their enzyme targets (*in italics*). The rate limiting enzyme HMG-CoA reductase catalyzes the conversion of hydroxymethylglutaryl to MVA, which is further converted to FPP. FPP is the precursor for both, squalene and GGPP. Proteins are prenylated with either FPP or GGPP, and the reaction is catalyzed by FTase and GGTase, respectively. These enzymes are inhibited by specific inhibitors, FTi 277 and GGTi 286, respectively. U 18666A represents an inhibitor of 2,3-oxidosqualene oxidase and desmosterol reductase, two enzymes involved in the biosynthesis of cholesterol

triphosphate to MVA 5-diphosphate, which then is decarboxylated to form isopentenyl pyrophosphate (IPP) and equilibrates with its isomer dimethylallyl pyrophosphate (DMAPP) that is catalyzed by the IPP isomerase. IPP or DMAPP then undergo subsequent condensation reactions to produce a 10-carbon lipid GPP, the 15-carbon FPP, and the 20-carbon GGPP. The synthesis of GPP and FPP is catalyzed by FPP synthase (FPPS), whereas GGPP is synthesized by GGPP (GGPPS) [4]. FPP is the precursor of GGPP and cholesterol. Furthermore, FPP and GGPP are substrates for posttranslational prenylation of the small GTPases by farnesyltransferase (FTase) and geranylgeranyltransferase I (GGTase I). Prenylation of these proteins is required for proper localization in cell membranes in order for the proteins to function properly [5]. FPP and GGPP also serve as precursors of longer chain isoprenoids like dolichol, ubiquinone, and heme A [1].

FPP is the branch point of the pathway leading to squalene catalyzed by the farnesyldiphosphate FTase (FDFT), also known as squalene synthase. Squalene is synthesized by a head-to-head reaction. Subsequent reactions result in the production of lanosterol, which represents the structure of all steroids and production of desmosterol, 7-dehydrocholesterol, and finally cholesterol (Fig. 1).

Pharmacologic manipulation of the MVA pathway by statins has been intensively studied [6], and there is growing evidence that interfering with the biosynthesis of FPP and GGPP may open the field for novel therapeutic indications for Alzheimer's disease (AD) in the future.

The role of FPP and GGPP in protein prenylation and cell function has generated great interest in AD [7]. Recent experimental evidence indicates that isoprenylated small GTPases are involved in AD pathogenesis [8, 9]. A straightforward prediction is that FPP and GGPP levels would be elevated in AD brains as compared with normal neurologic controls. Using a newly developed and validated high performance liquid chromatography with fluorescence detection (HPLC-FD) method [10], we recently determined FPP and GGPP levels and gene expression of their respective synthases in the frontal cortex of AD patients as compared with control samples [11]. This study showed for the first time FPP and GGPP levels in brain tissue of AD patients and normal neurologic controls. GGPP levels were significantly higher in brain tissue of AD patients (56%) as compared with control samples. FPP levels were also significantly higher (36%) in the AD brain tissue (Table 1). In both AD patients and controls, GGPP levels were markedly higher than FPP levels and that finding was consistent with two recent reports in mouse brain and normal human brain [10, 12].

Gene expression of FPPS and GGPPS was determined in AD and control brain samples by quantitative real-time

Table 1 FPP, GGPP, and cholesterol levels in postmortem gray matter tissue isolated from the frontal cortex of male AD patients and normal neurologic controls (data were adapted from Eckert et al [11])

	Control	AD
FPP (pmol/mg protein)	3.22±0.85	4.399±1.32*
GGPP (pmol/mg protein)	7.357±2.12	11.70±4.74*
Cholesterol (nmol/mg protein)	60.08±16.76	63.78±19.06^{ns}

Values are expressed as mean ± SD (n=13).

*$p<0.05$ (*t* test); ns=not significant.

polymerase chain reaction [11]. The increase in FPP levels in the frontal cortex of AD brain was associated with a significant up-regulation of FPPS gene expression. GGPPS mRNA levels were increased, but differences did not reach significance [11].

FPP serves as a precursor of both GGPP and cholesterol (Fig. 1). FPP and GGPP levels were significantly higher in brain tissue of AD patients (Table 1). However, cholesterol levels (Table 1) and gene expression of HMGR were similar in AD and control samples [11]. Taken together, one conclusion drawn from these results is that homeostasis of FPP and GGPP but not cholesterol is specifically targeted in brain tissue of AD patients [11].

Evidence indicates that FPP and GGPP are involved in the cellular production of Aβ which is thought to be one of the prime causes of neuropathology in AD. Rab-6 is increased in AD brain, and membrane association of this geranylgeranylated protein is dependent on presenilin 1 (PS-1) [9, 13]. PS-1 represents an important physiological facilitator of γ-secretase activity, which promotes Aβ production [13–15]. Action of γ-secretase is stimulated by addition of geranylgeraniol to SH-SY5Y cells [16]. Incubation of H4 neuroglioma cells, expressing the Alzheimer relevant amyloid precursor protein APP695NL with FPP or GGPP increased Aβ levels [17]. The present study determined if changes in endogenous levels of FPP, GGPP, and cholesterol alter Aβ production in human neuroblastoma SH-SY5Y cells expressing APP695 (SH-SY5Y-APP695).

Materials and Methods

Chemicals and Reagents

FTase and GGTase I were obtained from Jena Bioscience (Jena, Germany) and D*-GCVLS (dansyl gly-cys-val-leu-ser) and D*-GCVLL (dansyl gly-cys-val-leu-leu) from Calbiochem (Darmstadt, Germany). NH_4OH solution (28%–30%) was purchased from Alfa Aesar (Karlsruhe, Germany), the phosphatase inhibitors Halt® from Fisher/Piercnet (Bonn, Germany), and Phosstop® as well as the Complete® proteases inhibitor cocktail tablets from Thermo and Roche Diagnostics GmbH (Mannheim, Germany). All chromatography solvents were of analytical grade or higher quality. Acetonitrile was obtained from Carl Roth GmbH (Karlsruhe, Germany), 1-butanol, ethanol, ammonium acetate, and assay buffer compounds: Tris-HCl, $MgCl_2$, $ZnCl_2$, and Na_2CO_3 were obtained from Merck (Darmstadt, Germany). FPP, GGPP, octyl-β-D-glucopyranoside, and dithiothreitol were from Sigma-Aldrich (Schnelldorf, Germany). Millipore water was used for all solutions (Schwalbach, Germany). The inhibitors U 18666A, lovastatin, FTi 277, and GGTi 286 were from Calbiochem. Phenylmethylsulfonylfluorid (PMSF) was from Sigma-Aldrich (Schnelldorf, Germany). Unless otherwise stated, all cell culture reagents were obtained from Gibco/Invitrogen (Karlruhe, Germany).

Cell Culture

SH-SY5Y cells were stably transfected using the mammalian expression vector pCEP4 harboring the APP695wt construct into SH-SY5Y (cells were a kind gift from Dr. Tobias Hartmann, University Homburg, Germany). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) at 37°C and 5% CO_2. The medium was supplemented with 1% of glutamine, MEM-vitamins, pyruvate, and nonessential amino acids plus 10% fetal calf serum from (Sigma-Aldrich, Schnelldorf, Germany). Hygromycin B (3 μg/ml) was used as a selection antibiotic. For incubation with MVA-pathway inhibitors, cells were kept in serum-free OptiMEM medium supplemented only with hygromycin B to ensure the cell dependence on endogenous cholesterol synthesis. Effects of 5 μM lovastatin (dissolved in ethanol), 1 μM U 18666A (dissolved in sterile water), 1 μM FTi 277, and 1 μM GGTi 286 (dissolved in dimethyl sulfoxide) were investigated. Cells were incubated for 24 hours. Media were collected and supplemented with PMSF (1 μM). Cells were centrifuged and washed twice with phosphate-buffered saline (PBS). Pellets were resuspended and homogenized in PBS containing Complete® protease-inhibitor cocktail and the two phosphatase inhibitors Halt® and Phosstop®.

Isoprenoid Extraction from SY5Y Cells

Following protein determination of the respective cell homogenate samples, 600 μg of total protein was transferred into a reaction vial. The following extraction of FPP and GGPP from cell homogenate samples was conducted according to the protocol described by Tong et al. [18], with the following modifications: all reaction vials for analysis contained the same amount of protein in the same volume of PBS buffer. After the extraction procedure, the solutions were dried under reduced pressure. For precolumn dansyl-

labeling, the dried residue was dissolved in 44 µl Tris-HCl assay buffer [18] and spiked with 2 µl of a 50-µM solution of D*-GCVLS and D*-GCVLL (dansyl-labeled peptides) and 250 ng FTase and GGTase I, respectively. The mixture was incubated at 37°C in an Eppendorf thermomixer comfort (Wesseling-Berzdorf, Germany) programmed for 90 minutes (per minute: 5 seconds; 500 rpm). After stopping the reaction, the mixture was centrifuged (4°C; 15,000×*g*; 5 minutes) before HPLC-FD analysis.

HPLC Analysis

The chromatographic separation was carried out on a Jasco HPLC-system (Gross-Umstadt, Germany) with a gradient elution on an Ascentis® Express C-18 reversed-phase analytical column from Supelco (100×2.1 mm, 2.7 µm; Munich, Germany) protected by a Phenomenex Security guard column (C-18, 4×2.0 mm; Aschaffenburg, Germany). Two solvents were used for gradient elution: solvent A, 20 mM ammonium acetate in 40% acetonitrile, and solvent B, 20 mM ammonium acetate in 90% acetonitrile. The gradient was initiated at 35% solvent B for 1.5 minutes, subsequently ramped linearly to 100% within 6.5 minutes, kept for 6 minutes, and then brought back to 35% solvent B within 2 minutes. The total run time was 20 minutes with a constant flow rate of 0.5 ml/min at 30°C. The labeled analytes were monitored by a Gilson fluorescence detector (Middleton, WI, USA) set at an excitation wavelength of 340 nm and emission wavelength of 525 nm. The retention times for the labeled FPP and GGPP were 4.1 and 11.0 minutes, respectively.

Protein and Cholesterol Assays

Protein concentrations were measured using the BCA Protein Assay Kit from Thermo-Fisher/Pierce. Samples were measured in triplicates. Total cholesterol levels were determined enzymatically, using the CHOD-PAP method from Roche Diagnostics GmbH.

GGTase and FTase Activity Measurement

Transferase inhibition after treatment with FTi 277 and GGTi 286 was detected individually by means of fluorescence spectroscopy. Dansyl-GCVLL and dansyl-GCVLS were used as fluorescence dyes leading to shifts in the fluorescence maximum after prenylation. Following incubation, cells were sonified and centrifuged at 12,000×*g* for 30 minutes. The supernatant was then centrifuged for another 60 minutes at 30,000×*g*. All steps were performed at 4°C. All samples were adjusted to the same protein concentration. 500 µl of each sample were heated to 30°C and mixed with 10 µl of octyl-β-D-glucopyranosid. After addition of 5 µl of the respective dansyl labels and 2 µl FPP and GGPP, respectively, time-dependent measurements were performed with an excitation wavelength of 360 nm and an emission wavelength of 500 nm.

Aβ-levels

Secreted $A\beta_{(1–40)}$ and $A\beta_{(1–42)}$ levels were determined using enzyme-linked immunosorbent assay (ELISA) kits (KHB3481 and 3544) from Invitrogen. The medium was collected after incubation and supplemented with PMSF (1 µM). The ELISA was conducted according to the manufacture's instructions.

Cytotoxicity

Cytotoxicity was determined using the nondestructive ToxiLight® BioAssay Kit from Lonza (Rockland, ME). Cells were seated in a 96-well plate over night and incubated with different inhibitors as described above. The assay was conducted according to the manufacture's instructions. Inhibitors used in this study were not cytotoxic at any concentration investigated (data not shown).

Statistics

All experiments were done in triplicate and repeated a minimum of three times. Statistical analysis was performed using Student's *t* test. All calculations were performed with GraphPad Prism version 5.00 for Mac, GraphPad software (San Diego, CA).

Results

Despite their fundamental roles in cellular functions [19], FPP and GGPP levels have only been reported for a few peripheral cell lines [12, 18, 20, 21]. This study shows for the first time FPP and GGPP levels in neuronal cells that express picomolar levels of $A\beta_{1–40}$ (Table 2). $A\beta_{1–42}$ levels

Table 2 Endogenous concentrations of FPP, GGPP, cholesterol, and Aβ measured in SY5Y-APP695 control cells

	SY5Y-APP695 cells
FPP (pmol/mg protein)	17.2±1.2
GGPP (pmol/mg protein)	13.4±1.3
Cholesterol (nmol/ mg protein)	381.4±8.5
$A\beta_{1–40}$ (pg/mg protein)	86.8±10.9
$A\beta_{1–42}$ (pg/ml)	<1.56

Values are expressed as mean ± SD (n=9).

were below the detection limit (Table 2). FPP and GGPP levels were determined in SH-SY5Y-APP695 cells by HPLC-FD, and a representative chromatogram is shown in Fig. 2. Table 2 demonstrates that GGPP levels were significantly lower (22%) than FPP levels in SH-SY5Y-APP695 cells. Cellular concentrations of FPP and GGPP are in the picomolar range, whereas cellular cholesterol levels are in the nanomolar range (Table 2).

To establish if FPP and GGPP regulation is more susceptible to perturbation than cholesterol, we determined FPP, GGPP, and cholesterol levels in SH-SY5Y-APP695 cells treated with the HMG-CoA reductase inhibitor lovastatin. It has been previously shown that statin treatment reduces FPP and GGPP levels in human multiple myeloma and mouse embryonic fibroblast cells [18, 20], but such data have not been reported for neuronal cells. Data in Fig. 3a show that inhibition of HMRG significantly reduced FPP and GGPP by 62% and 96% in SH-SY5Y-APP695 cells, respectively. Furthermore, cholesterol levels were also significantly reduced but to a lower extent (41%) as shown in Fig. 3b. $A\beta_{1-40}$ levels were significantly reduced by 38% (Fig. 3c).

Data in Fig. 4a show that inhibition of oxidosqualene cylase significantly enhanced FPP levels (57%) and reduced GGPP levels (20%). Cholesterol levels were also significantly reduced by 26% as shown in Fig. 4b. Aβ levels were significantly reduced by 22% (Fig. 4c).

Since reduction of Aβ levels was accompanied by reductions in FPP, GGPP, and cholesterol levels (Figs. 3 and 4), we next examined the role of protein prenylation on cellular Aβ production. SH-SY5Y-APP695 cells were

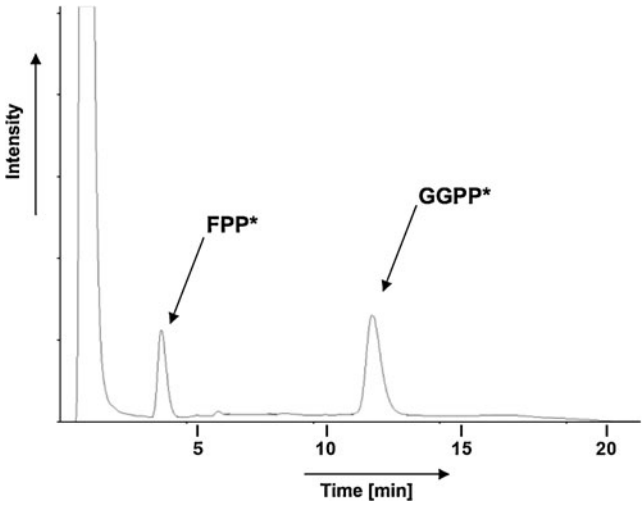

Fig. 2 An exemplified HPLC fluorescence chromatogram showing the dansyl-labeled FPP (FPP*) and GGPP (GGPP*) detected in SY5Y-APP695 cell matrix. Details of the chromatic settings are described in "Materials and methods."

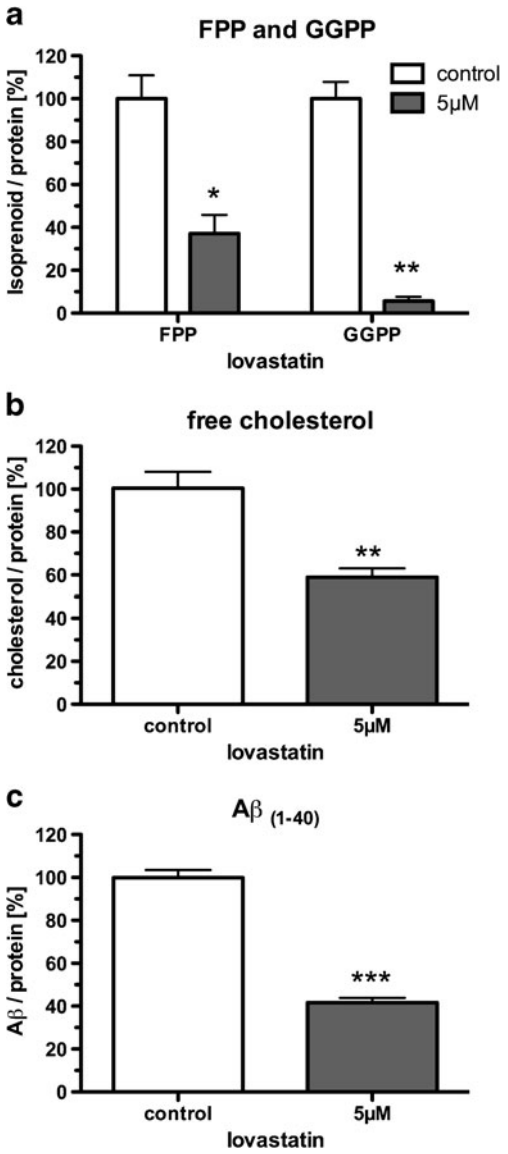

Fig. 3 Relative changes in FPP, GGPP (**a**), cholesterol (**b**), and $A\beta_{1-40}$ (**c**) levels in SY5Y-APP695 cells after incubation with lovastatin (5 μM) for 24 hours. Data are shown as means±SEM, $*p<0.05$ and $**p<0.01$; $n=6$ for each group

incubated with transferase inhibitors, which block protein prenylation. Figure 5a shows that FTi 277 inhibited FTase activity. This reduction in activity was associated with a small reduction in FPP levels and a slight increase in GGPP levels, but those differences were not significant compared with controls (Fig. 5b). Cholesterol levels (Fig. 5c) and Aβ levels (Fig. 5d) were not altered by FTi 277 treatment. Inhibition of GGTase I using GGTi 286 (Fig. 6a) resulted in a small, but significant reduction in FPP levels, but GGPP levels were not significantly changed (Fig. 6b). Cholesterol and Aβ levels were not altered by incubation with GGTi 286 (Fig. 6c, d).

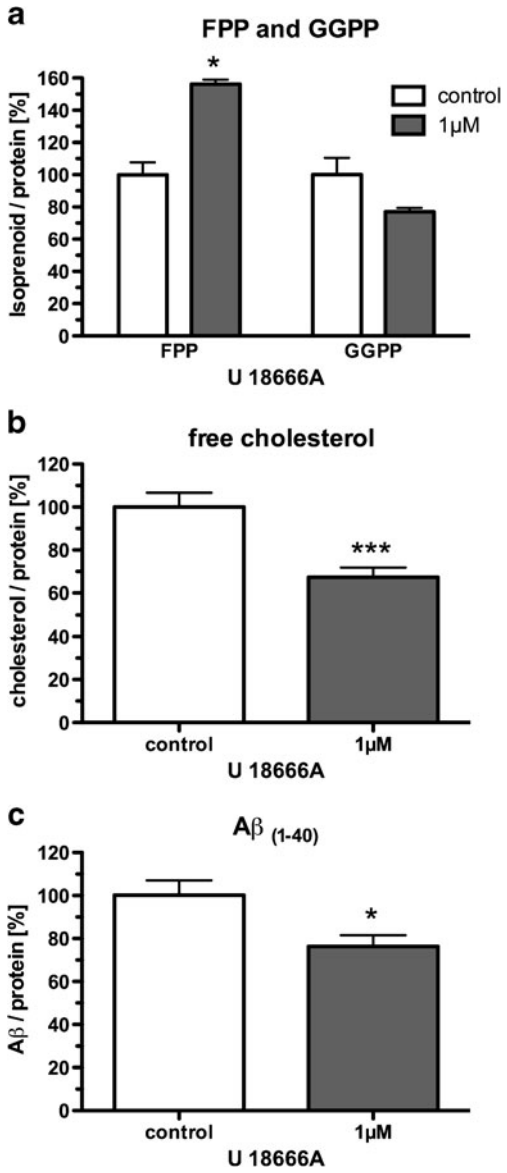

Fig. 4 Relative changes in FPP, GGPP (**a**), cholesterol (**b**), and $A\beta_{1-40}$ (**c**) levels in SY5Y-APP695 cells after incubation with U 18666A (1 μM) for 24 hours. Data are shown as means±SEM, *$p<0.05$ and ***$p<0.001$; $n=6$ for each group

Discussion

Posttranslational modification of proteins with a C-terminal CaaX motif by FPP and GGPP is critical for enabling proteins to be inserted into membranes, thus determining their localization and function [19]. Prenylated proteins include the subunits of trimeric G proteins, protein kinases, and more than 150 members of the Ras GTPase superfamily [22]. These proteins are integral components of complex signaling networks and control diverse cellular activities including intracellular vesicle transport, cell adhesion, endocytosis, cytoskeletal organization, receptor signaling, cell cycle progression, and gene expression [22]. Despite those critical roles in a wide array of fundamental cell processes, our knowledge on the cellular regulation of the two isoprenoids FPP and GGPP is limited. Preventing advancement was mainly due to analytical difficulties concerning a robust isolation technique and a sensitive detection method. Recent analytical progress in detecting FPP and GGPP now allows investigations of isoprenoid regulation *in vitro* and *in vivo* [10, 18, 23].

Tong et al. [24] described the first method for the simultaneous determination of FPP and GGPP in cultured cells. Up to now, FPP and GGPP were determined in mouse embryonic fibroblasts and human cancer cells such as immortalized colorectal adenocarcinoma, myelogenous leukemia, and multiple myeloma cells (Table 4) [12, 18, 20, 21]. The current work describes for the first time levels of FPP and GGPP in immortalized SH-SY5Y neuroblastoma cells. Cellular levels of FPP and GGPP are generally in the picomolar range as depicted in Table 4; however, significant differences in isoprenoid abundance can be observed between different cell lines (Table 4). Moreover, the relative distribution between FPP and GGPP varies between different cell lines. We report that GGPP levels are 22% lower, as compared with FPP in SH-SY5Y-APP695 cells. Lower levels of GGPP have also been reported for U266 cells (−59%). However, in other cell lines, GGPP levels are equal or higher as compared with FPP (Table 4). Such variances were also observed in mammalian tissue. Compared with FPP, GGPP levels are lower in mouse kidney and liver tissue, equal in mouse heart tissue, and higher in mouse and human brain tissue [10–12]. It can be speculated that depending on the demand for intermediates in the MVA pathway, specialized cells may differentially regulate FPP and GGPP production.

The current work shows that inhibition of HMG-CoA reductase by lovastatin significantly reduced FPP and GGPP levels by 62% and 96%, respectively, in SH-SY5Y-APP695 cells. Our data are in line with recent findings, which demonstrated a statin-induced reduction in FPP (69%) and GGPP levels (77%) in NIH3T3 cells [18]. Moreover, lovastatin treatment of human multiple myeloma cells and Caco-2 cells also significantly reduced both, FPP and GGPP- levels [20, 21]. Inhibition of HMGR in vivo also impaired the production of FPP and GGPP. Treatment of mice with simvastatin reduced brain levels of FFPP and GGPP by 41% and 32%, respectively [11].

It is well established that the reduction of cellular cholesterol levels using inhibitors of the MVA pathway or extraction by methyl-β-cyclodextrin (MβCD) results in lower Aβ levels in vivo and in vitro (reviewed in Refs [24–27]). However, evidence exists that FPP and GGPP are also involved in the cellular production of Aβ. It has been

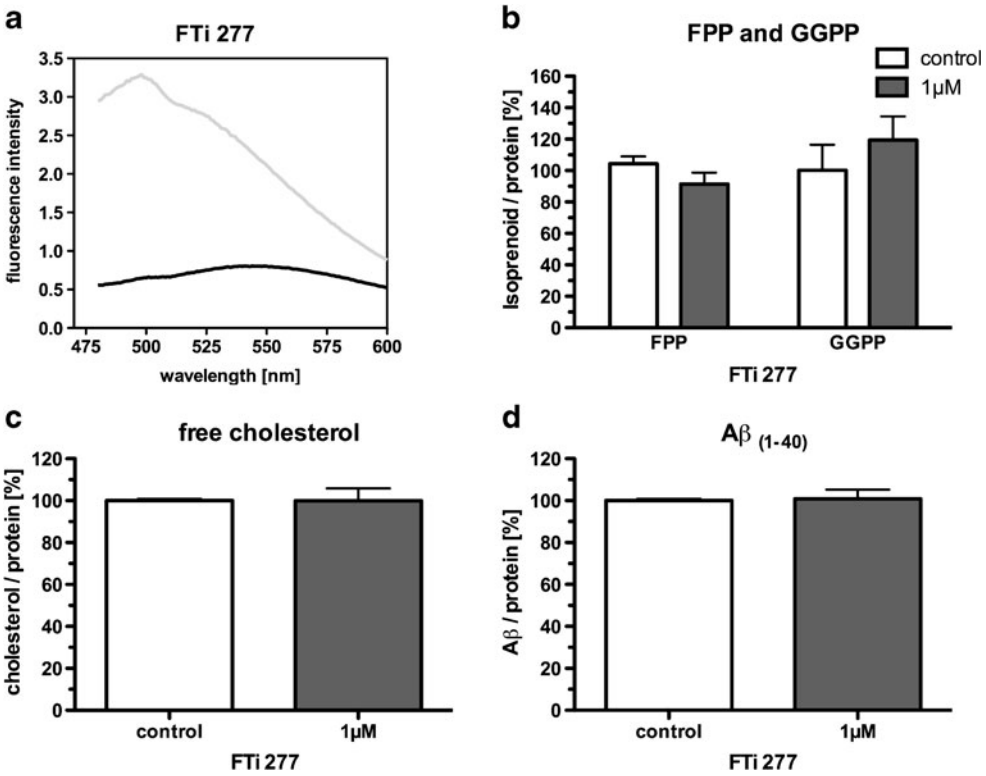

Fig. 5 **a** Changes in fluorescence activity and in the fluorescence peak after incubation of SY5Y-APP695 cells with FTi 277 (1 μM; *gray line*) compared with control SY5Y-APP695 cells (*black line*). Relative changes in FPP, GGPP (**b**), cholesterol (**c**), and $A\beta_{1-40}$ (**d**) levels in SY5Y-APP695 cells after incubation with FTi 277 (1 μM) treatment for 24 hours. Data are shown as means±SEM; n=4–6 for each group

previously reported that γ-secretase is stimulated by geranylgeraniol [16], and incubation of H4 neuroglioma cells with FPP or GGPP was found to increase Aβ levels [17]. In the aforementioned studies, APP processing was modified after incubation of cells with 10 μM geranylgeraniol, FPP, or GGPP, whereas we determined endogenous FPP and GGPP levels in the picomolar range. The data presented in the current work demonstrate that drug-

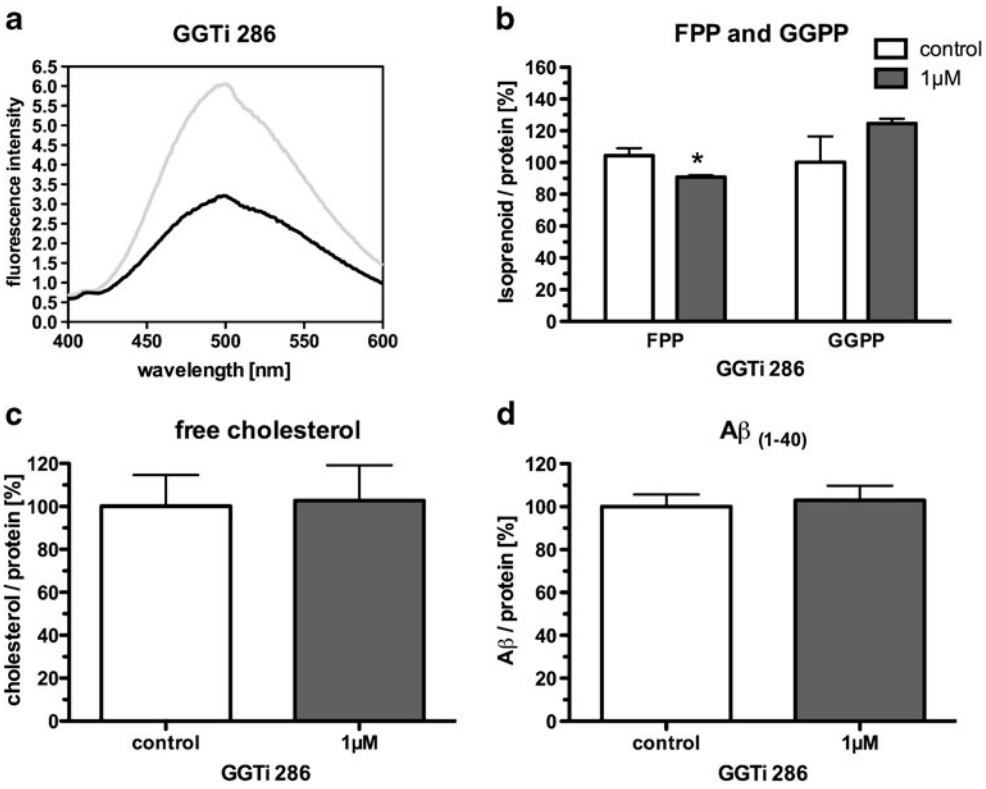

Fig. 6 **a** Changes in fluorescence activity after incubation of SY5Y-APP695 cells with GGTi 286 (1 μM; *gray line*) compared with control SY5Y-APP695 cells (*black line*). Relative changes in FPP, GGPP (**b**), cholesterol (**c**), and $A\beta_{1-40}$ (**d**) levels in SY5Y-APP695 cells after incubation with GGTi 286 (1 μM) treatment for 24 hours. Data are shown as means±SEM, *p<0.05; n=4–6 for each group

Table 3 Effect table summarizing the effects of the different inhibitors of the mevalonate/isoprenoid/cholesterol pathway

	Cholesterol	FPP	GGPP	FPP-transfer	GGPP-transfer	Aβ
Lovastatin	⇓	⇓	⇓	-	-	⇓
U 18666A	⇓	⇑	⇔	-	-	⇓
FTi 277	⇔	⇔	⇔	⇓	⇔	⇔
GGTi 286	⇔	⇓	⇔	⇔	⇓	⇔

Individual symbols: ⇧, significantly elevated levels; ⇩, significantly reduced levels; ⇔, unchanged levels; –, not measured.

induced, specific inhibition on various levels of the MVA/isoprenoid/cholesterol pathway resulted in distinct changes in endogenous FPP, GGPP, and cholesterol levels in human neuroblastoma cells. Aβ levels were specifically reduced when cholesterol levels were lowered by inhibitors of cholesterol synthesis, whereas selective inhibition of either farnesylation or geranylgeranylation did not affect Aβ production (Table 3). The findings of the present study indicate that changes in endogenous cholesterol levels rather than in FPP and GGPP levels account for the observed modifications in Aβ production. It is well established that APP processing is influenced by cholesterol abundance [28–31].

If FPP and GGPP do not affect Aβ metabolism, the question arises as to how elevated levels of FPP and GGPP contribute to AD progression [11]. The Rho family of GTPases (e.g., RhoA, Rac1, and Cdc42 monomeric G-proteins) is the major regulator in synaptic plasticity, both in dendrite morphogenesis and stability as well as in growth cone motility [32–36]. Moreover, a small G protein Rac-dependent forgetting mechanism, which contributes to both passive memory decay and interference-induced forgetting, was discovered in *Drosophila* recently, and it was suggested that Rac's role in actin cytoskeleton remodeling may contribute to memory erasure [37]. Synaptic degeneration is a striking characteristic of AD, and there is evidence that certain small GTPases are associated with AD [9, 38–40]. Rac/Cdc42 protein levels were increased in hippocampal membrane fractions and abnormal distributed in hippocampal slices isolated from AD brain [40]. The antibody used in that study detects endogenous levels of Rac1/cdc42 only when phosphorylated at serine 71. Phosphorylation at this site may inhibit GTP binding of Rac1, attenuating the signal transduction pathway downstream of Rac1 [41]. Rab-6, which is involved in the cellular regulation of vesicular transport, is increased in AD brain [9, 13, 22]. These proteins cannot function properly unless prenylated by FPP or GGPP, and we are proposing that FPP and GGPP are up-regulated in AD resulting in a surplus of prenylated proteins, which may contribute to synaptic dysfunction [11]. AD elevated abundance of prenylated Rac1, for example, could possibly stimulate Rac1-NADPH oxidase-regulated generation of reactive oxygen species, which may contribute to oxidative stress in AD brain [42, 43].

Conclusion

Our data show that modulation of the MVA/isoprenoid/cholesterol pathway results in changes of FPP, GGPP, and cholesterol levels in human neuroblastoma cells. Aβ levels were selectively reduced by early-stage and late-stage inhibitors of cholesterol synthesis. Inhibition of isoprenylation did not affect Aβ metabolism, indicating that changes in cholesterol levels rather than in FPP and GGPP levels accounted for the effects on Aβ production.

Table 4 Leves of FPP and GGPP in different cell lines

Cell line	FPP	GGPP	Unit	Reference
NIH3T3	0.125±0.010	0.145±0.008	pmol/10^{-6} cells	Tong et al. [18]
NIH3T3	0.131±0.008	0.133±0.003	nmol/g wt	Tong et al. [12]
K562	0.112±0.008	0.238±0.003	nmol/g wt	Tong et al. [12]
Caco-2	0.65±0.02	–	pmol/well	Murthy et al. [21]
RPMI-8226	0.19±0.001	0.29±0.02	pmol/10^{-6} cells	Holstein et al. [20]
H929	0.16±0.001	0.16±0.02	pmol/10^{-6} cells	Holstein et al. [20]
U266	1.4±0.001	0.58±0.03	pmol/10^{-6} cells	Holstein et al. [20]
SY5Y-APP695	17.2±1.2	13.4±1.3	pmol/mg protein	

FPP and GGPP levels were determined using a fluorescence HPLC method [18] in mouse embryonic fibroblast cells (NIH3T3), in human immortalized myelogenous leukemia cells (K562), in human immortalized colorectal adenocarcinoma cells (Caco-2), in human multiple myeloma cells (RPMI-8226, H929, U266), and in human neuroblastoma cells (SH-SY5Y-APP695). Values are expressed as means±SD.

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