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Review article:

The possible implication of clusterin-dependent serum cholesterol trafficking in Alzheimer's disease

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Abstract

Hypercholesterolemia is known as a major risk of atherosclerosis. However, recent studies revealed that elevated level of LDL ("known as bad cholesterol") predisposes also to dementia, stroke and neuropathies including Alzheimer's disease (AD). The relationship between the blood cholesterol level and AD has not been completely explained, however, the clusterin/ApoJ role seems to be pivotal. Clusterin is a glycoprotein expressed by various cell types. Moreover, Clu/ApoJ is secreted into body fluids and its expression is stimulated in response to raised sterol levels. In blood, Clu/ApoJ interacts with other lipoproteins, plasma proteins or lipids, including cholesterol, and is also capable to translocate through the blood-brain barrier. Additionally, the high blood plasma levels of cholesterol promote formation of plasma membrane lipid rafts, enriched in β-secretase enzyme. Consequently, the abnormal amyloid β precursor protein (AβPP) cleavage may occur and neurotoxic amyloid β is produced. Thus, the intensity of amyloid β processing is apparently related to cholesterol concentration, and could be limited by cholesterol-depleting agents such as statins. Interestingly, Clu/ApoJ is not merely transported from blood plasma into brain, but at the same time it is secreted by astrocytes to extracellular fluid. Clu/ApoJ interacts with $A\beta_{1\text{-}40}$ and is accumulated in peptide plaques. To maintain Aβ in soluble form, the extracellular Clu/ApoJ is simultaneously complexed with Aβ. Finally, intracellular variant of clusterin is admitted to mediate the activation of Ca^{2+}-permeable ion channels, which initiate Aβ-induced neuronal cell apoptosis. Taken together, we hypothesize, that Clu/ApoJ plays an important role in cholesterol-dependent AD pathogenesis and should be seriously considered as a component in AD pathology.

Keywords: Hypercholesterolemia, atherosclerosis, cholesterol, neuropathies, Alzheimer's disease, glycoprotein

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Introduction

It is known that during aging, several physiological disturbances occurred. Hypertension, hypercholesterolemia, insulin resistance, inflammation and neurodegeneration are all the most popular hallmarks of the senescence. The correlation between cholesterol level and hypertension is well documented. Furthermore, epidemiologic studies revealed that a decline in total cholesterol levels suspends the diagnosis of dementia, including Alzheimer's disease (AD), by at least 15 years. Noteworthy, the molecular mechanism of the relationship between cholesterol and AD remains ambiguous. Herein, we describe the possible scenario of this phenomenon.

Clusterin

Clusterin, also termed apolipoprotein J (Clu/ApoJ), is a multifunctional protein, which is known to be involved in distinct physiological processes, such as lipid transport, cell-cell and cell-matrix adhesions, cell differentiation, membrane recycling, cell membrane protection and programmed cell death (for more information please refer to Advances in Cancer Research, Elsevier, 2009 [1]). Interestingly, Clu/ApoJ has been reported both as a pro- and anti-survival agent. These, apparently contradictory functions could be related to the specific proteomic profile, that results from complex regulation of clusterin expression. In spite of coding by a single gene located on human chromosome 8 [2], clusterin appears at different isoforms in the respective cell compartments. The main product of human *CLU* gene expression is 60

kDa peptide, which is processed by removal of N-terminal signal peptide, subsequent glycosylation in ER (endoplasmic reticulum) and cleavage to α and β units, bound together by five disulfide bonds [3]. The mature, ~80 kDa form is secreted to the extracellular space and body fluids, including cerebrospinal fluid [3,4]. Hitherto, secretory clusterin (sClu) is believed to act as a chaperone molecule. The biochemical structure of clusterin contains three amphipathic and two coiled-coil α-helices, which are characteristic for chaperons, such as heat shock proteins [5]. Moreover, there are also three intrinsic disordered regions i.e. molten globule domains in sClu, determining the protein-protein interactions [5]. The main functions of the sClu are based on molten globule domains, which bind sClu via its flexible structures, to various targets, including lipids and proteins. The issue whether the sClu-protein interactions are only protective for the cells is questioned if one reminds that sClu had been found in senile plaques and neurofibrillary tangles in patients suffering from Alzheimer's disease (AD) [6-8]. In addition, some reports demonstrated the increased expression of sClu in AD brains [9, 10]. Since then, the numerous studies investigated the role of clusterin in AD pathology (for more information refer to the review by Nuutinen et al. [5]).

Alzheimer's disease

Alzheimer's disease is a complex disorder that shows a definite but limited familial component. Several genes and their mutations have been associated with AD, such as amyloid beta precursor protein (*AβPP*), apolipoprotein E (*ApoE*), presenilin [11]. However, the epigenetic regulation of other, undefined genes could explain the sporadic AD cases. One of the known risk factors of AD development is hypercholesterolemia. It is apparent, that the elevated level of serum cholesterol fraction may influence the brain function. Recent studies revealed that people with total cholesterol levels between 249 and 500 milligrams were one-and-a-half times more likely to develop Alzheimer's disease in elderly than those with cholesterol levels of less than 198 milligrams. People with total cholesterol levels of 221 to 248 milligrams were more than one-and-a-quarter times more likely to develop AD. A total cholesterol level below 200 is generally considered healthy [12]. Previously, similar effect of diet-induced hypercholesterolemia on AD incidence was reported in transgenic mouse model of AD [13]. Biochemical analysis showed that, compared to control, the hypercholesterolemic mice had significantly decreased sAPPalpha and increased levels of C-terminal fragments (beta), suggesting alterations in amyloid precursor processing in response to high dietary cholesterol [13]. How the blood cholesterol can affect the AβPP processing in not fully understood. The possible mechanisms which could explain cholesterol-AD relationship is the ability of clusterin to bind lipids, including cholesterol. It was demonstrated that Clu/ApoJ forms are linked with ApoA-I lipoprotein and with lipids, where lipids were composed of 54% total cholesterol, 42% phospholipids and 4% triglycerides (mol/mol). The molar ratio between unesterified and esterified cholesterol amounted to 0.58.

Thus, the ApoA-I-clusterin complex is a lipoprotein complex of unusual composition in that it has a very high protein content and is rich in free cholesterol [14]. More importantly, Clu/ApoJ levels could be elevated in response to atherogenic diet, containing oxidized LDL and lipids [15].

The next cholesterol transporter, which is also relevant to AD is apolipoprotein E (ApoE). The Clu/ApoJ, ApoE or ApoJ might be transported individually or in complexes through the blood-brain barrier (BBB) by megalin/gp330 receptor [16]. Megalin/gp330 receptor is a member of the low density lipoprotein (LDL) receptor family, expressed in various tissues, including brain, reproductive organs, epithelia of tracheal, mammary, kidneys and other organs [17].

It is possible that BBB-localized megalin/gp330 receptor promotes ApoA-I-ApoJ-cholesterol transit, or ApoJ-cholesterol, and ApoE-cholesterol complexes [18]. Our assumption is supported by the results reported by Assemat et al. [19], who found that megalin is capable to endocytose dietary sterols such as cholesterol, and this function is important both in adult and during embryogenesis. Thus, the relationship between serum and brain cholesterol seems to be functional. The hypothesis is illustrated on Figure 1.

Cholesterol role in the brain

In the brain, cholesterol plays an important role in cell signaling, being the main component of lipid rafts (LR). Upon uptake, apolipoproteins (A-I, J, E) are shuttled through the early endosomes and next, the cholesterol is transported to the ER via a vesicles that contains on the surface Niemann-Picks proteins (NPC) [20]. As cholesterol is inserted into the plasma membrane, it becomes concentrated in small microdomains, where it facilitates packing of the sphingomyelin. The more cholesterol is delivered, the more LR is formed. Noteworthy, many transmembrane receptors or enzymes

act best in the cholesterol-rich domains. One of such enzymes is β-secretase, the constitutive LR protein, involved in AβPP processing. AβPP processing is physiologically mediated by α- or γ-secretase which are both LR-independent. As

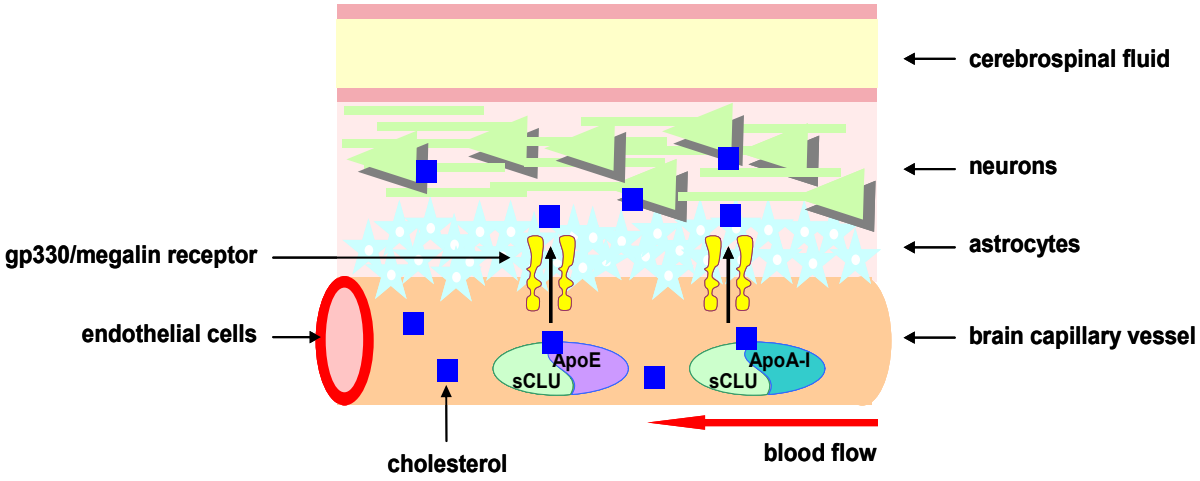

Figure 1: *Clusterin (ApoJ) binds to lipids, including cholesterol and it translocate them alone or with ApoA-I or/and ApoE-complexes, through the blood-brain barrier. The BBB-localized gp330/megalin receptor promotes transit of ApoA-I-ApoJ-cholesterol, or ApoJ-cholesterol, or ApoE-cholesterol complexes.*

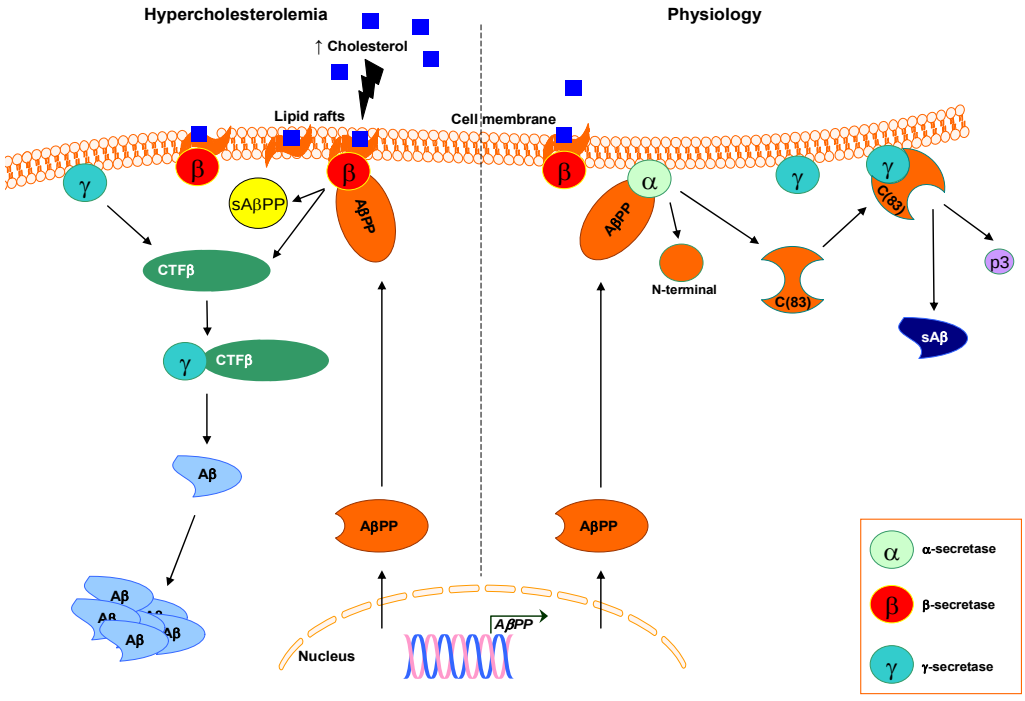

Figure 2: *Cholesterol levels affect the AβPP cleavage. The schematic representation of AβPP protein cleavage: in physiological conditions (right), and in hypercholesterolemia (left). The detailed description of AβPP processing is included in the manuscript.*

a consequence of α-secretase activity, the soluble N-terminal, fragment of AβPP and an 83-residue C-terminal fragment (C83) are generated. Alternatively, a small potion of AβPP is cleaved by β-secretase to produce a slightly shorter N-terminal fragment and a 99 residue C-terminal fragment (C99). Both C83 and C99 are subsequently cleaved by γ-secretase to generate p3 and Aβ, respectively [21]. The other peptide derived from γ-secretase cleavage of C83/C99 is called amyloid intracellular domain (AICD) and has been shown to translocate into the nucleus and regulate downstream genes. Since α-secretase cleaves AβPP at the site corresponding to the middle of the AβPP region, AβPP molecules un-

dergoing α-secretase activity do not lead to the formation of Aβ [22]. On the other hand, when more cholesterol is delivered, β-secretase which resides in lipid rafts is predominantly engaged in AβPP processing. β-Secretase (BACE, Asp-2) is a transmembrane aspartic proteinase responsible for cleaving the amyloid beta precursor protein to generate the soluble ectodomain sAβPP and its C-terminal fragment CTFβ. CTFβ is subsequently cleaved by γ-secretase to produce the neurotoxic/synaptotoxic amyloid-β peptide (Aβ) that is accumulated and initiates Alzheimer's disease [23] (Figure 2). This effect could be reversed when the lipid rafts are disrupted by depleting cholesterol. These observations suggest that processing of AβPP to the amyloid-β peptide occurs predominantly in lipid rafts and that BACE is the rate-limiting enzyme in this process [23]. The balance between raft and non-raft AβPP processing is crucial for AD development. Bearing in mind the above mentioned results, the protective role of statins, drugs used in therapy of hypercholesterolemia, against AD seems to be reasonable. It was proved recently by two independent retrospective studies, that incidence of AD and dementia in patients treated is markedly reduced (by almost 80%) with inhibitors of 3-hydroxy-3-methylglutaryl-CoA-reductase [24, 25].

Aβ and protective role of Clu/ApoJ

$A\beta_{1\text{-}40}$ formed via lipid rafts-dependent β-secretase aggregates with other cellular proteins inside neurons. It is well documented, that clusterin could bind to Aβ peptides. Yerbury et al. [26] observed that clusterin can either prevent or enhance Aβ oligomerization. The authors hypothesized that the final biological effect of Clu/ApoJ is determined by the balance between clusterin and Aβ peptides. The clusterin-dependent increase in fibrillar Aβ formation was observed when more than 10-fold concentration of Aβ was used. Otherwise, clusterin increased the Aβ solubility and prevented its aggregation [26]. Similar effect was demonstrated previously by Matsubara et al. [27], who found that Clu/ApoJ and peptide homologous to the main forms of sAβ ($A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$) are featured by saturable and specific high-affinity binding interactions.

Furthermore, the formation of Clu/ApoJ- sAβ complexes significantly prevented peptide polymerization and aggregation, but once the complex is formed, peptides became resistant to the proteolytic degradation. These data suggest that above-mentioned interaction may preclude sAβ aggregation in biological fluids and point to a protective role of Clu/ApoJ [27]. Despite the possible protective role of clusterin in β-amyloidosis, in the case of hypercholesterolemia, where great amount of toxic Aβ are formed in LR, the balance between Clu/ApoJ and Aβ is disrupted and clusterin does not capture Aβ peptide any longer. Consequently, the Aβ accumulates within the cells, and Clu/ApoJ is included in plaques [10]. Interestingly, also apolipoprotein E was found to be associated with neurofibrillary tangles [28].

Aβ and activation of cation channel

Cytotoxic Aβ accumulates within the cell interior, however at the same time the Aβ is secreted from the cells in association with exosomes and intraluminal vesicles of multivesicular bodies (MVBs), which fuse with plasma membrane [29]. Thus, Aβ is present in the intraneuronal space, as well as cerebrospinal fluid (CSF). According to Nilselid et al. [4], CSF contains Clu/ApoJ, which is able to form complexes with Aβ. On the other hand, the intracellular Aβ is the major cause of neuronal cell death, which occurs through apoptosis. It was shown that the formation of $A\beta_{1\text{-}40}$ correlates with an increased intracellular calcium concentration resulted from calcium influx through the cation-permeable channels [30].

Furthermore, Fonfria et al. [31] revealed, that the calcium permeant, non-selective cation channel, triggered by ADP-ribose, NAD and H_2O_2, TRPM2 (melastatin-like transient receptor potential 2), is also activated by Aβ. TRPM2 activity may contribute to neuronal cell death, mediated by upregulated levels of ROS and Ca^{2+} [31]. Elevated levels of calcium ions are known to trigger intrinsic apoptotic pathway, as well as caspase-independent, calpain-dependent cell death [32]. The second clusterin isoform, so-called nuclear clusterin (nClu), could participate in this process. It is believed, that nClu is a truncated transcript from alternative splicing of *CLU* out of exon II [33]. nClu lacks the ER signal peptide sequence, thus it is not intensively glycosylated, and it is not cleaved to α and β chains which undergo extracellular secretion.

The ~49 kDa Clu variant is localized mainly in cell cytoplasm, however, in response to various cytotoxic stimuli, such as oxidative stress or apoptosis-inducing factors, nClu translocates to the cell nucleus. Calcium ions are potent modulators of intracellular clusterin activity [34], thus the Aβ-induced TRPM2 activation could influence nClu activity. The schematic representation of Aβ-induced TRPM2 activity is illustrated on Figure 3.

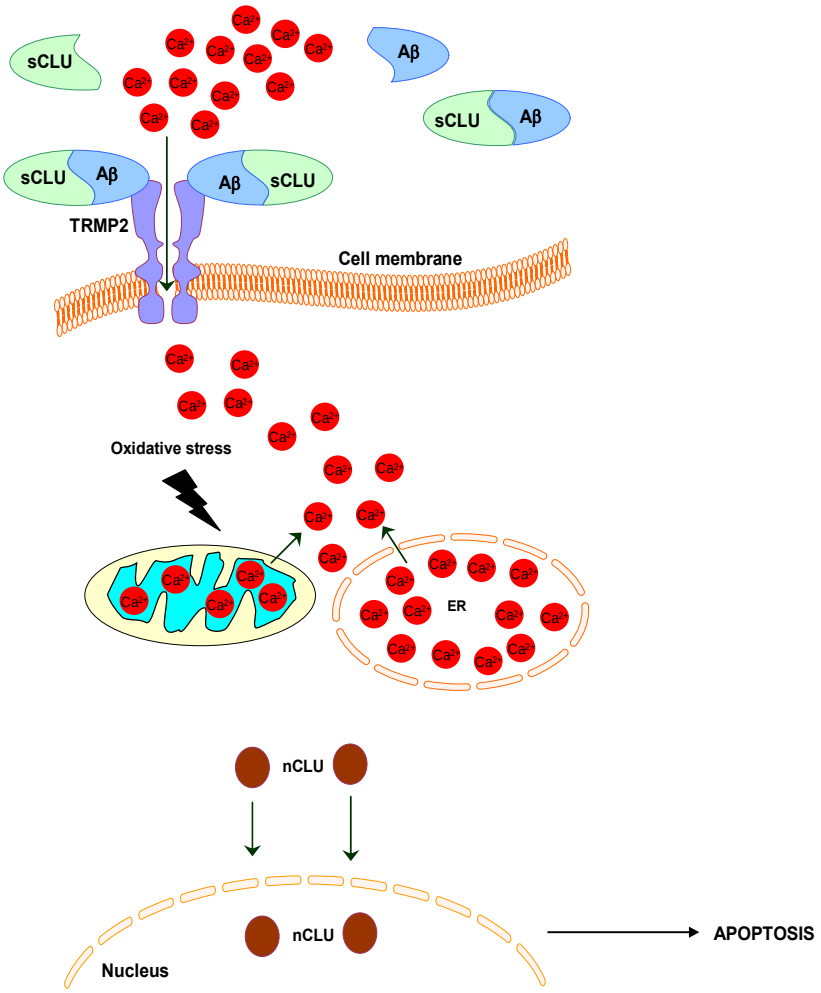

Figure 3: *Clu(ApoJ)-Aβ complexes activate TRPM2 cation channel, leading to Ca^{2+} intracellular influx. Elevated concentrations of calcium ions induce nClu intranuclear translocation. Finally, the intrinsic apoptosis is activated.*

Conclusions

Taken together, we hypothesize that clusterin accompanies both extra- and intracellular Aβ-dependent regulations. Furthermore, based on cited reports we suppose, that Clu/ApoJ could play a role of the blood-sensor, with the expression, activities and localizations correlated with the cholesterol level. On the other hand, due to interaction with Aβ, clusterin could be seriously considered as important player in AD pathogenesis and disease progression. Apparently, Clu/ApoJ might provide a link between hypercholesterolemia and Alzheimer's disease. Further studies are urgently needed if one wishes to elucidate the definite role of Clu/ApoJ in AD development and progression.

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