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Review Article

The decline of the expression of low density lipoprotein receptor-related protein 1 (LRP1) during normal ageing and in Alzheimer's disease

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Abstract

Considering the importance of the low-density lipoprotein receptor-related protein 1 (LRP1) as the scavenger of harmful waste products from the brain, especially amyloid beta (Aβ), and as the mediator in a number of signal events, LRP1 is increasingly becoming the object of intensive researches. Alzheimer's disease (AD), as proved, is the result of the disordered Aβ intracerebral homeostasis, characterized by intensive Aβ accumulation in the brain, induced primarily by the Aβ disordered efflux from the brain, and not by its increased production. This elevated Aβ accumulation in the brain structures leads to the increase of the local oxidative stress with intensively harmful effects of the generated free radicals, especially hydroxyl radicals (*OHs). This condition is accompanied by severe oxidative damages of a number of vital brain structures, especially endothelial cell membranes. LRP1, at the level of blood-brain barrier (BBB) endothelial cells, initiated by Aβ binding to its ligand binding domains, especially domains II and IV, induces specific conformational changes in both receptor domains, which causes the onset of pulling forces that, starting from the point of Aβ binding to LRP1, spread along the receptor structure in both directions. Especially important is the spreading of these forces towards the abluminal membrane, their passing across this membrane and entering the receptor tail region and the compound structure of its molecules. The activation of these molecular systems leads to endocytosis, transcytosis, and exocytosis of Aβ bound to the receptor, into the capillary blood of the BBB. The drained Aβ travels further on by the blood stream, exits from the brain, and arrives at the positions of its degradation and elimination, liver, kidneys, spleen, and skin. Investigations have shown that generally, the expression of LRP1 in the BBB endothelial cell system declines during normal ageing, and especially in AD. It is evident that this phenomenon weakens the Aβ drainage from the brain. The actual question is what causes the LRP1 expression drop in these conditions, and if it is possible to slow down this drop. In this respect, this review paper intends to explain the problem of the LRP1 expression decline, and its possible increase, i.e., the retardation of the AD pathophysiological course.

KEY WORDS: Lipoprotein receptor-related protein 1 (LRP1), intracerebral Aβ homeostasis, Aβ drainage from the brain, blood-brain barrier (BBB)

Introduction

*Oxidative stress and generation of hydroxyl radical *OH*

A whole range of compound biological and molecular investigations of the Alzheimer's disease (AD) pathophysiology has shown the importance of the cell surface receptor, low-density lipoprotein receptor-related protein 1 (LRP1), in the drainage of the amyloid beta (Aβ) peptide from the brain across the blood-brain barrier (BBB) endothelial cells into the brain capillary circulation. The accumulation of this peptide in the brain structures, induced primarily by its disturbed drainage, and not, according to a number of researchers by its elevated production, leads to evident oxidative damages of a number of important brain structures, which, as it has been proved, are extremely important for the pathophysiological events crucial in the AD development and course [1-5]. Oxidative stress in the organism of living beings, especially elevated in AD, according to a number of investigations, is mostly induced by the effects of free radicals, among them primarily by the hydroxyl radical (*OH), the neutral form of the hydroxyl ion ($^{-}$OH). This radical is generated in the brain during the aggregation and sedimentation of Aβ monomers at the point of interrelated contacts of Aβ strands with the transit of one electron ("e^{-} hop") from the sulphur (S) of methionine (MetS35, nonpolar essential amino acid) on the

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β2-strand of the incoming Aβ2, onto the ferric ion (Fe^{3+}) located on the β1-strand of the earlier fixed Aβ1 (metal binding domain, His13-His14 sequence on the β1-strand). MetS35 on the incoming Aβ2 monomer has the reductive-donator electron function. Oxidised ferric ion (Fe^{3+} on MBD of Aβ1) by reduction (electron gain) transforms into the redox active ferrous ion (Fe^{2+}) that reacts with, in AD, the abundantly present hydroxy peroxide (H_2O_2), with the generation of Fe^{3+}, hydroxyl ion (^{-}OH), and the extremely active and aggressive hydroxyl radical (*OH). These events are presented in the Fenton reaction: $Fe^{2+} + H_2O_2 = Fe^{3+} + {}^{-}OH + {}^{*}OH$. The generated *OH oxidatively attacks the surrounding molecules, primarily located in lipid cell membranes (***Fig. 1, 2***)[1,6-13].

Redox active metal ions, for example the above mentioned ferrous ion (Fe^{2+}), bound to the Aβ, catalyze the production of reactive oxygen species (ROS), among them particularly the hydroxyl radical *OH. *OH oxidatively damages the surrounding molecules, as well as Aβ peptide itself, inducing its conformational changes, and the decline in affinity for LRP1. These changes result in the impairment of Aβ clearance by LRP1. The accompanying destructive oxidation of LRP1, with the consecutive decline of its functions, additionally contributes to the Aβ accumulation in the brain[14,15].

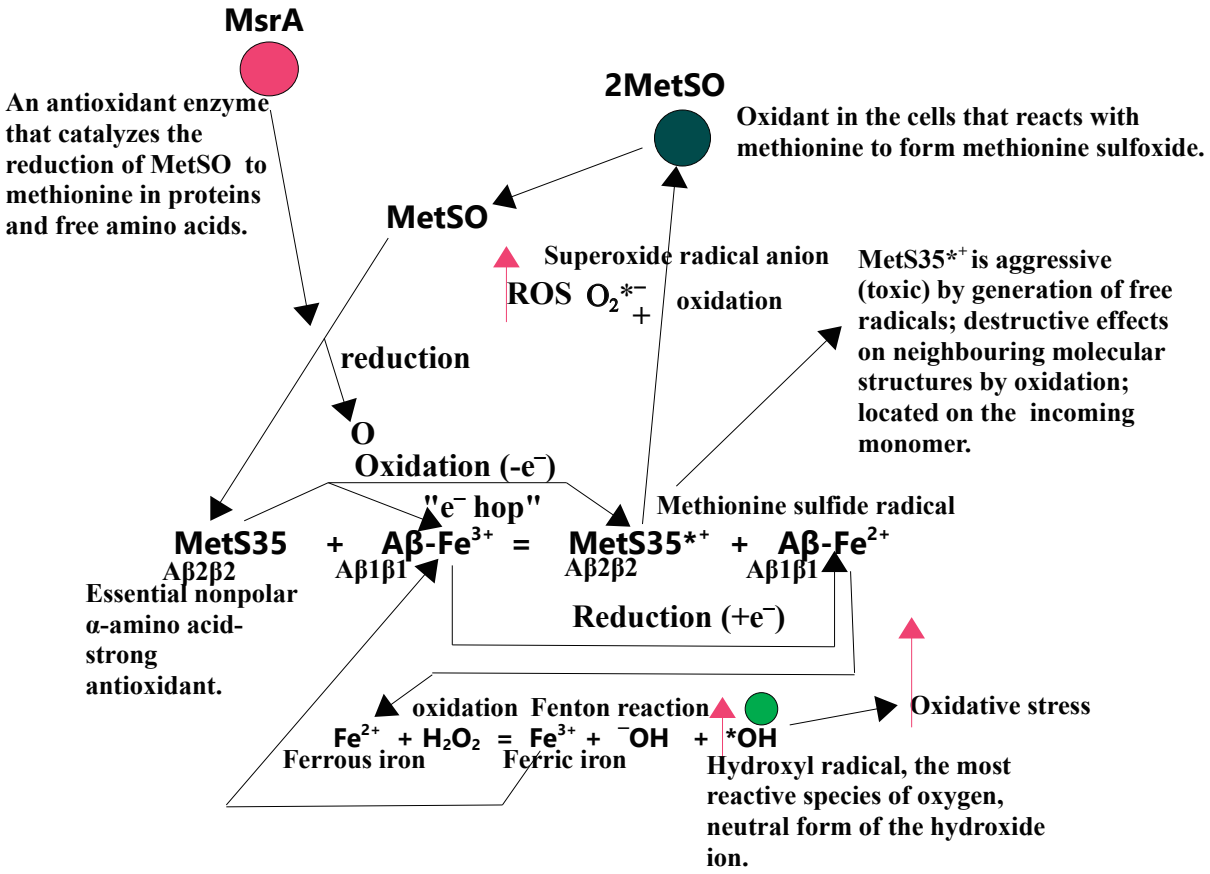

Fig. 1. **Aβ-mediated generation of destructive oxidation compounds.**

The figure presents the crucial biochemical events which result in hydroxyl radical (*OH) generation. Fenton reaction is particularly emphasized. It is a reaction between oxidatively very reactive ferrous iron (Fe^{2+}) and hydrogen peroxide (H_2O_2). Metal ions reduction (electron gain) is possible, not before the distance between MetS35 and their location on the metal binding domain (MBD, -His6, His13, His14) on β1-strand drops below 19Å; MetS35, methionine, location on Aβ2β2-strand, reductant-electron donor; MetS35*+, methionine sulfide cation radical of MetS35, very aggressive; ^{-}OH, hydroxyl ion; oxidation, electron loss; reduction, electron gain; "e⁻hop," electron crossing (without any mediator) through space from the reducing center to the acceptor; MsrA, methionine sulfide reductase type A; MetSO, methionine sulfoxide; $O_2{}^{*-}$, superoxide radical anion; Aβ, amyloid beta peptide; Fe^{2+}, ferrous iron; Fe^{3+}, ferric iron; ROS, reactive oxygen species.

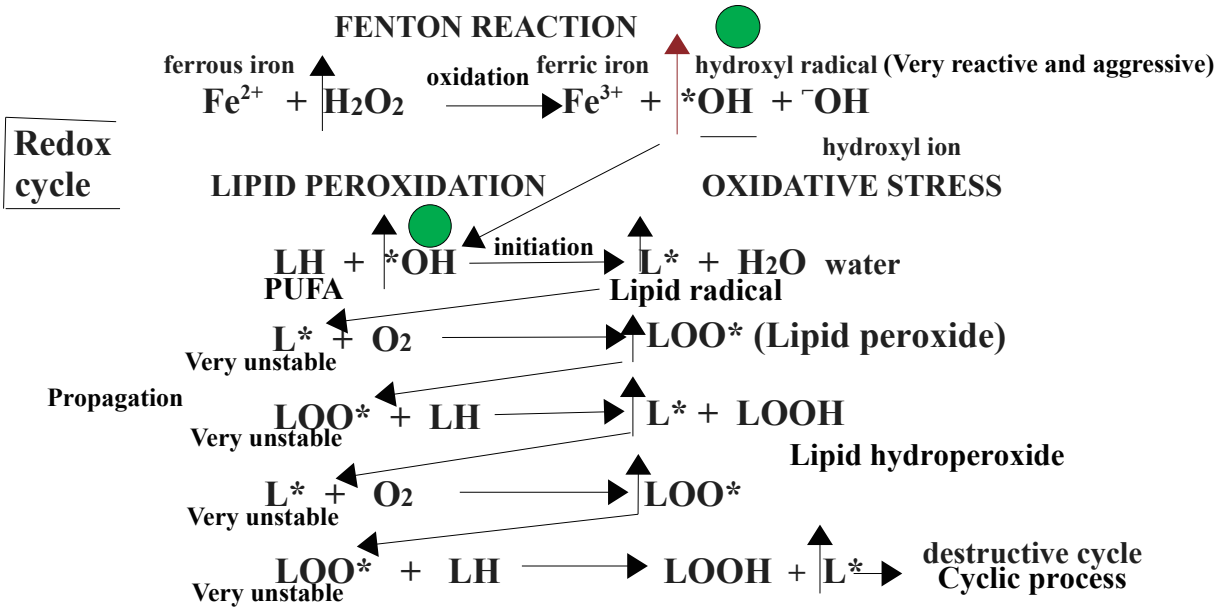

Fig. 2. **Schematic presentation of lipid peroxidation important biochemical processes related to destructive free radical effects on essential molecular structures.**

LH, PUFAs, and hydrocarbon chains containing two or more double bonds; lipid peroxidation, oxidative degradation of lipids; during this process free radicals (on this figure hydroxyl radical, *OH) steal electrons from the lipids in cell membranes with their consequent destruction; between multiple PUFAs double bonds are methylene bridges ($-CH_2$) with especially reactive hydrogen atoms; brain microvascular endothelial cells contain high proportions of PUFAs; PUFAs contain more than one double bond in their structure; at one end these acids have a carboxyl group (COOH); oxidation, electron loss; reduction, electron gain; endothelial cell membranes are specially vulnerable to the effects of *OH; the first step in the lipid peroxidation-production of a fatty acid radical, L^*; in lipid peroxidation it is necessary to distinguish initiation, propagation, termination, and final products; L^* is very unstable, so it reacts readily with molecular O_2 and creates LOO* (lipid peroxide); LOO* is also very unstable and reacts with a free LH; a new lipid radical is produced, and the destructive cycle continues; antioxidants can inhibit lipid peroxidation; *OH can damage virtually all types of macromolecules. It is evident that damaged endothelial cell membrane cannot effectively manage the LRP1/clathrin dependent endocytosis, transcytosis, and exocytosis; one of the final products of the lipid peroxidation is acrolein, a small aldehyde (C_3H_4O) which easily binds to lysine residues of protein; protein-bound acrolein is a potential marker of oxidative and glycative stress and damages to proteins in aging, atherosclerosis and AD; LH, non-oxidised lipids; PUFAs, polyunsaturated fatty acids; Fe^{2+}, ferrous iron; Fe^{3+} ferric iron; ROS, reactive oxygen species. LRP1, low density lipoprotein receptor-related protein; AD, Alzheimer's disease.

The structure and functions of the LRP1 receptor

LRP1, the transmembrane receptor, is the member of a great LRP receptor family. It is located on the cell membranes of a number of brain and other body cells. In the brain it is dominantly located on the abluminal cell membranes of the BBB endothelial cells. As mentioned earlier, it has the central role in the, by the transcytosis mechanism induced, Aβ drainage across the BBB endothelial cells into the capillary blood flow. LRP1 recognizes, binds, and transports over 40 different ligands, among them Aβ is the most significant. The LRP1 receptor in the human and animal organism exists in two forms. One form is bound to the cell membrane of a number of cells and is composed of two components: extracellular α-chain 50-70 nm (500-700Å) long, or magnitude of 515 kDa, and intracellular β-chain magnitude 85 kDa. The transmembrane part is markedly short. The receptor extracellular part consists of 31 cysteine-rich modules, 22 cysteine-rich EGF repeats, and 8 YWTD domains (β-propeller domains). All these modules are connected with covalent bonds, except the bond between the extracellular and intracellular LRP1 part. Using the nuclear magnetic resonance (NMR) spectroscopy, Daly NL *et al.*[16], first revealed the structure of the first cysteine-rich module (CR1) composed of 40 amino acids. The starting-point of the module consists of one β-hairpin structure which is followed by a series of β-turns. Subsequently, undertaken

crystallography analysis of the CR5 module has shown that this module is actually a cage in which the calcium ion stabilizes the module structure. The module consists of 6 cysteines bound by disulphide bonds. The accumulation of negatively charged amino acids is predominantly situated on one module side (Asp15, Asp26, Glu30, Asp23, Asp36, Glu37, Glu40). This paper does not address the description of the EGF-repeats (epidermal growth factor [EGF]-like repeats) and YWTD domain structures, however, it is important to present intracellular motifs which have a crucial role in the Aβ peptide drainage. Especially important are two NPXY motifs, two di-leucine motifs, and the essential YXXL motif. Although all these motifs have a role in the Aβ drainage process, the most important is the YXXL motif, which is activated by the phosphatidylinositol-binding clathrin assembly protein (PICALM), strongly activates endocytic and scaffold adaptors, causing the Aβ/LRP1 complex entrance into endosomes and the consequent clathrin-dependent transcytosis. All this indicates that the binding of Aβ to the receptor induces conformational changes on both receptor segments, enabling the PICALM access to the YXXL motif and its strong activation. The other form of LRP1 is free or bound with Aβ in the spinal liquid and in the blood. This form of receptor, sLRP1, transports Aβ to body locations where Aβ is disintegrated. The pulling forces generated by the binding of Aβ with the ligand binding cluster II and IV induce the spread of the PICALM access to the YXXL motif, their interaction, and strong YXXL motif activation. The result is the adaptor activation with the PICALM and clathrin accumulation in the region around the receptor tail with the beginning of PICALM/clathrin transcytosis and exocytosis. On the other hand, the NPXY and di-leucine shift toward the abluminal cell membrane induces the detachment of G-protein-coupled receptor (GPCR) α-segment, with the adenylate cyclase activation, transformation of adenosine triphosphate (ATP) into cyclic adenosine monophosphate (cAMP), the activation of protein kinase A (PKA), phosphorylation of serine 76 (SE76) on the receptor tail and a strong adaptor activation. All this has a favourable effect on the Aβ/LRP1 transcytosis and exocytosis into the capillary blood (***Fig. 3***)[4, 18-20].

A number of researchers emphasize that the binding of Aβ with the negative charges of ligand binding clusters II and IV amino acids on the extracellular segment of LRP1 induces conformational changes on the binding place, as well as on the receptor tail structures. These changes, induced by electrostatic forces, manifest themselves on the immediate binding place with the approach of two included CR modules, most probably, CR26 and CR27, with the onset of strong pulling forces which act from both sides of the contact point and pull the adjoining receptor segments towards the contact point. The pulling forces propagate with great velocity towards the apical NH_2 end and towards the receptor tail (COOH end). The velocity of the force propagation is incomparably greater than the velocity of activated receptor segments. The majority of published papers related to the mentioned conformational changes do not give their detailed description. Future investigations have to describe and explain these phenomena with exactitude (***Fig. 3***)[21, 22-24].

Before further analysis, it is important to emphasize that there exist two types of AD: early onset AD (EOAD, 5% of all cases) induced by amyloid precursor protein (APP) gene – APP gene (chr. 21Q21), BACE1 gene (chr. 11Q23.3), PSEN1 gene (chr. 14), and PSEN2 gene (chr. 1). Mutations in the APP gene induce its strong production and activity with a high generation of Aβ. Mutations of BACE1, PSEN1, and PSEN2 induce proteolysis and the fragmentation of APP with a strong accumulation of Aβ in extracellular space. The late onset AD (LOAD, 95% of all cases) is linked with two genes, ADAM10 gene (chr. 15Q21.3) and allele ApoEε4 of APOE gene (chr. 19Q13.32). Two mutations in the first gene induce a drop in the cleaving of the APP transmembrane protein, and the mutation in the second gene induces a drop of APOE protein lipidation and Aβ binding. In both AD types the role of LRP1 is the same[25].

The decline of LRP1 expression in the BBB endothelium during ageing and in AD. Following are research papers whose authors present the mentioned expression decline connected with life age.

Silverberg GD *et al.*[26, 27], based on experiments on rats, emphasize the evident capillary LRP1 expression decline during the ageing process. This decline is inversely proportional to the parallel expression elevation of the receptor for advancing glycation end products (RAGE). They emphasize that alterations in the LRP1 expression evidently contribute to the Aβ accumulation in senescence. This accumulation is also typical for AD. The explanation for this phenomenon lies in the RAGE gene, where, connected with aging and AD, there is decreased methylation in its promoter region. Otherwise, during the aging process and in AD, the increased accumulation of AGEs has been found, as well as increased values of Aβ, cytokines (interleukin 1, tumor necrosis factor), and of nuclear factor kB, which all can decrease the promoter methylation and RAGE gene transcription. The transport of Aβ across the BBB is carried out by LRP1 and at the same time, by the phosphoglycolate phosphatase receptor (P-gp), which during ageing also has a decreased expression. However, the authors of the study give preference to the LRP1 transport.

Kang DE *et al.*[28], by measuring the LRP 85 kDa light chain level in the section material from the midfrontal cortex of individuals deceased without AD signs, and by comparing this material with the material from AD brains, have found approximately a two-times higher LRP1 level in brains without AD signs. In the brain of individuals non affected by AD, the strong inverse correlation between life age and the LRP1 level ($n = 39$, $r = -0.4905$, $p = 0.0015$) has been found, indicating that with the rise of age the LRP1 expression normally declines. The average expression reduction is two times higher in the group aged 40-60 years than in the group aged 85 and more. The inverse correlation is even stronger in the bearers of alleles different from APOEε4. In AD individuals where the disease has begun in the later age, the authors have found greater LRP1 values, which indicates the possibly LRP1 protective effect against the disease.

Osgood D *et al.*[29], emphasize the drop of the possible toxic metabolite clearance, linked with the changes induced by ageing, as probably the strong factors of the Aβ and other metabolites accumulation in the brain of the elderly and in AD. According to these researchers, ageing is the strongest risk factor for the AD occurrence. In the course of ageing there is an increasing drop in LRP1 and P-glycoprotein (P-gp, besides LRP1, a very strong Aβ efflux receptor),

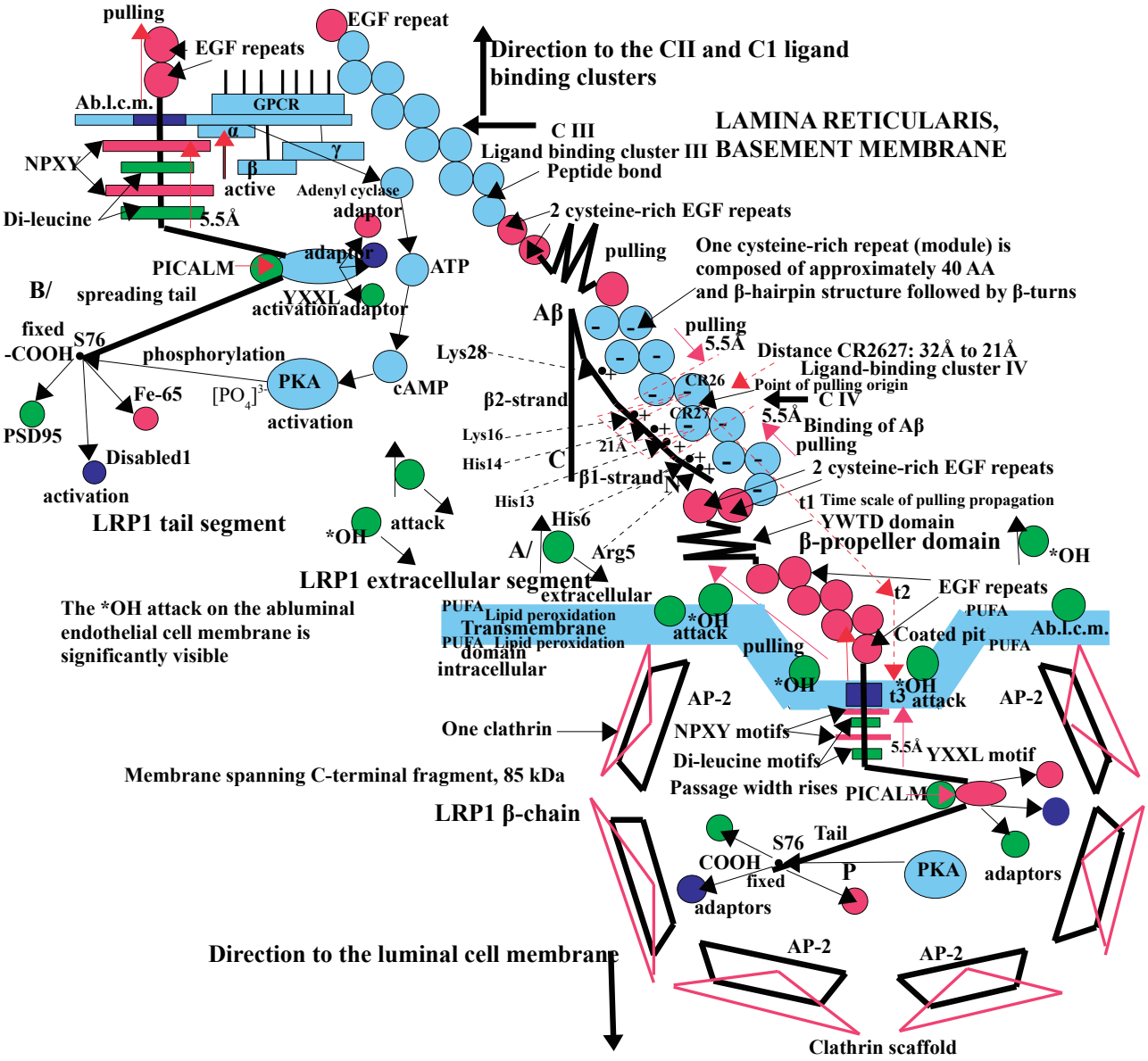

Fig.3. **Partial presentation of LRP1 (CD91) receptor molecule (CIII and C IV).**

Fourth ligand-binding cluster contains 11 cysteine-rich ligand-binding repeats (blue circles); in the direction to the abluminal cell membrane there are the positions of nine EGF repeats and one YWTD domain (β-propeller domain); in the tail there are visible 2 NPXY motifs, 2 di-leucine and 1 YXXL motif. Aβ binding to the LRP1 ligand binding domains II and IV (on the figure, only domain IV is visible) induces the structural changes of both, the extracellular ligand binding domain, and receptor cytoplasmic tail. The receptor tail has 2 NPXY motifs, 1 YXXL motif, and 2 di-leucine motifs. The binding of PICALM with YXXL motif induces the recruitment of clathrin and AP-2 on the cell membrane at the position of coated-pit formation. PICALM/clathrin-dependent transcytosis begins.

PICALM, phosphatidyl inositol binding clathrin assembly protein; AP-2, adaptor protein 2; EGF-repeats, epidermal growth factor (EGF)-like repeats; Aβ, amyloid beta peptide; LRP1, low density lipoprotein receptor-related protein; GPCR, G-protein-coupled receptor; PKA, protein kinase A; PSD95, postsynaptic density protein 95; PUFA, polyunsaturated fatty acids; ab.l.c.m,. abluminal lipid cell membrane.

as well as the receptor expression increase in the receptor for advanced glycation end products (AGEs). They have proved this by measuring the mRNA LRP1, P-gp, and RAGE expression from the isolated cerebral microvessels. In these events, the transcriptional changes connected with aging have a crucial role. Their experiments suggest that gene transcription (in endothelial cell nucleus) is altered by some upstream events rather than post-transcriptional events. Transcriptional alterations of the gene expression may occur via modification of the gene promoter or by histone modification. Their study included a hundred male F344/BN hybrid rats marked with similar characteristics. Histone modifications have been observed during aging connected with the stimulation or destimulation of a series of related genes. During the aging process, as well as in AD, acetylation (a reaction that includes an acetyl functional group CH_3CO into an organic chemical compound) has been observed, as well as methylation (the addition of the methyl group $^-CH_3$ on a substrate), of a histone with the consequent expression change of the important neurotropic brain factors. The authors of the study have also found, related to the LRP1 expression drop, a significant decline of the PICALM expression. The drop of the PICALM expression induces an evident decline in the clearance of Aβ across the BBB of laboratory rats. In the hypoxia and hypoglycemia conditions in tissue cultures, the LRP1 mRNA level is decreased and the mRNA RAGE level is elevated. These effects can be partially repaired by the use of *Ginko biloba* extract (***Fig. 4, 5***).

Liu X *et al.*[30], in their review paper emphasize that epigenetic mechanisms play a crucial role in the development and course of AD. Among them, the most important are the histone modifications and DNA methylation. Histone acetylation catalyzed by histone acetyltransferase (HAT) induces the decondensation of chromatin with the generation of euchromatine that is transcriptionally active. Condensate chromatine type, *i.e.* heterochromatin, is compact and transcriptionally inactive. This is the deacetylated type of chromatine obtained through deacetylation catalyzed by enzyme histone deacetylase (HDAC). Otherwise, chromatin, a nucleosome polymer, is composed of two almost complete DNA turns wrapped around the histone octamer. Chromatin is extremely flexible and dynamic and can exist as heterochromatin or euchromatin. In AD, a rise of specific types of HDACs has been found. The decline of their expression induces the rise of histone acetylation and the improvement of the AD clinical situation. The authors also point out that the elevated level of promoter methylation of certain genes related to AD, and a mild cognitive impairment (MCI) are correlated with the transition from MCI to AD, or with the deterioration of the AD clinical situation. This level is markedly elevated in MCI and in AD as compared to healthy individuals. The authors also emphasize that the global DNA methylation in mononuclear cells of the periphery blood in LOAD patients shows significantly higher values in relation to the healthy control examinees. They also emphasize that higher methylation levels are correlated with the presence of ApoEε4 allele (***Fig. 5***).

Yi SJ *et al.*[31], point out that the general loss of histones and nucleosomes as the mark of ageing exists in the range from yeast (yeast, single cell eukaryotic microorganisms) up to humans. Here, it is necessary to present a detailed analysis of the complex process of acetylation and deacetylation of the mentioned histone octamer, because it is certain that these two processes are included in the LRP1 receptor expression. By the HAT action, the electronegative acetyl group (CH_3CO) is inserted onto the electropositive histone tail, and after this inclusion, the tail becomes less electropositive. The consequence is the drop in the electrostatic attraction between positive histone N-termini located on the tail and negatively charged phosphate groups on the DNA strand. Condensed chromatin (heterochromatin) transform into relaxed euchromatin, which leads to gene transcription increase and to an adequate protein expression. HDAC moves the electronegative acetyl group from the positive histone tail, leading to histone deacetylation. Following, there is a rise of electrostatic interaction between histone N-termini on the histone tail and the negative phosphate group on the DNA strand. The level of condensed chromatin rises. Euchromatin transforms into heterochromatin that is more compact and transcriptionally inactive, and the gene transcription declines (***Fig. 5, 6***).

Xu K *et al.*[32], point out the crucial characteristics of histone acetylation and deacetylation, the two fundamental processes related to the AD pathophysiology. The first process is catalyzed by the enzyme HAT, and the second one by HDAC. Their experiments were performed on laboratory rats (mouse models of AD). For tissue analyses, autopsy material from the brain of individuals deceased from AD was used. The histone acetylation is characterised by the insertion of the CH_3CO electronegative group into the histone tail which is electropositive. The result is the decrease of the total positivity of the tail, and consequently, to the decrease of the interaction between the DNA phosphate and the tail. All this induces conditions for the generation of free noncondensed euchromatin and gene transcription. HDAC catalyzes deacetylation, *i.e.* the ejection of CH_3CO from the histone tail that becomes more positive. Its connection with DNA phosphate becomes stronger, and this leads to favourable conditions for chromatin condensation and the drop of gene transcription. The level of histone acetylation plays an important role in chromatin condensation and gene expression. HDAC inhibitors, presently under serious investigations, improve the memory and cognition in mouse models. However, their nonselective use can be inefficient, even dangerous, so their application on patients is still not safe.

DNA methylation is catalyzed by a family of DNA methyltransferases (Dnmts) that transfer a methyl group from S- adenosyl methionine (SAM) to the 5th C atom of cytosine residue, to form 5mC. The $^-CH_3$ group is not a dipole. It is a nonpolar group. The role of Dnmt is to separate the methyl group from SAM, to catalyze its transport to the C5 of the cytosine residue, and after the H deprotonation from C5, to attach it to the empty C5 place. These events induce the consequent decline of the DNA transcription. Recently, it became evident that DNA methylation and demethylation have a crucial role in epigenetic events related to gene activity [33].

Collins BE *et al.*[34], emphasize that the addition of methyl groups to the histone tails induces histone methylation. The consequence is the generation of the condensed state of chromatin, the heterochromatin that blocks the histone binding to DNA phosphate and decreases the rate of transcriptional expression. The bilaterally induced strong

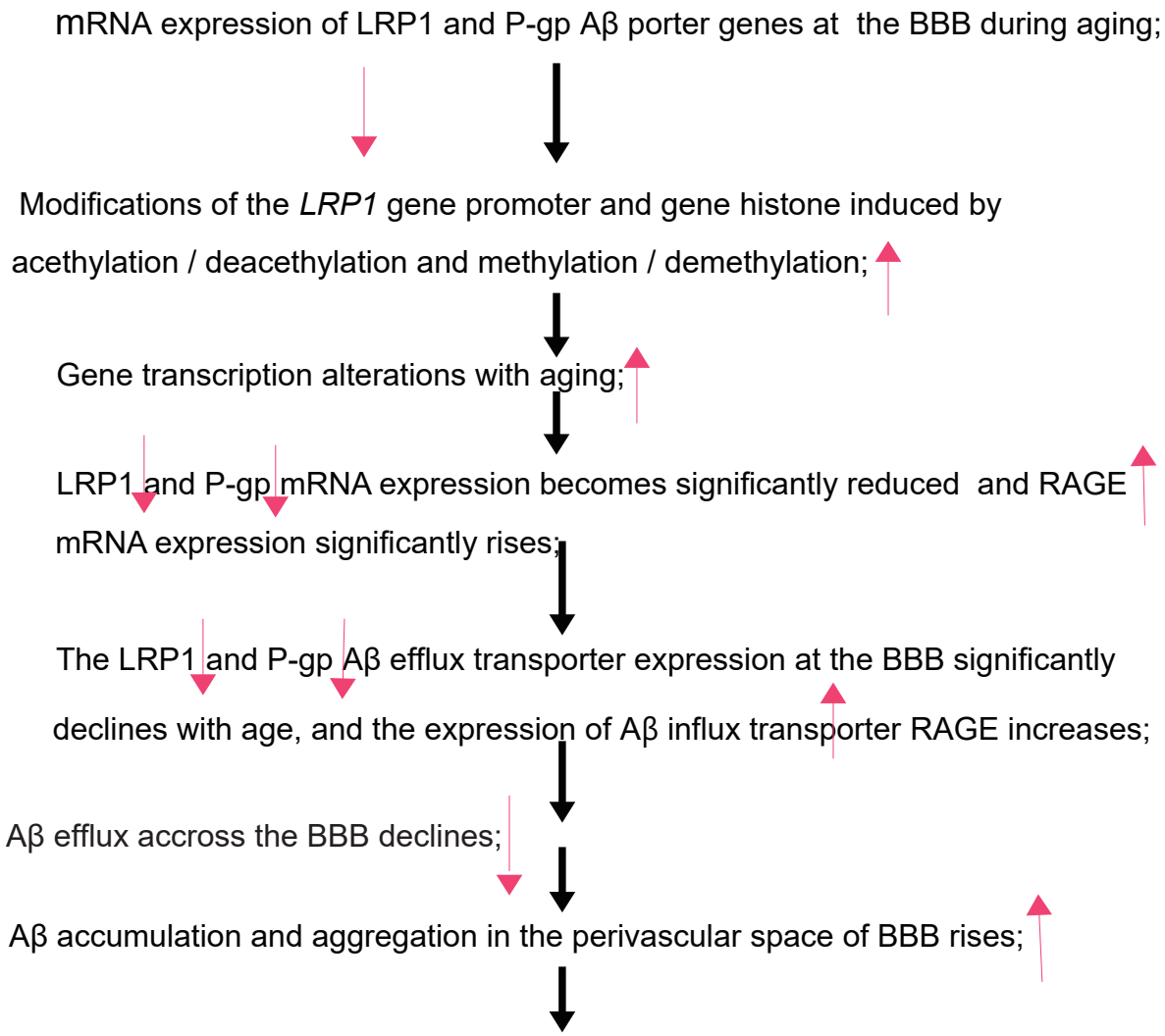

Fig. 4. **The course of events that precede the Aβ accumulation and aggregation in the perivascular space of BBB induced by aging.**

mRNA, messenger RNA; RNA (ribonucleic acid) contains sugar ribose; ribose, monosaccharide, carbohydrate with molecular formula $C_5H_{10}O_5$; P-gp, P-glycoprotein, ATP-binding cassette (ABC) transporter; gene promoter, the region of DNA where transcription of gene is initiated; gene histone, a component of a nucleosome that is composed of two DNA turns wrapped around a histone protein octamer, it is associated with the regulation of gene expression; gene transcription, the process of making a RNA copy of the gene sequence; acetylation, chemical reaction that introduces an acetyl functional group (CH_3-C(=)-O-); methylation, the addition of a methyl group (-CH3) on a substrate; perivascular space, crucial for Aβ drainage, intra-mural peri-arterial drainage pathway (IPAD), on the inner side, the space is limited with endothelium and inner basement membrane of smooth muscle cells (SMCs), on the outer side the space is limited with the outer basement membrane of SMCs; vessel pia, Virchow-Robin space (pial funnel), cortical pia, paravascular space, and astrocyte processes of glia limitans, in the region of BBB there is no perivascular space because both SMC membranes are joined in one membrane; in this vascular segment there exist only endothelium, joined SMC membrane, and basement membrane of glia limitans, inducing Aβ accumulation; LRP1, low density lipoprotein receptor-related protein; Aβ, amyloid beta protein; BBB, blood-brain barrier; RAGE, receptor for advanced glycation end products. DNA, deoxyribonucleic acid; SMC, smooth muscle cell;

Chromatin is a complex of DNA and proteins (histones). HAT catalises histone acetylation in decondensated form, euchromatine, which is transcriptionally active. By HDAC euchromatin transforms into heterochromatin, which is compact and transcryptionally inactive.

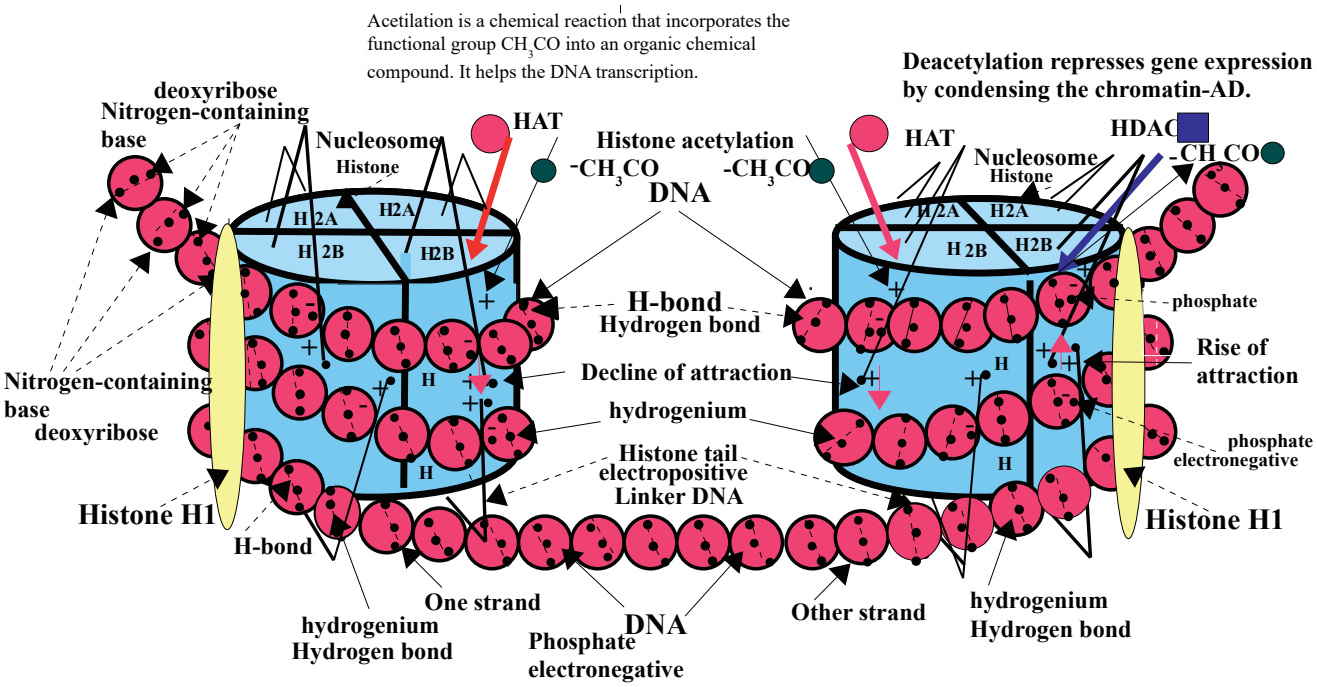

Fig.5. **Schematic presentation of the DNA with histone acetylation and deacetylation – LRP1 gene.**

DNA is a complex molecule composed of two chains that coil around each other to form a double helix carrying genetic information for the development functioning, growth, and reproduction of all known organisms and many viruses. Two DNA strands are composed of simpler monomeric units called nucleotides; Each nucleotide contains one of four nitrogen-containing nucleobases (cytosine, C, guanine, G, adenine, A, or thymine, T). Two DNA chains are joined to one another by H-bonds; Nucleosome is a specific structure that belongs to the cell nucleus; it is a functional unit of chromatin; lying along the DNA strand, a nucleosome is composed of eight histone molecules and approximately two DNA turns wrapped around a set of histones. Histones associate with DNA and help the DNA to condense into chromatin; a nucleosome height is about 6 nm; each DNA turn around the histone octamer complex has the diameter of about 2 nm; histones are positively charged proteins and strongly adhere to negatively charged DNA phosphates; histones protect and regulate gene expression; histone tails project from the nucleosome and some residues in these tails after modification, can affect transcription; HAT can catalyze its acetylation and as a consequence the enhanced rates of gene transcription; HDAC represses the gene expression by condensing the chromatin; histone modifications have a crucial role in the development and course of AD; LRP1, low density lipoprotein receptor-related protein 1; DNA, deoxyribonucleic acid; HAT, histone acetyltransferase; HDAC, histone deacetylation catalyzed by histone deacetylase; AD, Alzheimer's disease.

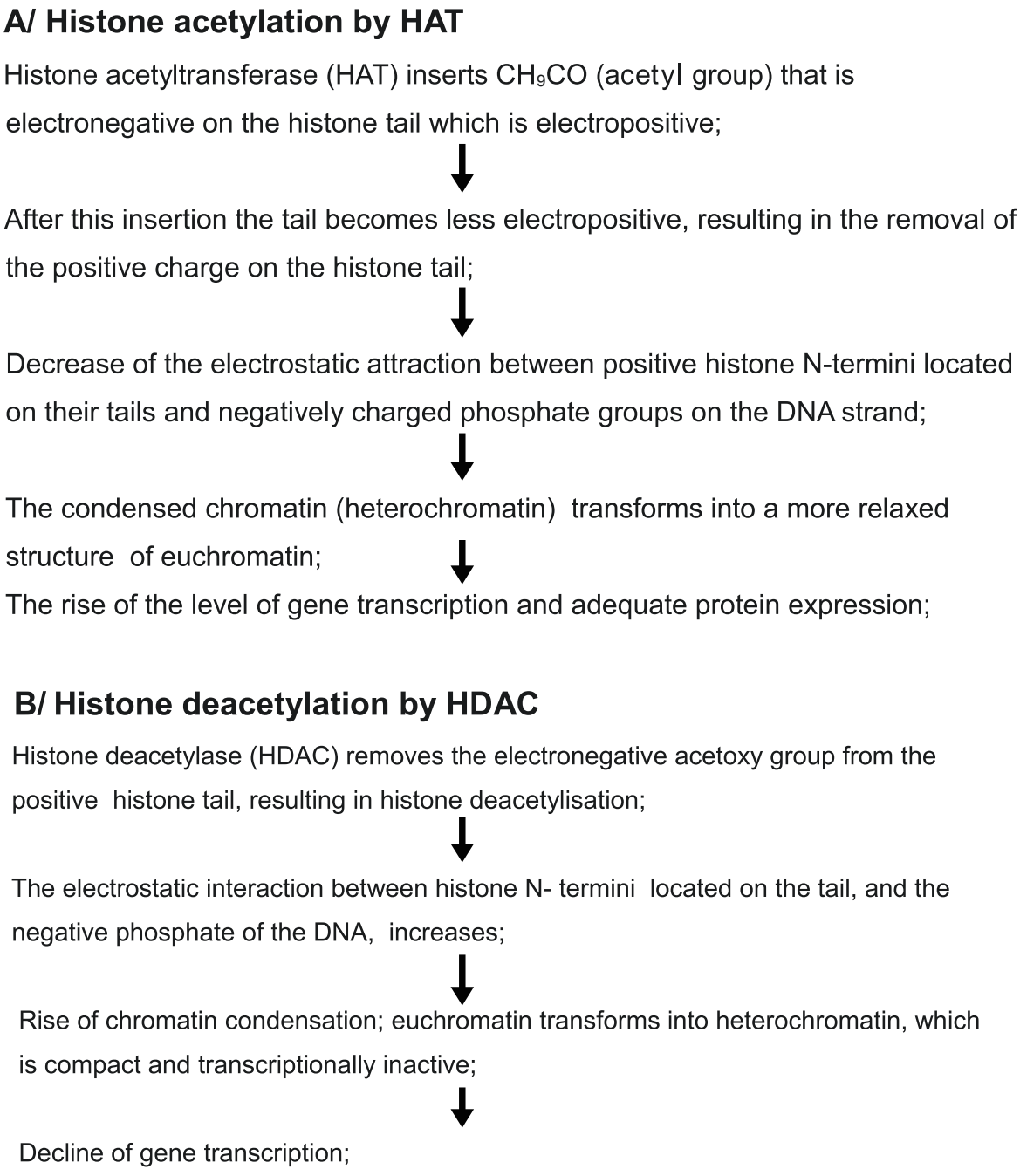

Fig.6. **Presentation of the histone acetylation and deacetylation.**

Due to the HAT action, the electronegative acetyl group (CH_3CO) is inserted on the histone tail that is electropositive. After this insertion, the tail becomes less electropositive. Consequently, there is a drop of electrostatic attraction between the positive histone N-termini located on the tail and negatively charged phosphate groups on the DNA strand. The condensed chromatin (heterochromatin) transforms into relaxed euchromatin, resulting in the rise of gene transcription and adequate protein expression. HDAC moves the electronegative acetyl group away from the positive histone tail, leading to histone deacetylation. The consequence is the rise of electrostatic interaction between histone N-termini on the histone tail and the negative phosphate group on the DNA strand. The chromatine condensation rises. Euchromatin transforms into heterochromatine which is more compact and transcriptionally inactive. The gene transcription declines; HAT, histone acetyltransferase; HDAC, histone deacetylase; DNA, deoxyribonucleic acid.

connection blocks the link between histone and DNA and reduces the transcriptional expression. Histone methylation is induced by histone methyltransferases (HMTs). However, it is found that lysine 4 on histone H3 (H3K4) methylation activates gene expression, and lysine 27 on histone H3 (H3K27) methylation represses gene expression. DNA methylation (induced by DNA methyltransferases), located in the gene promoter, usually represses gene transcription.

Storck SE *et al.*[35], emphasize that according to the neurovascular hypothesis, the decreased expression of LRP1 in brain capillaries of BBB contributes to neurotoxic Aβ brain accumulation and accelerates AD pathology. In their experiments, the authors have developed a transgenic mouse strain on which they have investigated tamoxifen-inducible selective deletion of LRP1 in the mouse brains, accompanied by a strong efflux reduction across BBB, of the previously injected Aβ1-42 in the brain. Their results show that receptor-mediated Aβ clearance can be a potential target for the treatment and prevention of Aβ accumulation in the AD brain.

Shibata M *et al.*[36], in their study present experimental results related to the intracerebral microinjections of 1251-Aβ1-40 in young and adult wild-type mice. LRP1, abundant in microvessels in young mice, was downregulated in older experimental animals, and this downregulation correlated with regional Aβ accumulation in brains of Alzheimer's units. The authors conclude that the BBB removes Aβ from the brain largely via age-dependent LRP1-mediated transport that is influenced by α2M and/or ApoE, and may be impaired. The importance of the optimal LRP1 expression should be seen in the context of significantly declined lipidation (covalent binding of the lipid group to the peptide chain) of the lipoprotein APOEε4 as related to the other two alleles, APOE2 and APOE3. The lipidation of APOE lipoprotein increases its interaction and binding with Aβ, leading to the APOE/lipid (LDL or HDL) Aβ complex. The generated complex, by effects of vasomotion and two receptors, LRP1 and ABCB1 (P-glycoprotein, ATP-dependent translocase ABCB1), is promptly transported along the perivascular space and drained in the angular neck lymphatic nodes into the venous blood. ABCB1 is located on the the luminal and abluminal sides of the BBB endothelial cell membranes. It is an active mediator for the Aβ transport out from the brain.

APOEε4 (the dominant allele in LOAD) lipidated by ABCA, but significantly weaker in relation to the other two alleles, has evidently weaker linking with Aβ, and the generated complex Aβ/APOEε4/HDL or LDL transport is also weaker. The result is stronger Aβ accumulation and aggregation in the perivascular space (PVS). Otherwise, a drop of the ABCB1 and of the LRP1 expression has been established in normal ageing, as well as in the AD patients. All this indicates that due to the presence of APOEε4 allele (typical for LOAD) and its unfavourable effects on the perivascular drainage, the LRP1 effects would be of crucial importance for the AD course retardation. However, the decreased LRP1 expression, typical for the aging process and AD, cannot adequately compensate Aβ accumulation and aggregation, nor the accompanying oxidative stress[13].

LRP1 as a therapeutic factor in AD

Sagare AP *et al.*[37], point out that LRP1 is the main cell surface receptor involved in the brain and in the systemic clearance of AD toxin Aβ. There are two forms of LRP1: the plasmatic soluble form sLRP1, which is the major transport protein for peripheral Aβ, and the brain endothelium LRP1, the critical factor for the drainage from the brain by clathrin-coated transcytosis across BBB endothelial cells. The soluble form sLRP1 obstructs the possible return of free plasma Aβ into the brain. In the liver, the local LRP1 performs the degradation and clearance of Aβ that has arrived by the blood stream. The LRP1 receptor is encoded by the gene located on chromosome 12q13-q14. As Kang DE *et al.*[28], Shibata M *et al.*[36], and Silverberg GD *et al.*[26, 27], emphasize, the LRPI expression at the BBB is reduced during both, normal aging and AD. To correct the decreased LRP1expression in the brain, Sagare AP *et al.*[37], suggest three sets of actions: (a) lifestyle changes (*e.g.*, diet, exercise, and enriched environment), (b) pharmacological agents (*e.g.*, statins, plant-based active principles), and (c) gene therapy. They emphasize that potential points of intervention are: (1) restoration and/or enhancement of BBB LRP1 expression, (2) replacement of dysfunctional sLRP1, and (3) restoration and/or enhancement of the hepatic LRP1 expression. Analyzing the first set of actions (a), Sagare AP *et al.*[37], emphasize the great importance of antioxidant nutrients (polyphenol-rich foods, vitamin A, C, and E, green tea, extra virgin oil) which could protect LRP1 from oxidative damage. Physical activity can improve cerebral angiogenesis in rodents and humans. Otherwise, in AD, cerebral angiogenesis is retarded and decreased. Elevated cholesterol levels can increase the risk of AD. To reduce these levels, the optimal solution is to use statins which are cholesterol lowering drugs. Among them, simvastatin and atorvastatin are especially effective in the brain. Statins also increase the LRP1 expression in the liver. The authors point out that effective gene transfer can be achieved by viral based systems which are very effective in mediating cell entry and transfer of genes to the target cells. Among vectors for gene therapy, Adeno-associated virus (AVV) is frequently used as a vector for these therapies due to its possibility to induce the long-term gene expression in different cells. This virus is unable to autonomously replicate, and so is not pathogenic. The AAV-2 serotype has been used in neurological studies in mouse models of lysosomal storage disease and Parkinson disease. The authors prefer the direct targeting of the gene transfer vector to the BBB. In the direct approach, vector targeting is mediated by a small peptide that has been inserted into the viral capsid sequence with the modification of viral tropism. It is possible to enhance the mentioned targeting by identifying molecular signature epitopes in the cerebral endothelial cells of AD brains, and by presenting these epitopes on the capsid of AAV. It is evident that the use of AAV-2 which carries LRP1 DNA or its smaller fragments, leads to the restoration of LRP1 expression in the brain vascular endothelial cells in AD.

Shinohara M *et al.*[38], emphasize that statins could reduce the risk of AD. Fluvastatin at clinical doses significantly reduces Aβ and amyloid precursor protein C-terminal fragment (APP-CTF) levels in the brain of C57BL/6 mice. This effect is blocked by LRP1 antagonists.

Qosa H *et al.*[39], show the results of their experiments where the elevated Aβ clearance from the wild-type mice brain across the BBB is obtained with rifampicin and caffeine. Both preparations have induced an elevated expression of P-glycoprotein (P-gp) in the BBB endothelium. The elevated LRP1 expression was obtained only by rifampicin.

Tamaki C *et al.*[40], present the results of their investigations related to the effects of insulin in the Aβ (1-40) clearance by the liver. The portal infusion of insulin leads to the evident rise of LRP1 expression on the hepatocyte plasma membrane. The insulin in the plasma accelerates the LRP1 crossing from the intracellular stores onto the liver cell membranes.

This leads to significant influx of Aβ from the circulating blood into the hepatocytes and its adequate degradation. This explains why the type II diabetes is an evident risk factor for AD.

Shinohara M *et al.*[41], in their study discuss potential mechanisms underlying the LRP1 effects on the AD pathogenesis through Aβ-dependent and independent pathways, reviewing both clinical and preclinical studies. They also discuss potential therapeutic strategies for AD by targeting LRP1. In this respect, ***Fig. 3*** as part of their comprehensive presentation, shows the potential therapeutics for AD which are mediated through LRP1. They present pharmacological and nonpharmacological approaches which could be promising therapeutic strategies for AD. The pharmacological approaches include: statins (a class of drugs that lower cholesterol levels in the blood), *Withania somnifera* (Indian ginseng, a rejuvenating herbal drug, important in the Indian subcontinent traditional medicine), oleocanthol, cannabinoid, intravenous immunoglobulin (IVIG), and α-secretase inhibitors. The nonpharmacological approach includes environmental enrichment, electroacupuncture, and physical exercises. According to the authors, environmental enrichment can ameliorate cognitive function and amyloid pathology in amyloid AD mouse models. This enrichment improves the vascular dysfunction by increasing angiogenesis accompanied by upregulation of LRP1, ApoE, and α2-macroglobulin, as well as the downregulation of RAGE, so that this reduces Aβ deposition and restores cognitive impairment in APPV7171 transgenic mice. At the same time, an increase of LRP1 was found in the hippocampus. Exercise ameliorates brain Aβ deposition and cognitive decline in APP transgenic mice with upregulation of LRPI. Oral administration of statins promotes Aβ clearance by increasing cerebrovascular LRP1 in wild type mice and APP transgenic mice. Oleocanthol (a type of natural phenolic compound) from extra virgin olive oil upregulates LRP1 at the BBB, and enhances Aβ clearance. Cannabinoid (naturally occurring compounds from *Cannabis sativa* plant) treatment also enhances Aβ transit at the BBB with increased LRP1 levels at the BBB. Antioxidant N-acetylcysteine combats oxidative stress and improves the clearance of Aβ at the BBB. It is evident that pharmacological approaches to increase LRP1 in the cerebral vasculature are more promising strategies for AD therapy. Statin treatment can also upregulate LRP1 in the liver as well as in the brain. These findings are obtained from cellular and animal models, so it is extremely important to carefully explore their effects on humans.

At the end of this presentation, it is necessary to mention the study by Kanekiyo T and Guojun B[5], where they present possible therapies for AD by the use of LRP1. Presently, a number of researchers also work on the problem of the LRP1 expression modifications in AD therapy. It is evident that the improvement of existing approaches and finding new ones related to the mentioned modifications will increase the LRP1 expression in AD therapy. This would be one of the serious challenges in the elimination of AD[42,43].

Conclusion

The comprehensive analysis of the LRP1 receptor role in Aβ drainage from the brain shows its crucial role in this vital physiological process. Optimal Aβ homeostasis, *i.e.* optimal balance between the production and elimination of Aβ from the brain structures and from the blood, is crucial for the regular function of a range of physiological events whose disorder leads to brain metabolic catastrophe. In AD patients, this disorder of the mentioned homeostasis causes the acceleration of AD rapid destructive pathology, accompanied with the evident destruction of the patient's personality, and at the end, the patient's complete helplessness, and inevitable death. The LRP1 receptor dominantly located on the BBB endothelial cell membranes, with strong expression and activity, decelerates the course of this chronic and lethal neurodegenerative disease, and gives justified hope that this disease could potentially be eliminated in the near future. This hope is supported by a number of cell and animal experimental models, which reveal new diagnostic and therapeutic possibilities. In the scientific community, particularly in highly developed countries, it is necessary to activate all available resources with the aim to improve AD therapy, especially by the therapeutic application of LRP1 receptors.

Conflicts of Interest

The author states that there is no conflicts of interest.

References

1) Mawuenyega KG, Sigurdson W, Ovod V, et al. Decreased clearance of CNS amyloid-β in Alzheimer's disease. *Science*. 2010; 330: 1774.
2) Zuroff L, Daley D, Black KL, et al. Clearance of cerebral Aβ in Alzheimer's disease: reassessing the role of microglia and monocytes. *Cell Mol Life Sci*. 2017; 742167-2201.
3) Hawkes CA, Sullivan PM, Hands S, et al. Disruption of arterial perivascular drainage of amyloid b from the brain of mice expressing the human APOE e4 Allele. *PloS One*. 2012; 7(7): e41636.
4) Ramanathan A, Nelson AR, Sagare AP, et al. Impaired vascular-mediated clearance of brain amyloid beta in Alzheimer's disease: The role, regulation and restoration of LRP1. *Front Aging Neurosci*. 2015; 7: 136.
5) Kanekiyo T, Guojun B. The low-density lipoprotein receptor-related protein 1 and amyloid-β clearance in Alzheimer's disease. *Front Aging Neurosci*. 2014; 6: 93.
6) Barić N, Cetina M. Initial bonding of ferric ion (Fe^{3+}) and amyloid beta (Aβ) peptide as the precondition for oxidative stress in Alzheimer's disease. *Glycative Stress Res*. 2017; 4: 250-265.
7) Persson T, Popescu BO, Cedazo-Minguez A. Oxidative stress in Alzheimer's disease: Why did antioxidant therapy fail? *Oxid Med Cell Longev*. 2014; 2014: 427318.
8) Huang WJ, Zhang X, Chen WW. Role of oxidative stress in Alzheimer's disease. *Biomed Rep*. 2016; 4: 519-522.
9) Barić N. Role of advanced glycation end products (AGEs) on the reactive oxygen species (ROS) generation in Alzheimer's disease amyloid plaque. *Glycative Stress Res*. 2015; 2: 140-155.
10) Arslan J, Jamshed H, Qureshi H. Early detection and prevention of Alzheimer's disease: Role of oxidative markers and natural antioxidants. *Front Aging Neurosci*. 2020; 12: 231.
11) Misrani A, Tabassum S, Yang L. Mitochondrial dysfunction and oxidative stress in Alzheimer's disease. *Front Aging Neurosci*. 2021; 13: 617588.
12) Cioffi F, Adam RHI, Broersen K. Molecular mechanisms and genetics of oxidative stress in Alzheimer's disease. *J Alzheimers Dis*. 2019; 72: 981-1017.
13) Barić N. Pathophysiological reasons for the failure of the cerebral perivascular drainage and progression of Alzheimer's disease. *RAD 544. Medical Sciences*. 52-53. 2020; 544: 42-54.
14) Rivas-Arancibia S, Rodriguez-Martinez E, Badillo-Ramirez I, et al. Structural changes of amyloid beta in hippocampus of rats exposed to ozone: A Ramon spectroscopy study. *Front Mol Neurosci*. 2017; 10: 137.
15) Cheignon C, Tomas M, Bonnefont-Rousselot D, et al. Oxidative stress and the amyloid beta peptide in Alzheimer's disease. *Redox Biol*. 2018; 14: 450-464.
16) Daly NL, Scanlon MJ, Djordjevic JT, et al. Three-dimensional structure of a cysteine-rich repeat from the low-density lipoprotein receptor. *Proc Natl Acad Sci USA*. 1995; 92: 6334-6338.
17) Zlokovic BV, Deane R, Sagare AP, et al. Low-density lipoprotein receptor-related protein 1: A serial clearance homeostatic mechanism controlling Alzheimer's amyloid β-peptide elimination from the brain. *J Neurochem*. 2010; 115: 1077-1089.
18) Barić N. The drainage mechanism of beta amyloid (Aβ) from the intra-arterial perivascular space by LRP1 receptors of the blood brain barrier endothelial cells. *Glycative Stress Res*. 2021; 8: 8-19.
19) Barić N. Conformational changes in the LRP1 receptor related to the amyloid beta binding on the receptor's II and IV ligand binding domains. *Glycative Stress Res*. 2021: 8: 136-147.
20) Zhao Z, Sagare AP, Ma Q, et al. Central role of PICALM in amyloid-β blood-brain barrier transcytosis and clearance. *Nat Neurosci*. 2015; 18: 978-987.
21) De Nardis C, Lössl P, van den Biggelaar M, et al. Recombinant expression of the full-length ectodomain of LDL receptor-related protein 1 (LRP1) unravels pH-dependent conformational changes and stoichiometry of binding with receptor-associated protein (RAP). *J Biol Chem*. 2017; 292: 912-924.
22) Barić N. Conformational changes in the LRP1 receptor related to the amyloid beta binding on the receptor's II and IV ligand binding domains. *Glycative Stress Res*. 2021; 8: 136-147.
23) Sheridan S, Gräter F, Daday C. How fast is too fast in force-probe molecular dynamics simulations? *J Phys Chem B*. 2019; 123: 3658-3664.
24) Young HT, Edwards SA, Gräter F. How fast does a signal propagate through proteins? *PLoS One*. 2013; 8(6): e64746.
25) Barić N. Role of advanced glycation end products in Alzheimer's disease. *Glycative Stress Res*. 2014; 1: 68-83.
26) Silverberg GD, Messier AA, Miller MC, et al. Amyloid efflux transporter expression at the blood-brain barrier declines in normal aging. *J Neuropathol Exp Neurol*. 2010; 69: 1034-1043.
27) Silverberg GD, Miller MC, Messier AA. Amyloid deposition and influx transporter expression at the blood-brain barrier increase in normal aging. *J Neuropathol Exp Neurol*. 2010; 69: 98-108.
28) Kang DE, Pietrzik CU, Baum L, et al. Modulation of amyloid β-protein clearnce and Alzheimer's disease susceptibility by the LDL receptor-related protein pathway. *J Clin Invest*. 2000; 106: 1159-1166.
29) Osgood D, Miller MC, Messier AA, et al. Aging alters mRNA expression of amyloid transporter genes at the blood-brain barrier. *Neurobiol Aging*. 2017; 57: 178-185.
30) Liu X, Jiao B, Shen L. The epigenetics of Alzheimer's disease: Factors and therapeutic implications. *Front Genet*. 2018; 9: 579.
31) Yi SJ, Kim K. New insights into the role of histone changes in aging. *Int J Mol Sci*. 2020; 21: 8241.
32) Xu K, Dai XL, Huang HC, et al. Targeting HDACs: A promising therapy for Alzheimer's disease. *Oxid Med Cell Longev*. 2011; 143269.
33) Moore LD, Le T, Fan G. DNA methylation and its basic function. *Neuropsychopharmacology*. 2013; 38: 23-38.
34) Collins BE, Greer CB, Coleman BC, et al. Histone H3 lysine K4 methylation and its role in learning and memory. *Epigenetics Chromatin*. 2019; 12: 7.
35) Storck SE, Meister S, Nahrath J, et al. Endothelial LRP1 transports amyloid-β(1-42) across the blood-brain barrier. *J Clin Invest*. 2016: 126: 123-136.

36) Shibata M, Yamada S, Kumar SR, et al. Clearance of Alzheimer's amyloid-β1-40 peptide from brain by LDL receptor-related protein-1 at the blood-brain barrier. *J Clin Invest*. 2000; 106: 1489-1499.
37) Sagare AP, Deane R, Zlokovic BV. Low-density lipoprotein receptor-related protein 1: A physiological AB homeostatic mechanism with multiple therapeutic opportunities. *Pharmacol Ther.* 2012; 136: 94-105.
38) Shinohara M, Sato N, Kurinami H, et al. Reduction of brain β-amyloid (Aβ) by fluvastatin, a hydroxymethylglutaryl-CoA reductase inhibitor, through increase in degradation of amyloid precursor protein C-terminal fragments (APP-CTFs) and Aβ clearance. *J Biol Chem*. 2010; 285: 22091-22102.
39) Qosa H, Abuznait AH, Hill RA, et al. Enhanced brain amyloid-β clearance by rifampicin and caffeine as a possible protective mechanism against Alzheimer's disease. *J Alzheimers Dis*. 2012; 31: 151-165.
40) Tamaki C, Ohtsuki S, Terasaki T. Insulin facilitates the hepatic clearance of plasma amyloid β-peptide (1 40) by intracellular translocation of low-density lipoprotein receptor-related protein 1 (LRP-1) to the plasma membrane in hepatocytes. *Mol Pharmacol*. 2007; 72: 850-855.
41) Shinohara M, Tachibana M, Kanekiyo T, et al. Role of LRP1 in the pathogenesis of Alzheimer's disease: Evidence from clinical and preclinical studies. *J Lipid Res*. 2017; 58: 1267-1281.
42) Seok H, Lee M, Shin E, et al. Low-dose pioglitazone can ameliorate learning and memory impairment in a mouse model of dementia by increasing LRP1 expression in the hippocampus. *Scientific Rep*. 2019; 9(1): 4414.
43) Nikolakopouloul AM, Wang Y, Ma Q, et al. Endothelial LRP1 protects against neurodegeneration by blocking cyclophilin A. *J Exp Med*. 2021; 218(4): e20202207.