Effect of APP Isoforms on Cholesterol Metabolism in Alzheimer’s Disease
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ABSTRACT

Increasing evidence shows that cholesterol plays a role in the pathogenesis of Alzheimer’s disease (AD). Recent studies have shown that aberrant cholesterol metabolism can affect mitochondrial function and facilitate the production and accumulation of amyloid-beta (Aβ) deposits. However, the cause of this perturbation is unclear. Aβ is proteolytically derived from amyloid precursor protein (APP), and there are three major spliced forms of APP in human brain APP695, APP751 and APP770. In brain, the predominant isoform transcript appeared to be APP695, along with moderately high levels of APP751 and APP770. The APP751 and APP770 isoforms contain a Kunitz protease inhibitory (KPI) motif that APP695 lacks, and they are collectively known as APP-KPI(+). In AD-inflicted human brain tissues, the expression of APP-KPI(+) were elevated. The objective of this project is to explore the functional connection between APP-KPI(+) isoforms and cholesterol metabolism in AD cell models. We examined the contribution of APP-KPI(+) isoforms on the cholesterol metabalome in AD cell models. In addition, we also examined the contribution of APP-KPI(+) isoforms on Aβ and ATP formation, an indirect measure of glucose metabolism. In our results, we observed that the amount of Aβ40, Aβ42, ATP, cholesterol and cholesterol metabolites were not significantly different in wild type human APP695 (APP-KPI(-)) and APP770 (APP-KPI(+))-transfected cells. In cell and animal models, mutation in APP695 leads to an increase in Aβ42 formation, increase cholesterol metabolism and a decrease in ATP formation (due to hypoglycemia), in human however, even without a mutation, KPI expression is upregulated and an increase in Aβ42 formation, increase in cholesterol metabolism and a decrease in ATP formation is observed. Hence we can conclude that a mutation might be needed in APP-KPI(+) in cell model in order to observe significant increase in Aβ42 formation, cholesterol metabolism and ATP formation when compared to mutated APP-KPI(-)-transfected cell.

INTRODUCTION

AD is a neurodegenerative disorder characterized by cognitive and memory decline. Mounting genetic and biochemical evidence strongly supports the amyloid casade hypothesis that Aβ accumulation is the primary event in the pathogenesis in AD (Hardy and Selkoe 2002). The aggregated Aβ results in neuronal injury, leading to neurodegeneration and ultimately AD. In addition, mutations APP result in the greater production of Aβ (Aβ40) or increased production of a longer form of Aβ — Aβ42 that aggregate more rapidly as compared to Aβ40 (Hardy and Selkoe 2002). Many previous studies have provided mass information on the structural and biochemical co-factors favouring Aβ deposition in AD patient (Haass and Selkoe 1993). Cholesterol is an essential component of the cell membranes and myelin sheathes, it is important for the integrity of synaptic transmission n and the proper function of neurons

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(Pfrieger 2003). Particularly, attention has been devoted towards the association between brain cholesterol metabolism and the risk of developing AD. However, studies on cell lines and animal models often show conflicting results. In contrast, not much information is known on the affect of APP isoforms on cholesterol level. There are three major spliced forms of APP in human brain — APP695, APP751 and APP770. The APP751 and APP770 isoforms contain a Kunitz protease inhibitory (KPI) domain, and are expressed mostly in non-neuronal glial cells (Zheng and Koo 2006). The amount of APP751 and APP770 protein and mRNA are elevated in AD brain and they are associated with increased Aβ, in particularly Aβ42 production (Menéndez et al. 2005). In the present study, we examined the contribution of APP-KPI(+) isoforms on the cholesterol metabalam in AD cell models by using two major splice forms of human APP — APP695 (APP-KPI(-)) and APP770(APP/KPI(+)), on the cholesterol metabalam, in cell models of AD — APP-knockout cells transfected with wild type human APP695 and APP770. In additional, we also examined the contribution of APP-KPI+ isoforms on Aβ and ATP formation.

METHODS AND MATERIALS

Cell culture
Immortalized mouse neuronal cell line derived from the APP knock-out mice (Tan et al. 2008) transfected with wild type human APP695 and APP770 were maintained in Dulbecco’s modified Eagle’s Medium (DMEM), supplemented with 10% fetal calf serum and 1% penicillin/streptomycin/amphotericin B (PSA) and 1.5µg/ml Blasticidin (Invitrogen). For protein and sterol extraction, the cells were grown to about 90% confluency, harvested using either trypsin or through scrapping, washed with PBS and collected by centrifugation. The cell pellets were than immediately frozen at -80οC for further use.

Sterol Analysis on Gas Chromatography/Mass Spectrometry
For sterol extraction, the cell samples (n=3 per cell line) were dried and weight. 40µg of internal standard mixture containing 24S-OH-D7-cholesterol (5µg/µL) and 27-OH-D5-cholesterol (2.5µg/µL) mixture in ethanol were added as internal standard (total amount of 24S-OH-D7-cholesterol added was 200ng and 27-OH-D5-cholesterol was 100ng). Gas chromatography/mass spectrometry (GC/MS) was performed to determine the total levels of 24S-hydroxycholesterol and 27-hydroxycholesterol.

Amyloid β-peptide (Aβ)-40/42 ELISA
Cells sample were incubated with 5M Guanidine HC1 in 50mM Tris HCl for 2 hours. Amount of Aβ40 and Aβ42 were analyzed by using Human Aβ (1–40) (N) Assay Kit and Human Aβ (1–42) (N) Assay Kit — IBL (Immuno-Biological Laboratories Co., Ltd.) respectively, according to the manufacturer’s instructions. Protein concentrations in cells were measured using BCA (bicinchoninic acid) Protein Assay Reagent (Pierce) according to manufacturer’s instructions. Amount of Aβ40 and Aβ42 in cells were then normalized with their protein concentration.

ATP Extraction and Assay
The cells were grown to about 90% confluency, harvested through scrapping, washed with PBS and collected by centrifugation. Proteinase K was added into to extract ATP. Amount of ATP in cell samples were then measured using ENLITEN® ATP Assay System Bioluminescence Detection Kit (Promega) according to manufacturer’s instructions.
RESULT AND DISCUSSION

In this study, we compared between the cholesterol metabolism, energy metabolism and Aβ formation in APP-KPI(+)(APP770) and APP-KPI(-)(APP695)-transfected cells. The analysis of the amount of Aβ40 revealed that there was no significant different in the Aβ40 formation in the APP695 and APP770-transfected cell (P=0.805 > 0.05; APP695 1.490 ± 0.378 pg/µg protein, APP770 1.609 ± 0.190 pg/µg protein). The level of Aβ42 (P=0.105; APP695 0.188 ± 0.048 pg/µg protein, APP770 0.333 ± 0.019 pg/µg protein) and Aβ42/Aβ40 ratio (P=0.198; APP695 0.133 ± 0.038, APP770 0.211 ± 0.030), showed a non-statistically significant increase in APP770-transfected cell line (P=0.105; APP695 0.188 ± 0.048 pg/µg protein, APP770 0.333 ± 0.019 pg/µg protein). The amount of APP751 and APP770 mRNA and protein are elevated in AD, a functional linkage between APP770 isoform and Aβ might be present. In addition, experiment has shown that the expression of KPI domain reduces α-secretase cleavage and hence contribute to increase amount of full length Aβ produced (Ho et al. 1996). However, this functional linkage is not observed in the result.

Hypoglycaemia is another characteristic of AD. This decline in cerebral glucose metabolism early in the disease process (Costantini et al. 2008). Analysis of the amount of ATP revealed that there was no significant different in the amount of ATP in the APP695 and APP770-transfected cell line (P=0.516 > 0.05; APP695 13.15 ± 1.947 nmol/ng cells, APP770 15.21 ± 2.000 nmol/ng cells), indicating that the KPI domain did not contribute to the decrease amount of ATP in AD brain due to hypoglycaemia.

In addition, results also show that the total cellular cholesterol and cholesterol metabolites, 24S-OH-cholesterol and 27-OH-cholesterol were not significantly different between the APP695 and APP770-transfected cells. Cholesterol exits the brain by conversion into 24S-OH-cholesterol, the amount of 24S-OH-cholesterol exiting can be thought to equal the amount of cholesterol synthesized. Since the amount of cellular cholesterol and cholesterol metabolites were not significantly different, it must indicate that APP-KPI(+) and hence KPI domain did not contribute to an increase in cholesterol (hypercholesterolemia) in AD patients.
Figure 3. Amount of (A) cellular cholesterol (nmol/µg protein), (B) 24S-OH-cholesterol (ng/mg cells) and (C) 27-OH-cholesterol (ng/mg cells) in APP695 and APP770-transfected cells were determined.

In cell and animal models, mutations in APP695 leads to an increase in Aβ42 formation, increase cholesterol metabolism (hypercholesterolemia) and a decrease in ATP formation (due to hypoglycemia), in human however, even without a mutation, KPI expression is up-regulated and increase in Aβ42 formation, increase in cholesterol metabolism and a decrease in ATP formation is observed. Hence, an up-regulation in KPI in human might be an important factor causing the increase in Aβ42 formation, increase in cholesterol metabolism and a decrease in ATP formation observed. In our result, however, we observed that the amount of Aβ40 and Aβ42, ATP, cholesterol and cholesterol metabolites are not significantly different in APP695 (APP-KPI(-)) and APP770 (APP-KPI(+))-transfected cells. Hence we can conclude that a mutation might be needed in APP-KPI(+) in cell model in order to observe significant increase in Aβ42 formation, cholesterol metabolism and ATP formation when compared to mutated APPKPI(-)-transfected cell. Measurement of ATP and cholesterol and cholesterol metabolites alone is not a very accurate measurement of glucose and cholesterol metabolisms, other metabolome need to be considered measured so as to be able to more accurately determined the functional relation of APP-KPI(-) and APP-KPI(+) on cholesterol and glucose metabolism.

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