Expression of LXR target genes decreases cellular amyloid β peptide secretion

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Running title: LXR activation decreases Aβ
**Abbreviations:** AD, Alzheimer's disease; Aβ, amyloid β peptide; APP, amyloid precursor protein; LXR, liver X receptor; ABCA1, ATP binding cassette transporter A1; apo, apolipoprotein; SCD, stearoyl CoA desaturase; RXR, retinoid X receptor; cAPP, cellular APP; C99, 99-residue COOH-terminal fragment of APP; 9-cis RA, 9-cis retioic acid; 22(R)OHchol, 22(R)hydroxycholesterol; TO, TO901317; LPDS, lipoprotein deficient serum; IP, immunoprecipitation; ACAT, acylCoA:cholesterol acyl transferase; mk, mock.
SUMMARY

A hallmark of Alzheimer’s Disease (AD) is the deposition of plaques of amyloid β peptide (Aβ) in the brain. Aβ is thought to be formed from the amyloid precursor protein (APP) in cholesterol-enriched membrane rafts and cellular cholesterol depletion decreases Aβ formation. The liver X receptors (LXR) play a key role in regulating genes that control cellular cholesterol efflux and membrane composition, and are widely expressed in cells of the central nervous system. We show that treatment of APP-expressing cells with LXR activators reduces the formation of Aβ. LXR activation resulted in increased levels of the ATP binding cassette transporter A1 (ABCA1) and stearoyl CoA desaturase (SCD), and expression of these genes individually decreased formation of Aβ. Expression of ABCA1 led to both decreased β-cleavage product of APPsw (i.e. C99 peptide) and reduced γ-secretase-cleavage of C99 peptide. Remarkably, these effects of ABCA1 on APP processing were independent of cellular lipid efflux. LXR and ABCA1-induced changes in membrane lipid organization have favorable effects on processing of APP, suggesting a new approach to the treatment of AD.
Introduction

The deposition of plaques of amyloid β-peptide (Aβ) in the brain is the hallmark of Alzheimer’s Disease (AD). The generation of Aβ requires sequential cleavage of the type I integral membrane amyloid precursor protein (APP) by β- then γ-secretase. β-secretase cleaves APP extracellularly leaving a 99-residue COOH-terminal fragment (C99) that remains membrane-bound. γ-secretase then mediates an intramembranous cleavage, yielding the Aβ peptide. (For review, see (1,2).) An alternative initial cleavage of APP by α-secretase precludes subsequent Aβ formation. Simons and co-workers have proposed that the initial cleavage of APP by β-secretase occurs in cholesterol-rich liquid ordered domains of the plasma membrane, known as ‘rafts’ (3). The basis of this hypothesis was the finding that depletion of cellular cholesterol by cyclodextrin treatment decreases raft formation and markedly reduces Aβ formation (4). Interestingly, presenilins, important components of γ-secretase, have also been found in cholesterol-rich domains (5).

Cellular lipid homeostasis is controlled by SREBPs, transcription factors regulating cholesterol and fatty acid synthesis pathways, and by liver X receptors (LXRs), oxysterol-activated nuclear receptors that induce a battery of genes involved in cellular lipid efflux and transport (6,7). The two forms of LXR, α and β, are both expressed in the brain. LXRβ is broadly expressed in the developing and adult brain and is present in both neurons and glial cells (8). Recent studies show an essential role of LXRs in brain structure and function as aging LXRα/β KO mice develop cellular lipid inclusions, abnormalities of the choroid plexus and closure of the lateral ventricles (8). Although this
pathology is different to that of AD, LXR{\textregistered}s could potentially have a role in modulating the course of chronic neurodegenerative diseases.

An important target of LXR{\textregistered}s is the ATP binding cassette transporter A1 (ABCA1) (9). ABCA1, the defective molecule in Tangier Disease, mediates efflux of cellular phospholipids and cholesterol to lipid poor apolipoprotein, including apolipoproteinA-I (apoA-I) and apoE that are present in the cerebrospinal fluid (10). Treatment of mice with LXR agonists resulted in increased expression of LXR target genes in the brain, especially ABCA1 (8), and LXR activation induces lipid efflux from glial cells (11). These observations led us to hypothesize that activation of LXR{\textregistered}s in neurons might induce ABCA1, resulting in cholesterol efflux and reduced secretion of Aβ.
Materials and Methods

Cell culture -- Mouse neuroblastoma Neuro2a cells stably expressing Swedish APP695 were previously described (12). Neuro2a-APPSw cells were grown in Dulbecco's modified Eagle's medium (DMEM)/OptiMEM supplemented with 5% fetal bovine serum at 37° C in a humidified 5% CO₂ incubator. Tissue culture reagents were from Invitrogen. Transient and stable transfections were performed with Lipofectamine 2000 (Invitrogen). 22(R)-hydroxycholesterol (22(R)-OHchol) and the synthetic LXR activator, TO-901317 were purchased from Sigma (MO). 9-cis Retinoic Acid (9-cis RA) was from Biomol (PA), apoA-I from Biodesign (ME) and apoE isoforms from Calbiochem.

Immunoblots -- Cells were lysed in buffer (10 mM Tris PH7.5, 150 mM NaCl, 1% Triton-X100, 0.25% NP-40, and 2 mM EDTA), supplemented with protease inhibitor cocktail (Roche). Postnuclear lysates were collected by spinning the lysed cells at 8000Xg for 5 minutes. Postnuclear lysates were fractionated in 4-15% SDS-polyacrylamide gel electrophoresis, and transferred to 0.2-um nitrocellulose membranes (Bio-Rad). Polyclonal anti-ABCA1 antibody was purchased from Novus (Littleton, CO). Monoclonal anti-actin antibody was purchased from Sigma (MO). Polyclonal anti-SCD antibodies were raised in rabbits (13). Cellular APP was detected by monoclonal antibody 22C11 (Chemicon).

Detection of secreted Aβ -- In experiments with LXR activation, cells were first induced with activators for 24 hours in DMEM/5% lipoprotein deficient serum (LPDS). The cells were then incubated in fresh medium for 4 hours for Aβ measurements. In experiments
with ABCA1 transient transfections, 24 hours after transfection, and the cells were incubated with DMEM/1% LPDS with or without apolipoproteins for 6 h for Aβ measurements. After indicated treatments, conditioned medium was collected on ice and centrifuged at 6000Xg for 10 minutes to remove cell debris. Immunoprecipitation (IP) of Aβ or C99 was performed with monoclonal antibody 4G8 (Signet, MA) and protein A/G conjugated agarose (SantaCruz, CA). Aβ and C99 were then extracted in NuPAGE sample buffer (Invitrogen) and fractionated in 4-12% NuPAGE Bis-Tris Gel (Invitrogen). Fractionated proteins were then transferred to PVDF membrane (Bio-Rad), and blotted with monoclonal antibody 6E10 (Signet) after boiling. Immunoblots were developed using the ECL system (Pierce) scanned and quantified by ImageQuant (Molecular dynamics). Quantitation of Aβ40 and Aβ42 was performed using a commercial Aβ ELISA kit (Biosource).

Cellular cholesterol efflux -- Efflux experiments were performed as described (13). Briefly, Neuro2a cells were labeled with 1 uCi/ml [1,2-3H(N)]-cholesterol (PerkinElmer Life Science) in DMEM containing 5 mM methyl-β-cyclodextrin:cholesterol at a molar ratio of 8:1 for 15 minutes at 37°C. After washing, the cells were equilibrated in DMEM/0.2%BSA for 30 minutes and then used for efflux experiments. The cells were incubated with 10 ug/ml purified human apoA-I or apoEs in DMEM/1% LPDS for 6 hours, medium was collected, centrifuged at 6000Xg for 10 minutes to remove cell debris and cholesterol crystals. The cells were lysed in 0.1M sodium hydroxide, 0.1% SDS and radioactivity was determined by liquid scintillation counting. Efflux was expressed as the
percentage of radioactivity in the medium relative to the total radioactivity in cells and medium.

Statistical Analysis -- The significance of differences between groups was assessed by the Student's $t$ test.
Results

To evaluate the effects of LXR activators on APP processing, we employed neuron-derived Neuro2a cells, stably transfected with human APP\textsubscript{Sw} containing a mutation that increases the formation of total Aβ (the Swedish mutation)\cite{14}. Cells were treated with increasing doses of the synthetic LXR\textsubscript{α/β} activator TO901317 (TO) and Aβ secretion into medium was measured by immunoprecipitation and Western blotting (Fig 1A). This revealed a decrease in the secretion of Aβ, with an approximate 50% reduction at 1 uM TO. The amount of the soluble form of APP (APP\textsubscript{sα}) in medium was not significantly changed by administration of the LXR activator (not shown). LXR acts in a heterodimeric complex with retinoid X receptor (RXR) and the response of genes to LXR activators is increased in the presence of RXR activators, such as 9-cis-retinoic acid\cite{15}. When cells were treated with 1 uM TO901317 plus 1 uM 9-cis-retinoic acid, there was a further reduction in Aβ secretion to 20% of control (Fig 1B). Aβ formation was reduced when cells were treated with the natural LXR activator, 22(R)-OH cholesterol, together with 9-cis-retinoic acid (Fig 1C). LXR/RXR activation also decreased the secretion of Aβ formed from endogenous APP in 293 cells (data not shown), indicating that these effects were not dependent on overexpression of mutant APP.

Aβ is secreted in several different forms. While Aβ\textsubscript{40} (40 amino acids) is the predominant species, Aβ\textsubscript{42} (42 amino acids) is a minor but more amyloidogenic form. Whereas Aβ\textsubscript{42} is formed predominantly in the endoplasmic reticulum and trans-Golgi, Aβ\textsubscript{40} is made in plasma membrane, endocytic compartments and trans-Golgi\cite{16,17}. Measurement of both forms of Aβ in medium of Neuro2a cells by ELISA revealed that
LXR/RXR activation (1μM TO/1μM 9-cis RA) decreased the secretion of Aβ40 by about 70%, while Aβ42 was more moderately reduced (Fig 2). Since the concentration of Aβ40 in medium was approximately 8-fold more than that of Aβ42, the marked decrease in Aβ signal in Fig 1 primarily reflects a reduction in Aβ40.

Neuro2a cells treated with LXR/RXR activators (1 μM TO and 1 μM 9-cis RA) showed a marked induction of ABCA1 protein (Fig 3A). To determine if induction of ABCA1 might be responsible for decreased secretion of Aβ, Neuro2a cells were transfected with ABCA1 and incubated with or without apoA-I. Transfection of ABCA1 resulted in comparable levels of ABCA1 protein to that induced by LXR/RXR activators (Fig 3A). Expression of ABCA1 decreased the formation of Aβ without affecting cellular APP levels (Fig 3B). Surprisingly, the major effect of ABCA1 expression was observed without addition of the extracellular acceptor apoA-I, and there was only a slight further decrease in Aβ when apoA-I was added to medium (Fig 3B). Measurement of cellular cholesterol efflux showed a small but significant increase in efflux (1.0 ± 0.1% of total cellular cholesterol) with expression of ABCA1 and addition of apoA-I. Similarly, there was an increase in efflux of cellular phospholipids (1.9% of total cellular choline-labeled lipids). However, expression of ABCA1 alone did not increase cholesterol or phospholipid efflux, compared to non-transfected controls. We also measured cellular cholesterol mass. Under our experimental conditions, there was no detectable cholesteryl ester in cells, either with or without expression of ABCA1.
As a control for possible non-specific effects of ABCA1 expression unrelated to functional activity, we transfected Neuro2a cells with a mutant form of ABCA1 (Walker motif mutant); this mutant is well expressed on the cell surface but inactive both in lipid efflux and binding of apoA-I (18). When expressed at similar levels to wild type ABCA1, the mutant failed to alter Aβ secretion by Neuro2a cells (Fig 3C), indicating that the effects of ABCA1 expression are related to activity of the transporter, even though they do not require lipid efflux.

Since apoE is a major apolipoprotein in the central nervous system, and the apoE4 isoform is associated with increased risk of AD (19,20), we also examined the effects of apoE on Aβ formation. Expression of ABCA1 with addition of apoE also resulted in a profound decrease in Aβ formation (Fig 4). Again, the major effect was attributable to ABCA1 expression alone. Addition of apoE2 resulted in a small but significant further decrease in Aβ formation, while apoE3 and apoE4 did not produce significant further reductions in Aβ secretion. Addition of apoE isoforms without ABCA1 expression did not affect Aβ secretion (not shown).

Both β- and γ-cleavage of APP are required for the generation of Aβ. We next carried out a series of experiments to determine which step is affected by ABCA1 overexpression. To measure β-cleavage, we immunoprecipitated the β-secretase cleavage product (C99) from cell lysates. There was an 85% decrease of cellular C99 in Neuro2a-APP<sub>sw</sub> cells transiently transfected with ABCA1 compared with mock transfected control (Fig 5A). γ-Secretase mediates the final step in Aβ generation (1). To assess the direct effects of
ABCA1 expression on γ-secretase cleavage of APP, Neuro2a cells were transfected with constructs encoding C-terminal APP fragments (C99) containing a myc epitope tag. ABCA1 expression was found to decrease the cleavage of C99 peptide, indicating a decrease in γ-secretase processing (Fig 5B. Addition of apoA-I did not provide a further significant decrease in Aβ generation (not shown).

In addition to targeting genes involved in cellular cholesterol efflux and transport, LXRs also activate synthesis of mono-unsaturated fatty acids (21). Stearoyl CoA desaturase (SCD) is a key LXR target gene that catalyzes the conversion of stearoylCoA to oleoylCoA and increases the content of mono-unsaturated fatty acids in cell membrane phospholipids (22). We have recently shown that SCD activity decreases the amount of liquid ordered domains in the plasma membrane (13). There are two forms of SCD, both LXR targets, with similar catalytic activity and cellular effects (22). Using an antibody that recognizes both forms of SCD, we showed that treatment of Neuro2a cells with LXR activators resulted in a modest 1.6 fold increase in SCD protein (Fig 6A). Transient transfection of SCD in Neuro2a cells resulted in a decrease in Aβ secretion into medium (Fig 6B). Since Neuro2a cells have high basal levels of SCD activity, we also carried out similar experiments in 293 cells that have much lower basal SCD expression (13). Transient overexpression of SCD resulted in an increase in APPsα formation (not shown) and a profound decrease in Aβ generation (Fig 7A) that was associated with a marked decrease in γ-secretase cleavage of the C99 peptide (Fig 7B).
Discussion

The idea that changes in cellular lipid metabolism might favorably influence APP processing has attracted considerable attention (23). Cellular cholesterol depletion, based on treatment with cyclodextrins or statins, reduces membrane rafts, increases $\alpha$-cleavage of APP and reduces $\beta$-cleavage, leading to less $\text{A}\beta$ secretion (24). One explanation for these observations is decreased partitioning or trafficking of APP into cholesterol-enriched membrane domains where the $\beta$-cleavage enzyme (BACE) resides (23,25). In contrast, in our study LXR activators and ABCA1 expression were observed to decrease $\text{A}\beta$ secretion in the absence of cellular lipid efflux, and this was not accompanied by changes in $\alpha$-cleavage, and was mediated in part by a decrease in $\gamma$-secretase cleavage of the C-terminal 99 amino acid peptide of APP. Our results suggest that ABCA1-mediated translocation of membrane cholesterol leads to a decrease in $\gamma$-secretase cleavage. This suggests either sensitivity of $\gamma$-secretase activity to membrane environment, or altered trafficking of C99 to the site of $\gamma$-cleavage, and represents a new mechanism to link alteration in membrane lipid organization and $\text{A}\beta$ production.

Our studies were undertaken as a test of the hypothesis that LXR activation would increase lipid efflux and thereby decrease $\text{A}\beta$ generation. However, LXR activators decreased $\text{A}\beta$ formation in the absence of extracellular acceptors (Fig 1) and addition of apoA-I or any of the three isoforms of apoE resulted in only minor further changes in $\text{A}\beta$ (Figs 3, 4). Serving as a control for non-specific effects related to ABCA1 expression, the Walker motif mutant of ABCA1 failed to decrease $\text{A}\beta$ formation (Fig 3C). These findings indicate that the decrease in $\text{A}\beta$ is related to an intrinsic cellular activity of
ABCA1. ABCA1 probably acts as a lipid translocase at the plasma membrane (26), and causes changes in plasma membrane morphology (27). Recently, Vaughan et al (28) showed that ABCA1 expression increases cell surface cholesterol oxidase-accessible domains, indicating a redistribution of cholesterol towards the outer membrane, independent of extracellular lipid acceptors. Decreased Aβ formation might result from an ABCA1-induced redistribution of membrane cholesterol either at the plasma membrane or in the Golgi or endocytic compartments.

While ABCA1-mediated lipid translocation could affect raft organization, it seems unlikely that an alteration in plasma membrane rafts fully accounts for our findings. ABCA1 appears to be localized in and to induce cholesterol efflux from non-raft membrane regions (29), and ABCA1 expression did not alter the distribution or amount of plasma membrane liquid ordered regions in 293 cells (unpublished). In contrast, SCD activity decreases membrane liquid ordered regions (13), and was associated with increased alpha-cleavage of APP, similar to the effects of cholesterol depletion, However, changes in SCD protein levels in Neuro2a cells were modest and unlikely to account for a major part of the effect of LXR activators.

Both ABCA1 and SCD expression were associated with a decrease in γ-secretase cleavage of the C-terminal 99 amino acids of APP (Figs 5B and 7). In the case of APPsw, ABCA1 decreased β-cleavage by 85% (Fig 5A), but the overall decrease in Aβ secretion was only around 60% (Fig 3B), which is comparable to the effect of ABCA1 on γ-cleavage (Fig 5B). This suggests that under these conditions γ-cleavage is the rate-
limiting step in Aβ secretion. Our findings suggest that this key enzyme of Aβ formation is highly sensitive to its membrane lipid environment, possibly reflecting the fact that γ-secretase mediates an intramembranous cleavage of APP. Alterations in membrane fluidity caused by lipid translocation or increased content of mono-unsaturated phospholipids could lead to a decrease in γ-secretase activity. While the cellular compartmentalization of γ-secretase activity is not completely understood (1), several reports suggest that Aβ formation mainly occurs in the endocytic compartments (30) where components of γ-secretase, including presenilins, are shown to be present (31). An alternative interpretation of our results is that induction of ABCA1 activity results in altered cellular trafficking of APP or the C-terminal fragment of APP, providing less substrate for γ-secretase cleavage.

The finding that cellular cholesterol depletion leads to less Aβ secretion has fostered the idea that statins could be useful in the treatment of AD. Epidemiological studies have suggested that statin therapy is associated with decreased prevalence of AD (32,33). However, brain cholesterol is derived by local synthesis (not from plasma LDL) and statins would have to be present in the brain at high levels in order to alter neuronal lipid metabolism. Although human studies show an association between statin treatment and decreased prevalence of AD, such associations can reflect the influence of confounding factors, as appears to be the case for statins and bone disease (34). A recent placebo-controlled prospective trial of statin therapy in the elderly failed to show any improvements in cognitive function (35).
Inhibition of cholesterol esterification by acylCoA:cholesterol acyl transferase (ACAT) inhibitors has also been proposed to favorably affect processing of APP (36). Since cholesteryl esters have minor solubility in membranes and are thought to be present in cells as inert lipid droplets, it is unlikely that these effects are related to cholesteryl ester accumulation. One possibility suggested by our studies is that ACAT inhibition leads to accumulation of cellular free sterol and conversion to LXR ligands via endogenous oxysterol synthesizing enzymes such as 24-cholesterol hydroxylase (37). Cholesteryl esters were not stored in cells in appreciable amounts under the conditions of our experiments, and changes in cellular ACAT activity are thus unlikely to account for the findings.

Our findings, along with another recent report (38), suggest that LXR activators, currently being developed for the treatment of atherosclerosis, might have therapeutic efficacy in Alzheimer’s Disease. LXR/RXR activation markedly decreased Aβ40 but had less effect on Aβ42 secretion. While Aβ42 is thought to be more amyloidogenic than Aβ40, much more Aβ40 is secreted by neurons and both forms are found in amyloid plaques (2). Therefore, controlling the predominant Aβ40 secretion could be beneficial. Our results differ from another report where a 65% increase in Aβ42 and no significant change in Aβ40 was found in Neuro2a cells treated with TO and 9-cis-retinoic acid (10uM) (39). The reasons for this discrepancy are not clear. However, our findings substantially agree with another report (38), which appeared while our paper was under review. Our results extend these latter studies by the direct demonstration that ABCA1
and SCD expression decreases Aβ formation, that these effects are observed without lipid efflux from cells and involve a decrease in γ-secretase cleavage.

In contrast to the more general approach of cholesterol biosynthesis inhibition, LXR activation may directly regulate genes that favorably modulate plasma membrane composition and structure in the brain (8). Tangier Disease patients have not been reported with premature dementia, suggesting that ABCA1 may not have an essential role in protecting against AD. However, this does not rule out the possibility that increased expression of ABCA1 has a protective role, just as it does in atherosclerosis (40).
Acknowledgements

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Figure Legends

**Figure 1. LXR activation reduces Aβ secretion from Neuro2a-APPsw cells.** Neuro2a-APP<sub>sw</sub> cells were treated with LXR activators (TO or 22-(R)OH cholesterol) and in some cases also with RXR activator (9-cis-retinoic acid). Secreted Aβ (sAβ) was detected in medium by immunoprecipitation and immunoblotting. Cellular APP (cAPP) levels were measured by immunoblotting cell lysates. Western blots were scanned and quantified by ImageQuant. The bar graphs show combined results (mean + SEM) from at least three independent experiments. (A) Cells were treated with increasing amount TO901317 (TO). (B) Cells were treated with mock (mk) transfection (control) or 1uM TO/1uM 9-cis retinoic acid (9-cis RA). (C) Cells were treated with mock transfection or 5uM 22(R)hydroxycholesterol (22(R)OHchol)/10uM 9-cis RA. #, p < 0.05 compared with control. *, p < 0.01 compared with control.

**Figure 2. LXR/RXR activation reduces Aβ40 and Aβ42 in medium as determined by ELISA assay.** Cells were treated as in Fig 1B; filled bar, mock transfected control; hatched bar, treated with 1uM TO/1uM 9-cis RA. The data are mean+SEM, normalized to control value (=1); n=4; *, p < 0.01 compared with control; #, p < 0.05 compared with control.

**Figure 3. ABCA1 overexpression inhibits Aβ secretion.** (A) ABCA1 protein in Neuro2a cells after LXR activation (top panel) or transient transfection (bottom panel), showing similar expression levels relative to actin. (B) and (C) Aβ peptide in medium was determined by immunoprecipitation and immunoblotting; cellular APP (cAPP) was
determined by immunoblotting cell lysates. Bar graphs show combined data from 3 or more independent experiments. *, p < 0.01 compared with control. (B) Neuro2a-APP<sub>Sw</sub> cells were transfected with either mock control plasmid or ABCA1 cDNA. ApoA-I (A1) was added for 6 h where indicated. (C) Neuro2a-APP<sub>Sw</sub> cells were transfected with empty vector (control) or vector containing ABCA1 cDNA or ABCA1 with a mutation in the ATP binding cassette (Walker motif mutation). Filled bar, no apolipoprotein added; hatched bar, apo A-I added.

Figure 4. The effect of apoE isoforms on Aβ secretion. Neuro2a-APP<sub>Sw</sub> cells were transfected with empty vector (control) or ABCA1 vector, and apoE isoforms were added during the last 6 h of the experiment; data are mean ± SEM for 5 separate experiments conducted in duplicate or triplicate; *, p < 0.01 compared with mk transfected; #, p < 0.05 compared with ABCA1 transfected without apolipoprotein.

Fig 5. ABCA1 overexpression decreases β-cleavage of APP<sub>Sw</sub> and γ-cleavage of the 99 amino acid C-terminal fragment of APP. (A) Neuro2a-APP<sub>Sw</sub> cells were transfected with either empty vector or vector containing ABCA1 cDNA. The β-cleavage product C99 was detected by immunoprecipitation of cell lysates with antibody 4G8 followed by immunoblotting with 6E10. Cellular APP (cAPP) level was measured by immunoblotting cell lysates. The data are mean± SEM for 5 separate experiments; *, p < 0.01 compared with mk transfected. (B) Neuro2a cells were transfected with vector containing either C99myc or C99myc and ABCA1. Aβ was detected as in Fig3. Cellular C99 was detected by IP with anti-myc antibody followed by western blot with 6E10. The data are shown
for three separate experiments conducted in duplicate; #, p < 0.05 compared with C99 transfected alone.

**Fig 6. Stearoyl CoA (SCD) overexpression inhibits Aβ secretion in Neuro 2a cells.**

(A) SCD protein in Neuro2a cells before and after LXR activation, as determined by Western blotting using an antibody that recognizes both forms of SCD (SCD1 and 2); (B) Neuro2a cells were transfected with wild type APP or APP with SCD expression plasmid. Aβ secretion was measured during 4 h incubation. Data are shown for 3 separate experiments conducted in duplicate; *, p < 0.01 compared with APP transfected alone.

**Fig 7. SCD overexpression inhibits Aβ secretion from APP and C99 in 293 cells.** (A) 293 cells were transfected with APP (wild type) or APP with SCD expressing plasmid. (B) 293 cells were transfected with C99 or C99 with SCD expressing plasmid. Aβ secretion was measured during 72 h incubation; *, p < 0.01 compared with C99 transfected alone.
Fig 1.

A

B

C

Aβ/cAPP (Arbitrary unit)

0 0.2 0.4 0.6 0.8 1 1.2

sAβ
cAPP

TO(uM)

control 1 10

* #

Aβ/cAPP (Arbitrary unit)

0 0.2 0.4 0.6 0.8 1 1.2

sAβ
cAPP

TO901317 9-cis RA

* *

Aβ/cAPP (Arbitrary unit)

0 0.2 0.4 0.6 0.8 1 1.2

sAβ
cAPP

control 22(R)OHchol 9-cis RA

*
**Fig 2.**

The figure shows a bar graph comparing the levels of Aβ/cAPP (Arbitrary unit) for control and TO+9-cis RA conditions. The graph indicates a noticeable difference in Aβ levels between the two conditions, with the TO+9-cis RA showing a higher level of Aβ at Aβ 40 and Aβ 42 compared to the control.

Legend:
- ■ control
- ☐ TO+9-cis RA
Fig 3.

A

ABCA1

Actin

To 901317  
- +

9-cis RA  
- +

ABCA1

Actin

mk ABCA1

B

sAβ

cAPP

![Image of Western blot](image1)

Aβ/cAPP (Arbitrary unit)

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C

Aβ/cAPP (Arbitrary unit)

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* p < 0.05
Fig 4.
Fig 5.

A

C99

C99,ABCA1

C99/cAPP (arbitrary unit)

B

sAβ

C99

Aβ/cellular C99 (arbitrary unit)

mk

ABCA1

C99

C99,ABCA1
Fig 7.

A

sAβ

APP  +  +
SCD  -  +

B

C99myc

sAβ

C99 +  +
SCD -  +

Aβ/cellular C99 (Arbitrary unit)

0  0.2  0.4  0.6  0.8  1  1.2

C99 +  +
SCD -  +

*