

Interaction between oxidative stress and epigenetics decreases the low density lipoprotein receptor related protein 1 (LRP1) expression on the blood-brain barrier (BBB) in Alzheimer's disease

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ABSTRACT:

The aim of the study is to present and critically evaluate recent knowledge about the interaction between oxidative stress and epigenetic mechanisms crucial for the development and course of Alzheimer's disease (AD), and it does not analyse the compound polygenetic AD etiology. While genetics investigates direct DNA (deoxyribonucleic acid) sequence alterations with hereditary changes of gene activities and functions, epigenetics is concerned with methylated changes without any changes in DNA sequences. Epigenetics does not deal with mutations, but exclusively with specific phenomena as histone acetylation and deacetylation, histone methylation and demethylation, and especially DNA cytosine methylation and demethylation. All these epigenetic phenomena are tightly associated with specific enzymes. Histone acetylation is induced by enzyme histone acetyltransferase (HAT), and deacetylation by enzyme histone deacetylase (HDAC). Histone methylation is induced by enzyme histone methyltransferase (HMT), and demethylation by enzyme histone demethylase (HDM). Especially important epigenetic phenomena are DNA methylation and demethylation, where the first mentioned phenomenon is induced by enzyme DNA cytosine methyltransferase (Dnmt), and the second by the combined effects of ten-eleven translocation (TET) family of dioxygenases and thymine DNA glycosylase (TDG). By the epigenetic DNA methylation mechanism, the Dnmt enzyme transports the methyl group ($-CH_3$) from the S-adenyl methionine (SAM) onto the C5 cytosine position with the 5-methylcytosine formation. On the DNA molecule especially accentuated are the so called CpG islands, where their methylation leads to the disturbances of transcription factors binding, to the accumulation of repressive methyl binding proteins and to the silencing gene expression. DNA demethylation, as it is now viewed, is very complicated and is achieved by the interplay of DNA oxidative reactions and repair mechanisms. Recent studies emphasize the importance of epigenetic mechanisms in the development and course of AD. This study has the aim to primarily analyse the *LRP1* gene methylation, as one of the crucial factors in AD pathophysiology. LRP1 receptor, dominantly located on the abluminal side of endothelial blood-brain barrier cells (BBB), is the major amyloid beta ($A\beta$) cleaner from the brain. According to a number of investigations, $A\beta$, accumulated and aggregated in the brain tissues, is the crucial factor in the onset and course of Alzheimer's disease, the chronic, severe and lethal neurodegenerative disease.

KEYWORDS: epigenetics, DNA methylation and demethylation, Alzheimer's disease, LRP1 receptor, oxidative stress

SAŽETAK:

MEĐUDJELOVANJE OKSIDATIVNOG STRESA I EPIGENETIKE SMANJUJE EKSPRESIJU RECEPTORA LIPOPROTEINA NISKE GUSTOĆE I (LRP1) NA KRVNO-MOŽDANOJ BARIJERI (KMB) U ALZHEIMEROVOJ BOLESTI

Ne ulazeći u analizu složene poligenetske etiologije Alzheimerove bolesti (AD), ova studija ima za cilj prikazati i kritički evaluirati najnovija saznanja o interakciji oksidacionog stresa i epigenetskih mehanizama presudnih za razvoj i tijek te kronične, teške i letalne neurodegenerativne bolesti. Dok genetika proučava direktne alteracije DNA (deoksiribonukleinska kiselina) sekvenci s nasljednim promjenama genske aktivnosti i funkcija, epigenetika se bavi navedenim promjenama bez ikakvih promjena DNA sekvenci. Ona ne obrađuje genske mutacije, već isključivo određene pojave tipa histonske acetilacije i deacetilacije, histonske metilacije i demetilacije, te posebno DNA citozinske metilacije i demetilacije. Sve te navedene pojave tijesno su vezane uz određene enzime. Histonska acetilacija uvjetovana je enzimom histonske acetiltransferaze (HAT), a deacetilacija enzimom histonske deacetilaze (HDAC). Histonska metilacija je uvjetovana enzimom histonske metiltransferaze (HMT), a demetilacija enzimom histonske demetilaze (HDM). Posebno važan epigenetski događaj je DNA metilacija i demetilacija gdje je prvo navedena pojava uvjetovana enzimom DNA citozinske metiltransferaze (Dnmt). Druga pojava je uvjetovana kombiniranim učincima "deset-jedanaest translokacijske (TET) obitelji dioksidogenaze i timin DNA glikosilaze (TDGs). Epigenetskim mehanizmom DNA metilacije navedeni enzim prenosi metilnu grupu ($-\text{CH}_3$) sa S-adenil metionina (SAM) na C5 poziciju citozina uz formiranje 5-metilcitozina. Tu se ističu posebni segmenti na DNA molekuli, tzv. CpG otoci, gdje njihova metilacija dovodi do poremećaja vezivanja transkripcijskih faktora, nakupljanja represivnih metil vezujućih proteina i do ušutkivanja genske ekspresije. DNA demetilacija, kako se aktualno smatra, dosta je komplicirana, a postiže se međuigrom DNA oksidativnih reakcija i mehanizama obnove. Recentne studije daju sve veći značaj epigenetičkim mehanizmima u razvoju i tijeku AD. Ova studija primarno nastoji učiniti analizu metilacije LRP1 gena, kao jednog od ključnih faktora u patofiziologiji AD. LRP1 receptor, dominantno lociran na abluminalnoj strani stanica endotelne krvno moždane barijere (KMB) je glavni amiloid beta čistač iz mozga. Prema nizu istraživanja, A β , akumuliran i agregiran u moždanim tkivima, je presudan čimbenik u nastajanju i tijeku Alzheimerove bolesti.

KLJUČNE RIJEČI: Epigenetika, DNA metilacija i demetilacija, Alzheimerova bolest, LRP1 receptor, oksidativni stres.

INTRODUCTION

Among all types of dementia, Alzheimer's disease is worldwide the most common form, especially found in old age, presently with a high and alarming rise of its incidence and prevalence. Marked with the progressive and irreversible decline of the complete spectrum of patients' intellectual and physical functions, it sometimes, faster or more slowly, leads to the total decay of personality and complete dependence on someone else's care and nursing. Actually, it is confirmed that the accumulation and aggregation of amyloid beta (A β) peptides in the brain, induce the development of complex and progressive neurodegeneration and dementia associated with Alzheimer's disease (AD). Yet, AD has a fairly unknown polygenetic etiology. Its two forms, the early form (EOAD, early AD onset, 5% of all cases) which develops before the age of 65, and the late form (LOAD, late AD onset, 95% of all cases) which develops after the age of 65, provide practically the same clinical picture. The mutations of gene complexes: APP (β -amyloid precursor protein gene, 21q21.3); PSEN1 (presenilin-1 protein coding gene, 14q24.2); PSEN2

(presenilin-2 protein coding gene, 1q42.13), and BACE1 (β secretase-1 protein coding gene, 11q23.3) lead to EOAD, and mutations of APOE (apolipoprotein E coding gene, 19q13.32) and ADAM10 (ADAM metalloproteinase Domain 10 protein coding gene-regulator of α -secretase activity-gene, 15q21.3) lead to LOAD. The analysis of pathophysiological events that cause the rise of intracerebral A β concentration, the essential factor in the development and course of AD, indicates the crucial importance of the decline of the A β drainage from the brain as related to A β increased production^{1,2,3}).

OXIDATIVE STRESS AND ITS ROLE IN ALZHEIMER'S DISEASE PATHOPHYSIOLOGY

Oxidative stress in the organism of living beings, especially elevated in AD, according to a number of researchers is mostly induced by the effects of free radicals, among them primarily by hydroxyl radical (*OH), the neutral form of the hydroxyl ion (OH). This radical is generated in the brain primarily during the aggregation and sedimentation of A β monomers at the point of in-

terrelated contacts of A β strands, with the transit of one electron ("e- hop) from the sulphur (S) of methionine (MetS35, nonpolar essential amino acid) on the β 2-strand of the incoming A β 2, and onto the ferric ion (Fe³⁺) located on the β 1-strand of the earlier fixed A β 1 (metal binding domain, His13-His14 sequence on the β 1-strand). MetS35 on the incoming A β 2 monomer has the reductive-donor electron function. Oxidised ferric ion (Fe³⁺ on MBD of A β 1) by reduction (electron gain) transforms into the redox active ferrous ion (Fe²⁺) that reacts with, in AD, the abundantly present hydroxy peroxide (H₂O₂), generating Fe³⁺, hydroxyl ion (-OH), and the extremely active and aggressive hydroxyl radical (*OH). These events are presented in the Fenton reaction: Fe²⁺ + H₂O₂ = Fe³⁺ + ⁻OH + *OH. The generated *OH oxidatively attacks the surrounding molecules, primarily located in lipid cell membranes (lipid peroxidation), as well as A β itself and the nearby LRP1 receptor (Fig. 1)^{1,3-10}.

Redox active metal ions, for example the above mentioned ferrous ion (Fe²⁺) bound to the A β , catalyze the production of reactive oxygen species (ROS), among them particularly the hydroxyl radical *OH. *OH oxidatively damages the surrounding molecules, as well as A β peptide itself, inducing its conformational changes and the decline in affinity for LRP1. These changes result in the impairment of A β clearance that is induced by the low density lipoprotein receptor-related protein 1 (LRP1). The accompanying destructive oxidation of LRP1, with the consequent decline of its functions, additionally contributes to the A β accumulation in the brain (Fig.1)^{2,11,12}.

THE PARALLEL, SIGNIFICANTLY IMPORTANT EXPRESSION ALTERATION OF AMYLOID TRANSPORT RECEPTORS LRP1, P-GP, AND RAGE, AND THEIR MRNA.

Osgood D, et al.¹³), in their detailed study emphasize that the A β drainage from the brain is primarily induced by certain amyloid transport receptors on the level of endothelial cells of tiny cerebrovascular blood brain barrier capillaries (BBB). Among these receptors, crucial are efflux transporters, the low-density lipoprotein receptor-related protein 1 (LRP1) and P-glycoprotein 1 (P-gp, ABCB1). Contrary to their strong drainage effects, on the same position there also is the influx transporter receptor for advanced glycation end products (RAGE), which from the local capillary network binds and injects A β into the endothelial cells and into the brain parenchyma. The complex interplay of these three receptors has the crucial importance in maintaining optimal brain A β homeostasis, and in the case of AD, its disturbed course and deterioration of the disease clinical state. The most recent investigations of the mentioned genes have shown in the isolated tiny brain vessels, related to ageing and AD, a significant reduction of the LRP1 and P-gp transporters mRNA expression, and an increase of the mRNA transporter RAGE expression. On the enclosed

graphs the authors show how the changes of the mentioned gene mRNA expression parallelly follow the receptor expression changes. All this indicates that adequate expression of these receptors and their genes is based on their regular activation and regular transcription. It seems that the determined alterations of these genes expression and transcription found in the process of ageing and old age, as well as in AD, according to the current perception, have a vital role in the A β accumulation in the brain.

The results of the Doreen Osgood, et al.¹³) research clearly show that the parallel, significantly important alteration of the expression of amyloid transport receptors LRP1, P-gp, RAGE, and their mRNA, is based on epigenetic mechanisms as the cause of these events related to age and AD. This does not annul the importance of the oxidative stress impact especially evident in AD, that leads to A β weaker binding to LRP1, and the consequently weaker A β drainage. Most probably it is the question of the mutual activity of both mechanisms.

Carrano A, et al.¹⁴), analyse the P-glycoprotein expression in the blood-brain barrier cells (BBB) in AD patients, using the advanced cerebral amyloid angiopathy (CAA). In these cells they have found a significant drop of this receptor concentration, accompanied by the obvious drop of P-gp mRNA. The mutual drop of the receptor level and its mRNA indicate the disturbed P-gp transcription, most probably induced by the epigenetic mechanism.

Kang DE, et al.¹⁵) and Yamanaka Y, et al.¹⁶) also emphasize that the drop of LRP1 receptor expression and the drop of its mRNA is closely related to old age. Both groups of researchers have used the obduction material from the frontal brain cortex of the diseased AD patients and age-matched non-demented controls. The first mentioned group obtained significant values of the decreased LRP1 in older patients (non-demented controls) as related to the younger control group (non-demented controls). The deceased AD patients had significantly lower values than the control AD group (non-demented controls). The second mentioned group of authors analysed the mRNA levels from the same region and obtained their significantly lower values in relation to the control group (non-demented controls). Both findings indicate lower LRP1 protein transcription probably caused by the epigenetic mechanism.

Castellano J, et al.¹⁷), analysed the influence of hypoxia on the LRP1 protein expression and mRNA LRP1 expression, and also found their close connection. In human vascular smooth muscular cells (VSMC), maximal upregulation of LRP1 by hypoxia occurs after 1 hour at the level of mRNA, and after 16 hours at the protein level. According to the authors, the delayed upregulation of the LRP1 protein by hypoxia seems to be due to the extremely long half-life of LRP1 protein in human VSMC. Their experiments have demonstrated that hypoxia-induced LRP1 overexpression depends on the transcription.

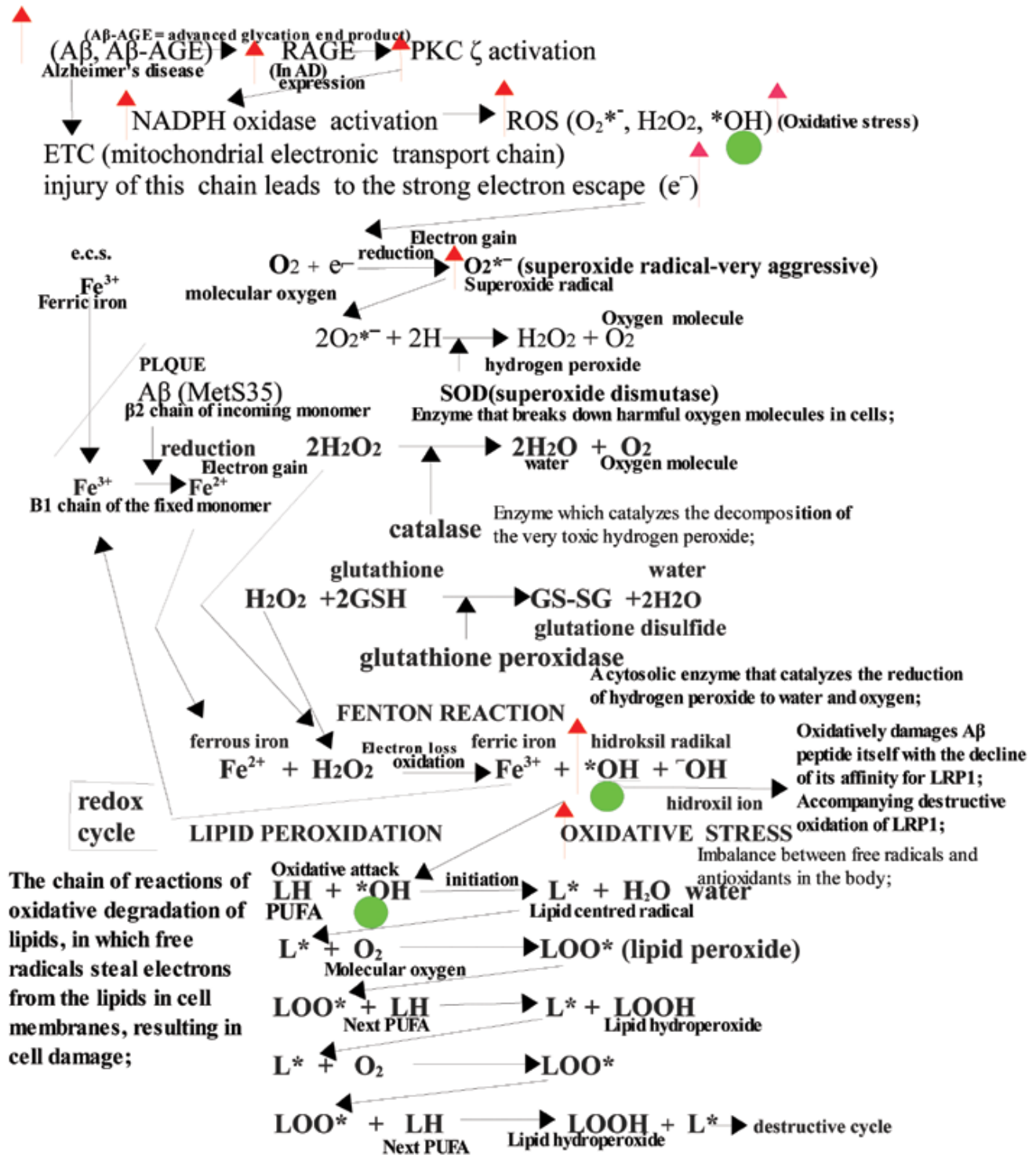


Fig. 1. Schematic presentation of important biochemical processes related to destructive free radical effects on essential molecular structures

PUFA, polyunsaturated fatty acid ; RAGE, receptor for advanced glycation end products; PKC protein kinase C; NADPH oxidase, nicotinamide adenine dinucleotide phosphate-oxidase; ROS, reactive oxygen species; O₂^{*-}, superoxide radical; H₂O₂, hydrogen peroxide; SOD, superoxide dismutase, strong antioxidant enzyme; catalase, enzyme, protects the cell from oxidative damage; GSH, glutathione peroxidase, enzyme, protection from oxidative damage; lipid peroxidation oxidative degradation of lipids; Fe²⁺, ferrous iron; Fe³⁺, ferric iron; e.c.s., extracellular space; oxydation, electron loss; reduction, electron gain.

HISTONE ACETYLATION AND DEACETYLATION

Before the detailed presentation of histone acetylation and deacetylation, it is necessary to emphasize that in this paper, these two important processes primarily relate to the LRP1 receptor and its gene (12q13.3).

Ke Xu et al.¹⁸⁾, point out the crucial characteristics of histone acetylation (inserting the electronegative acetyl group (CH_3CO^-) in the positive histone tail, and histone deacetylation (ejection of the CH_3CO^- group), the two fundamental processes related to the AD pathophysiology. The first process is catalyzed by the enzyme histone acetyltransferase (HAT), and the second one by histone deacetylase (HDAC). Their experiments were performed on laboratory rats (mouse models of AD). For tissue analyses, autopsic material from the brain of individuals deceased from AD was used. The histone acetylation is characterised by the inserting of the CH_3CO^- electronegative group into the histone tail that is electropositive. The result is the decrease of the total positivity of the tail and histone core, and consequently, the decrease of the interaction between the DNA phosphate (which is electronegative) and the tail. All this induces conditions for the easy approach of the RNA polymerase to the DNA chain and the generation of the free noncondensed euchromatin, and gene transcription. HAT can take the acetyl group from different compounds, for example acetic acid, acetylcholine, acetyl-CoA and acetylcysteine. HDAC catalyzes deacetylation i.e. the ejection of CH_3CO^- from the histone tail that becomes more positive. The tail connection with DNA phosphate becomes stronger, and this leads to unfavourable conditions for the RNA polymerase approach to the DNA chain, to chromatin condensation and the drop of gene transcription. The level of histone acetylation plays an important role in the decline of chromatin condensation and the rise of 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. Otherwise, it is necessary to emphasize that typically there is a labile equilibrium between acetylation and deacetylation, with the ascendancy of the latter. This ascendancy is especially evident in AD. With good reason we can suppose that it also occurs with LRP1 gene and LRP1 receptor (Fig. 2)

Xiaolei Liu et al.¹⁹⁾, in their review paper emphasize that epigenetic mechanisms play a vital role in the development and course of AD. Histone acetylation catalysed by histone acetyltransferase (HAT) induces the decondensation of chromatin with the generation of euchromatin that is transcriptionally active. The condensed chromatin type, i.e. heterochromatin, is compact and transcriptionally inactive. This is the deacetylated type of chromatin obtained through deacetylation (ejection of $-\text{CH}_3\text{CO}$ electronegative group) 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 (Class I HDACs, such as HDAC2 and HDAC3) has been found. This probably leads to the otherwise disordered labile equilibrium between levels of HAT and HDAC, with the ascendancy of the latter, and the tendency to tighter connections between the histone tail and pDNA. The decline of these HDACs expression induces the rise of histone acetylation and the improvement of the AD clinical situation. It is evident that the mentioned tighter connection leads to the transcription drop of several genes (Fig.2). According to the results of Osgood D et al.¹³⁾, it is possible to suppose that this can happen also with the LRP1 gene. It is important to emphasize that histone tails are typically tightly associated with the negatively charged pDNA. In AD this association is much stronger.

Krichevsky A, et al.²⁰⁾, in their study present the detailed review of the acetylation and deacetylation of histones H3 and H4 related to the root-specific gene LRP1 of Arabidopsis, the small flowering plant native to Euroasia, Africa, and North America. In their investigations they have found that SWP1/LDL1, a component of a corepressor complex is involved in the regulation of the root elongation by repressing the root-specific gene LRP1 via histone deacetylation. A null mutation in SWP1 results in the hyperacetylation of H3 and H4 in LRP1 chromatin, the elevation of LRP1 expression, and increased root elongation. Actually, the following happens: SWP1/LDL1 induces the ejection of negative CH_3CO^- groups from positive histone tails (analogous to HDAC), i.e. the appropriate histones become more positive and increasingly attract the negative pDNA. The free approach of RNA polymerase to pDNA is decreased, there is no transcription, the expression of root specific LRP1 drops, the plant root does not grow. The null mutation of SWP1 prevents the histone deacetylation (CH_3CO^- ejection), this leads to histone hyperacetylation of H3 and H4, the positivity (CH_3CO^- is electronegative) drops, the attraction between the tail and pDNA is lower, the approach for RNA polymerase becomes easier, the transcription is stronger, the expression of root specific LRP1 gene increases, the plant root becomes longer.

Sharmila Singh, et al.²¹⁾, analysed the fitohormone auxin signals transmitted by the auxin/indole-3-acetic acid (Aux/IAAs) and AUXIN RESPONSE FACTORS (ARFs) and found that these signals regulate the development of the lateral root (LR) of the plant Arabidopsis while controlling the expression of LATERAL ROOT PRIMORDIUM1 (LRP1) auxin-inducible gene. The exact developing role of LRP1 and the molecular regulation of its activity has not yet been explained. However the authors have concluded that the LRP1 expression is present in all development phases of the lateral root (LR). The LRP1 expression is regulated by histone deacetylation. LRP1 functions through auxin responsive Aux/IAAs-AREs module during the LR growth. Overexpression of LRP1 indicates that it is included in the LR growth. The results of the above mentioned authors' research show that auxin and histone deacetylation have a damaging effect on the LRP1 expression.

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

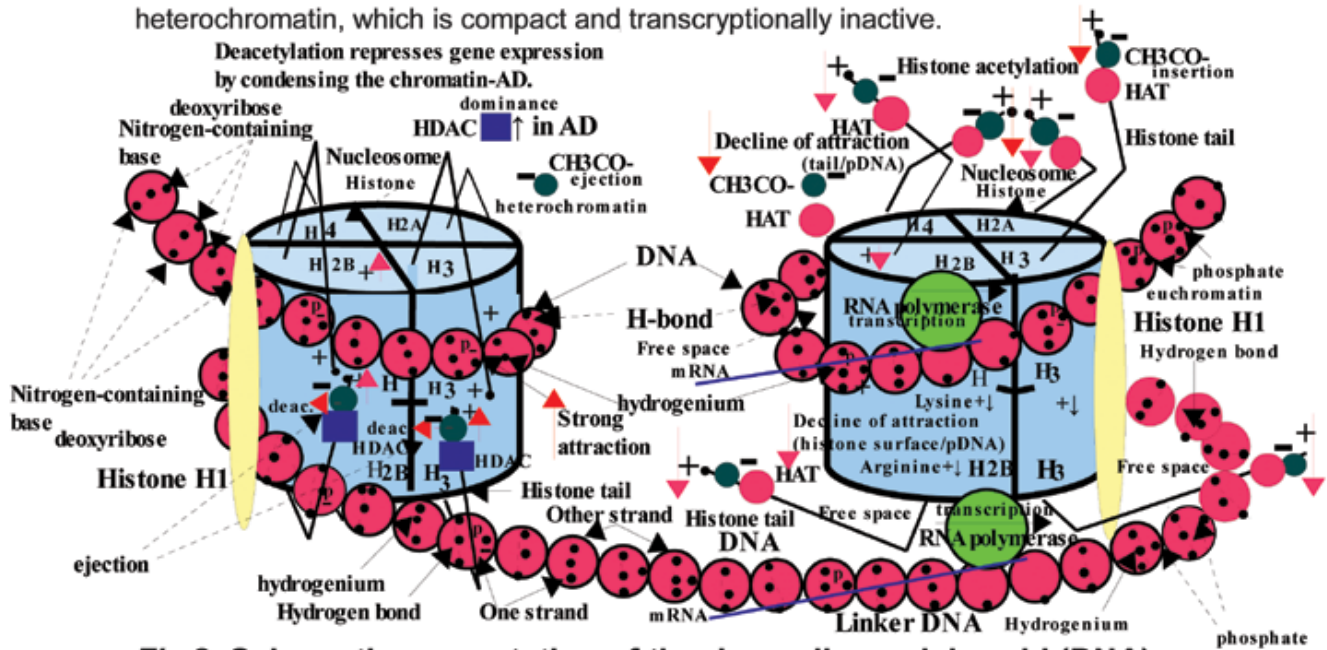


Fig.2. Schematic presentation of the deoxyribonucleic acid (DNA) with histone acetylation and deacetylation – LRP1 gene

Deoxyribonucleic acid (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, nucleosome is composed of 8 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; nucleosome height is about 6 nm; each DNA turn around the histone octamer complex has the diameter 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; histone acetyltransferase (HAT) can catalyze its acetylation and as a consequence the enhanced rates of gene transcription; histone deacetylation catalyzed by histone deacetylase (HDAC) represses the gene expression by condensing the chromatin; histone modifications have a crucial role in the development and course of AD.

The comprehensive histone analysis performed by Nativio R, et al.²²), in the brain (frozen postmortem) lateral temporal lobe has demonstrated the significant elevation of H3K27ac (acetylation) and H3K9ac (acetylation) in AD. These elevated levels were associated with the evident epigenetic and disease-related pathways in this disease. This elevation is especially related to the promotion of A β 42-induced neurodegeneration in vivo. Otherwise, it has already been found that the lateral temporal lobe was the region affected early in AD. By comparing the gene expression of AD and old age, the authors identified 421 genes with significant upregulation in AD, while 434 had significant downregulation. Overall, these multi-omic analyses have identified H3K27ac and H3K9ac as potential epigenetic drivers of AD, which spur disease pathways through the transcription dysregulation and chromatin gene feedback loops.

Ref.22 shows that the histone tail acetylation in the case of H3K27ac i H3K9ac does not lead to the fall of the positive tail charge, to the weakening of the tail and pDNA, and to the strengthening of the transcription. Actually, the opposite process takes place.

Wapenaar, et al. 23), emphasize that in the ping-pong mechanism of histone acetylation, the primary event is the binding of Ac-Co-A on the catalytic site. Afterwards occurs the separation of the acetyl group from Ac-Co-A and its transfer to the amino acid cysteine 143 that accepts the acetyl moiety. This cysteine is also present locally in the catalytic site of the enzyme. CoA leaves the enzyme. The histone tail (substrate) binds to the enzyme, and its ϵ -amino group on lysine accepts the transferred acetyl group. It is necessary to point out that some other mechanisms are being investigated (thendom-order ternary complex mechanism and the mpulsory-order ternary complex mechanism), but in order to understand the problem, the ping-pong mechanism is sufficient (Fig.2).

Suk-Youl Park, et al.24), In their study explain the structure and activity of the metal-dependent histone deacetylases. Their activity includes the hydrolysis of acetyl-L-lysine side chain in histones which yields l-lysine and acetate. In their investigations they emphasize class I, II, and IV HDACs (Fig.2)

HISTONE METHYLATION AND DEMETHYLATION

Collins BE et al.²⁵), point out that the addition of methyl groups (CH_3) to the histone tails induces histone methylation. The consequence is mainly the generation of the condensed state of chromatin, the heterochromatin that maintains the tail positive charge, making DNA more coiled. This hastens the positive histone binding to the negative DNA phosphate and decreases the rate of transcriptional expression. The bilaterally induced strong connection reinforces the link between histone and DNA and reduces the transcriptional expression. Histone methylation is induced by methyltransferase (HMTs). This type of methylation is a typical "gene silencer". It is a complex, more durable and

more stable relative to histone acetylation. However, it is found that lysine 4 on histone H3 (H3K4) monomethylation normally activates the gene expression, and lysine 27 on histone H3 (H3K27) methylation represses the gene expression. Experiments have been obtained on mice (Fig.3).

Uchiyama R et al.²⁶), using the droplet digital (PCR, polymerase chain reaction), have analyzed blood samples in stunted children and their mothers living in an urban slum in Dhaka, Bangladesh. In these samples they have found abnormal dominance of unfolding patterns (unfolding contributes to pathology) of histone H3 lysine 4 trimethylation (H3K4 me3). Otherwise, the histone tails have a positive charge and hence, typically associate tightly with the negatively charged DNA. Normally, the unfolding patterns of H3K4 and its trimethylation are not visible. In all stunted cases this was connected with the reduced expression of the LRP1 receptor. The authors emphasize a global decrease in methylation at canonical locations near gene start sites, and increased methylation at ectopic sites throughout the genome. They have concluded that reduced levels of one-carbon nutrients in their diet play a key role in stunting in this population. Experiments on mice with the deletion of LRP1 with tamoxifen, suggest that reduced LRP1 expression contributes to stunting in humans.

It is obvious that the histone tail methylation can increase or decrease the gene transcription, depending on the type of the amino acids methylated in histone and on the number of methyl groups used in each amino acid. The methylation that weakens the attraction of histone tail and pDNA, increases the possibility of RNA polymerase approaching the DNA chain and the transcription intensity (e.g. H3K4m). The methylation that increases the strength of the tail and pDNA connection enables the tail to wrap around DNA and prevents the RNA polymerase to approach the DNA chain, thus preventing the transcription (e.g. H3K27m). Generally, it is evident that the tail methylation maintains the tail positive charge, making DNA more coiled, with reduced transcription. Whether histone methylation will lead to the transcription activation or its repression actually depends on the methylated residue and on the presence of other methyl or acetyl groups in its immediate vicinity.

Bing Xiao, et al.²⁷), analysing the high resolution crystal structure of a ternary complex that includes human SET7/9 histone methyltransferase (HMT), histone peptide (amino terminal tail of histone H3, positively charged), and cofactor (S-adenosyl-L-methionine, AdoMet, methyl group donor), reveal that the peptide substrate and cofactor bind on opposite surfaces of the enzyme. Between two opposite surfaces of the enzyme there is a narrow channel that runs through the enzyme connecting distally both surfaces. SET7/9 is mono-methylase. The target lysine residue (Lys4) of the substrate (H3) is inserted into this channel so that its amine can access the methyl donor (AdoMet). Methylation of H3K4 is widely regarded as a mark of transcriptional activation.

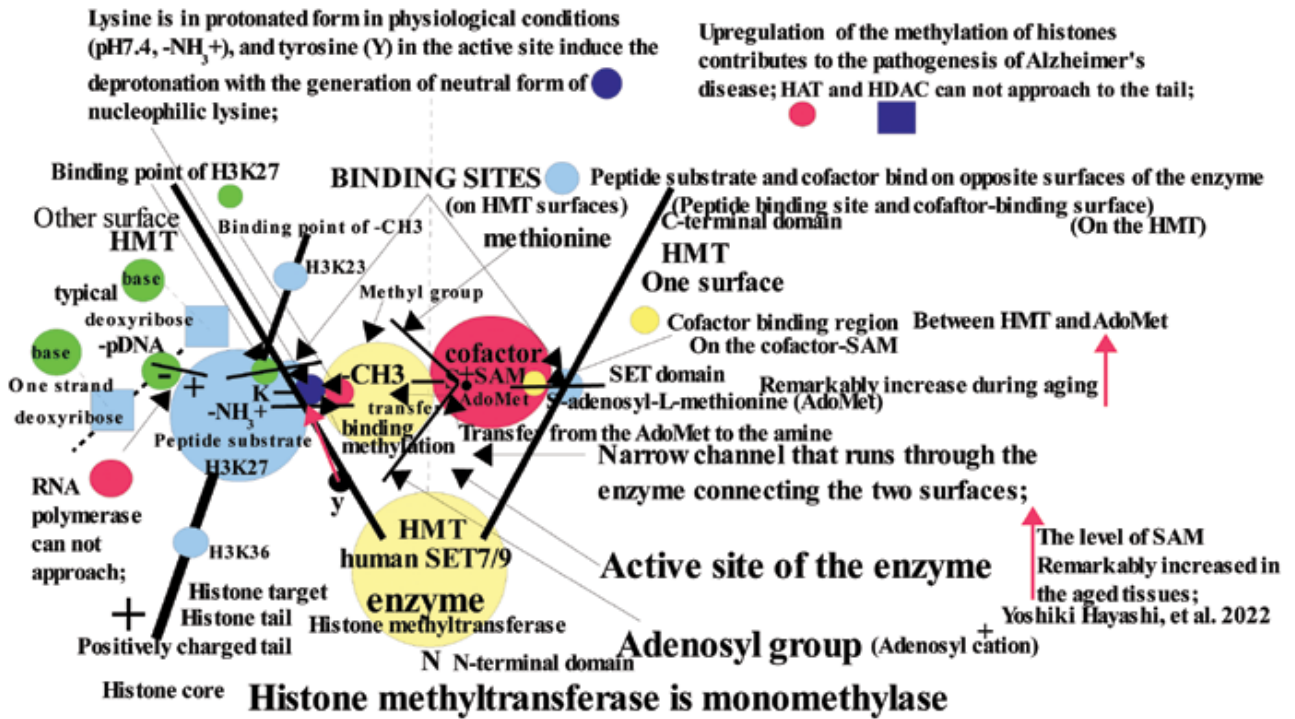


Fig.3. Presentation of the histone methylation—adding the methyl group to the histone tail suppresses the gene transcription

According to Ref. 27 and Ref. 29, adding the methyl group to the histone tail; Structure of the SET7/9 ternary complex; Catalytic mechanism of the human histone methyltransferase SET7/9; clearly visible is the histone H3 and lysine K27. HMT, histone methyltransferase SET7/9 has two sides; AdoMet is methyl (-CH₃) donor; generally, adding a methyl group to the tail (methylation) maintains the tail positive charge, making DNA more coiled and reducing transcription; side chain of Lys27 is methylated; -p, phosphate; DNA, deoxyribonucleic acid; K, lysine; the figure presents the situation with the dominance of heterochromatin; transcription is reduced; NH₂ of lysine, nucleophilic ε-amino group in histone tails and core histone; the nucleophilic ε-amino group of lysine is directed toward the electrophilic methyl group of AdoMet; lysine is in protonated form in physiological conditions (-NH₃⁺); it is considered that the enzyme active site tyrosine or a water channel induces the deprotonation of the ammonium ion of lysine with the generation of a neutral form of nucleophilic lysine; methionine is a non-polar amino acid; methionine has side chain that lacks polar bond; methylation keeps the positive histone charge; S, sulphur; AdoMet remarkably increased in the brain aged tissue (cerebellum, cerebrum, hippocampus); RNA polymerase cannot approach to the DNA; y, tyrosine, deprotonation of -NH₃⁺.

Keating S, et al.²⁸), emphasize that across eukaryotic genomes, lysine methylation at position 4 of histone H3 (H3K4) is commonly associated with transcriptional activation.

Bing Xiao, et al.²⁹), in their subsequent paper present the crystal structure of the enzyme Pr-Set7 ternary complex (also known as Set8, i.e. Histone H4K20 methyltransferase) that methylates Lys20 of histone H4 (H4K20). Set8 is exclusively mono-methylase, in other words it adds a single methyl group to H4K20. This enzyme is responsible for the methyl transfer from S-adenosyl-L-homocysteine (AdoHcy) cofactor to the histone lysine side-chain nitrogen Nε. Mono-methylated and di-methylated H4K20 are broadly distributed, but generally associated with euchromatin regions. Tri-methylated H4K20 is associated with transcriptionally inactive heterochromatin.

Xin Yi, et al.³⁰), point out that the histone lysine methylation plays a critical role in the epigenetic regulation of eukaryotes. They have found that lysine residues of K4, K9, K27, K36 and K79 in histone H3 and K20 in histone H4 can be modified by histone methyltransferases (HMTs) (Fig.3).

Yujiang Geno Shi, et al.³¹), emphasize that the first discovered histone demethylase (HDM, LSD1) catalyzes an amine oxidation by oxidative cleavage of the α-carbon bond of the methylated lysine to form an imine intermediate, which is hydrolyzed to form formaldehyde, releasing one molecule of hydrogen peroxide (H₂O₂) and the demethylated lysine. Histone demethylases belong to either the LSD family or the JmjC family. LSD demethylases can demethylate the mono and dimethylated states of histone lysine residues. The JmjC domain family demethylases can demethylate mono-, di-, and trimethylated histone lysine residues. Their demethylation reaction includes the oxygenation of a methyl group by a radical attack of a highly reactive oxoferryl species with the formation of an unstable carbinolamine intermediate, and the subsequent release of formaldehyde from the carbinolamine with the production of demethylated lysine. HDMs are involved in the gene transcription. Histone methylation is reversible (Fig.4).

The above mentioned text suggests the great importance of methylation and demethylation of a number of genes, especially the LRP1 gene and its receptor.

It generally seems that even without the participation of the mentioned enzymes (HAT, HDAC, HMT), there is typically a strong electrostatic attractive force between the positive histone tail and negative pDNA. HMT approaches this association and leads to the binding of its methyl group with the histone tail lysine residue. This binding (methylation) prevents the HAT approach and the histone acetylation. In this way, the added methyl group preserves the tail and pDNA association. The result is the drop of gene transcription and expression (Fig.3).

DNA CYTOSINE METHYLATION AND DEMETHYLATION

DNA methylation is catalysed 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 ·CH₃ group is not a dipole, in fact it is a non-polar group. The role of Dnmt is to separate the methyl group from SAM, to catalyse 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 has become evident that the DNA methylation and demethylation have the crucial role in the epigenetic events related to gene activity³²).

Jin Yang, et al.³³), emphasize that the vital events important for Dnmt (DNA methyltransferase Hhal, M.Hhal) binding with DNA and the methylation onset occur by the influence of intermolecular attractive electrostatic forces. These forces induce the initial approach of Dnmt to the DNA cytosine ring. The Dnmt binding to DNA begins with H-bonds breaking (three H-bonds) between cytosine (target base) and its pair guanine. Subsequently, the cytosine eversion occurs, so that it projects out of the double helix, and enters the pocket of the enzyme (Fig.5).

The first step in the methyl (·CH₃) transfer is the catalytic nucleophilic attack (Nfa) of deprotonated Cys81-S- thiolate (highly nucleophilic) from the Dnmt (M.Hhal) active site, on the C6 of the cytosine ring, with consequent formation of the covalent adduct (Michael adduct) via Michael addition (5-methyl-6-cys-81-S-5,6-dihydrocytosine). This methylation reaction is concerted with the methyl transfer from S-adenosyl-L-methionine (AdoMet) to cytosine C5. The covalent bond between Cys-81-S and cytosine C6 forms rapidly.

The formed adduct promotes the nucleophilicity of the cytosine C5 for the nucleophilic attack on the S-adenosyl-L-methionine (AdoMet) methyl group, with subsequent proton abstraction from C5 via a β-elimination reaction, and the methyl group transfer to C5. Subsequently, the β-elimination of the C5 proton utilizes as the proton abstracting base, an OH- derived from conserved crystal water that is a part of a proton wire water channel. This enables the formation of 5-methylcytosine.

At the same time, Dnmt Glu119, also located in the active site, forms a H-bond with cytosine N3 at the reactant. Arg163 and Arg 165 also form the H-bond with cytosine O2. Their crucial role is the stabilisation of a cytosine in the Dnmt reactive site pocket (Fig.5).

Yiyuan Liu, et al.³⁴), emphasize the importance of the AD-associated promoter DNA hypermethylation, which establishes an epigenetic barrier for transcriptional activation. They detail functional activities of selected neurons, microglia, and astrocyte-enriched genes (AGAP2, DUSP6 and GPR37L1), which are DNA hypermethylated at promoters in AD. According to the authors, in mammals, DNA methylation primarily occurs at the 5-carbon of the cytosine base in the form of 5-methylcytosine (5mC).

Xiaodong Cheng, et al.³⁵), describe the approach and binding of Dnmt to the DNA. This induces the eversion ("base flipping") of the target nucleotide with its projection out of the double helix and entrance into a Dnmt pocket. The catalytic process induces

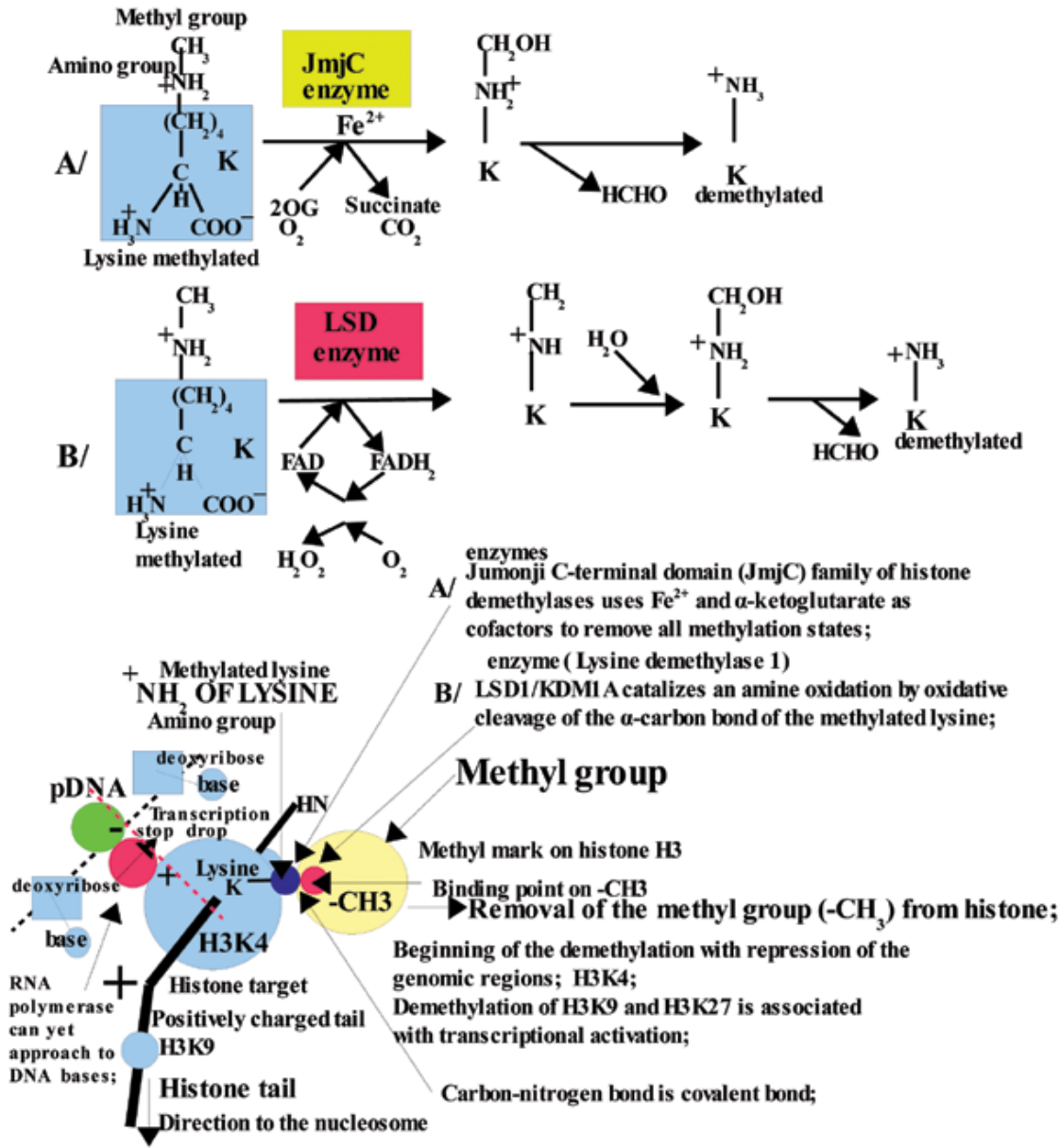


Fig.4. Presentaton of the histone demethylation

According to Ref. 30), histone demethylation is induced by enzyme histone demethylase which catalyzes the removal of methyl marks (-CH_3) from histones. Among demethylases the first is Lysine demethylase 1 (KDM1, LSD1) a flavin adenine dinucleotide (FAD)-containing enzyme that removes mono-/di-methylation. The second, the Jumonji C-terminal domain (JmjC) family of histone demethylases uses Fe^{2+} and α -ketoglutarate as cofactors to remove all methylation states. Both mechanisms of lysine demethylation involve the oxidation of the methyl group (with FAD or α -ketoglutarate and molecular oxygen as oxidizing agents) followed by the elimination of formaldehyde. Demethylation of H3K4 is linked with the repression of the genomic regions; K, lysine; Demethylation of H3K9 and H3K27 is associated with transcriptional activation.

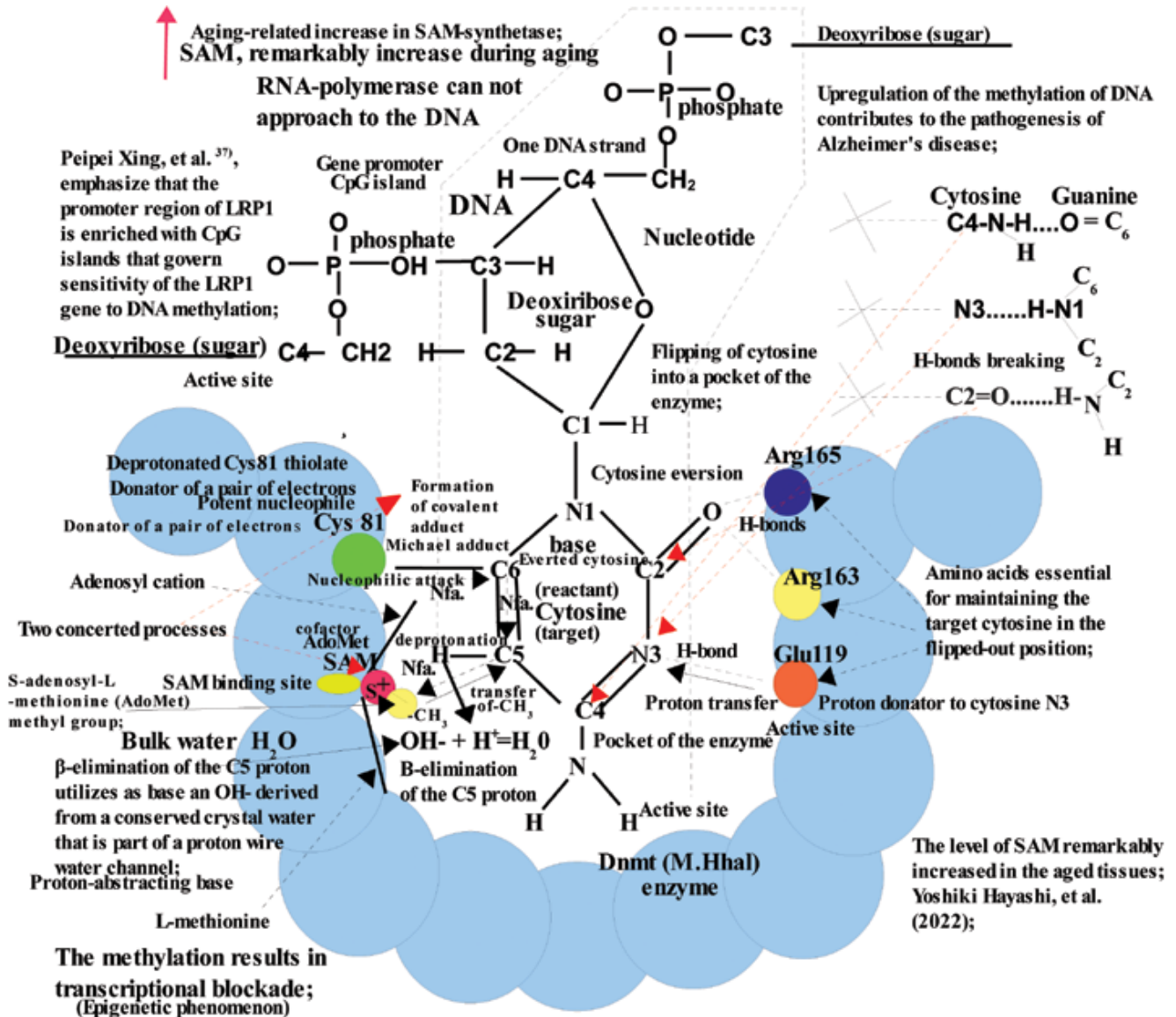


Fig.5. Schematic presentation of DNA methylation by human DNA cytosine methyltransferase M.HhaI (epigenetic event)

Dnmt M.HhaI, DNA methyltransferase M.HhaI, enzyme; cytosine, amino acid, cytosine ring, target base; Glu, glutamine, amino acid; cysteine, amino acid; SAM, S-adenosyl-L-methionine (AdoMet), methyl donor, cofactor; cys, cysteine, amino acid; -CH₃, methyl group; between cytosine and guanine there are three H-bonds; according to Jin Yang et al.³²⁾, and Xiadong Cheng, et al.³⁵⁾, the methyltransferase binding to DNA begins with three H-bonds breaking between cytosine (target base) and its pair guanine, cytosine eversion ("base flipping"), so that it projects out of the double helix, nucleophilic attack of Cys81 nucleophile on cytosine C6, and C6 attack on C5 with its activation and rise in nucleophilicity; C5 attacks the SAM methyl group and induces its abstraction.; at the same time three bases (Glu119, Arg163, Arg165) on the other Dnmt side, form the H-bonds between NH₂, N3 and O₂; Nfa, nucleophilic attack; Michael adduct; SAM, remarkably increase in the brain tissues (cerebellum, cerebrum, hippocampus) during aging; RNA polymerase cannot approach to cytosine.

the nucleophilic attack of Cys81 on cytosine C6 with the formation of the covalent Michael adduct. Cytosine C6 induces the nucleophilic attack on C5, which momentarily also induces the same attack on the S-adenosyl-L-methionine (AdoMet) methyl group, its absorption and transfer to C5.

Chien-Chu Lin, et al.³⁶), emphasize that DNA methyltransferases are primary enzymes for cytosine methylation at CpG sites of epigenetic gene regulation in mammals. DNMT3B methyltransferase uses two flexible loops to enclose DNA and employs its catalytic loop to evert the target nucleotide to project out of the double helix. DNMT3B recognizes DNA with CpGpG sites via residues Asn779 and Lys777 in its target recognition domain loop. This facilitates processive methylation of tandemly repeated CpG sites. They also emphasize the importance of the proton wire water channel essential for the C5 deprotonation.

According to Peipei Xing, et al.³⁷), the promoter region of LRP1 gene is enriched with CpG islands that govern the sensitivity of the LRP1 gene to DNA methylation. When CpG is completely methylated the transcriptional activity disappears completely and the expression of LRP1 is silenced. They think that the epigenetic mechanism might be involved as hypermethylation of the LRP1 gene promoter, especially as the promoter of this gene is rich in CpG islands.

Yoshiki H, et al.³⁸), in their experiments with tissues of young and aged mice, have found that the upregulation of the methylation of DNA and histones contributes to the pathogenesis of AD. The level of S-adenosyl-L-methionine (SAM) and SAM-synthetase (Sam-S) remarkably increase in the brain aged tissues (cerebellum, cerebrum, hippocampus). According to these findings they have concluded that the increase in SAM is an age-related phenomenon, common across the species and tissues. Everything indicates that in all species, the evolutionarily conserved fundamental biological program is active, which related to age, induces the generation of SAM-S and SAM.

It is necessary to point out that old age is a strong risk factor for the AD occurrence and course^{13,39,40}).

Tohgi H, et al.⁴¹), using the bisulfite method, polymerase chain reaction (PCR) and the direct sequencing of PCR products, have investigated the methylation status of cytosines in the promoter region of RAGE in the human cortex autopsy. They have found that the total number of methylcytosines in the mentioned region is significantly decreased with age. This reduction of methylcytosines at transcription factor binding sites can increase the expression of RAGE.

Silverberg GD, et al.⁴²), have found that connected with ageing and AD, there is decreased methylation in the RAGE promoter region. During the ageing process and in AD, there is an increased accumulation of AGEs, as well as increased values of A β , cytokines (interleukin 1, tumor necrosis factor), and of nuclear

factor kB, which all can decrease the promoter methylation and elevate the RAGE gene transcription.

Once again, it is evident that according to ref.37, ref.38, ref.41, and ref.42, in AD, the LRP1 expression decreases and RAGE expression increases.

Bayraktar G, et al.⁴³), in their mini-review analyse the current knowledge on the regulatory mechanisms controlling the active DNA demethylation. The first step of this process is 5mC oxidation induced by the ten-eleven translocation (TET) family of dioxygenases with the generation of 5-hydroxymethylcytosine (5hmC). In the second step, TET enzymes further hydroxylate 5hmC with the generation of 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC). Thimine DNA glycosylase (TDG), after recognising DNA, induces the 5fC and 5caC excision of the glycosidic bond, resulting in an apyrimidinic (AP) site. AP site can be efficiently repaired by Base Excision Repair (BER) enzymes with the generation of mark-free cytosine. At the end of their report the authors emphasize that the contribution of methylation and active DNA demethylation in Alzheimer's disease (AD) remains to be determined (Fig.6).

Pellegrini C, et al.⁴⁴), in their comprehensive analysis of AD, have particularly explored the characteristics of accelerated epigenetic ageing in this disease. They have found that AD-related epigenetic modifications are significantly enriched in probes whose DNAm varies with age. According to them, there is a high concordance between the direction of changes (hyper or hypo-methylation) in ageing and AD. This finding supports the accelerated epigenetic ageing in AD. They emphasize that several studies in post-mortem AD brains have identified a number of CpG sites that show robust changes in DNAm compared to non-demented controls.

Rezvan Noroozi, et al.⁴⁵), and Xueli Wei et al.⁴⁶), point out that DNA methylation is a vital component of the epigenetic machinery. Epigenetic modifications, particularly DNA methylation (DNAm), correlate with ageing and age-related diseases. A comprehensive analysis of both mechanisms that participate in the LRP1 expression drop in BBB related to AD, indicate their tight interrelation. It is difficult to say with absolute certainty which mechanism is primary. Oxidative stress with its components, especially hydroxyl radical (*OH), has been investigated for a number of years, and there are a lot of clinical and experimental verifications of its strong impact on AD pathophysiology and on the A β transport receptor interplay on the level of the BBB endothelial cells. The role of epigenetics represents a new research field which penetrates into a whole complex of events and relations that lie in the core essence of life. This study does not present therapeutic attempts related to both mechanisms, as this would require additional space. All information can be found in the comprehensive papers related to this problem^{47,48}).

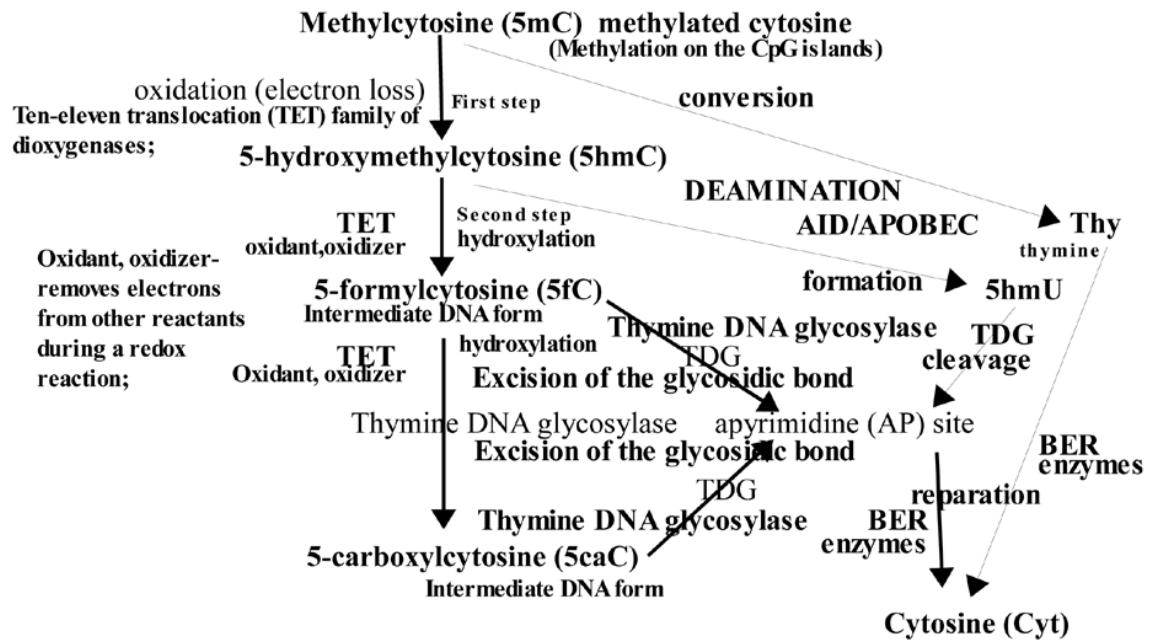


Fig.6. Schematic presentation of active DNA demethylation processes

According to Ref. 43 this figure presents two pathways of active DNA demethylation. In both pathways are included a number of enzymes and other compounds. 5-methylcytosine (5mC); -CH₃, methyl group; NH₂, amino group; N, nitrogen; O, oxygen; oxidation, electron loss; Thy, thymine; 5-hydroxymethylcytosine, 5hmC; ten-eleven translocation (TET) family of dioxygenases; 5-formylcytosine (5fC); 5-carboxylcytosine (5caC); thymine DNA glycosylase (TDG); apyrimidinic (AP) site, a location in DNA (also in RNA but much less likely) that has neither a purine nor a pyrimidinebase, either spontaneously or due to DNA damage; single-strand-selective monofunctional uracil-DNA glycosylase 1, SMUG1; Nei-Like DNA Glycosylase 1, NEIL1; methyl-CpG binding protein 4, MBD4; base excision repair enzymes, BER; (AID/APOBEC), activity-induced cytidine deaminase/ apolipoprotein B mRNA editing complex deaminase; deamination, removal of an amine group from a molecule; however, the active DNA demethylation in AD yet remains to be determined.

CONCLUSION

Alzheimer’s disease is a serious, chronic and lethal neurodegenerative disease. Its incidence and prevalence in the contemporary world has been increasingly growing. However, its complex etiology has not yet been completely explained. The role of LRP1 receptor in Aβ drainage from the brain is increasingly being investigated. The evident drop of the LRP1 expression in BBB in the course of ageing and in AD, associated with oxidative stress and epigenetics, is the main object of contemporary investigation

related to AD pathophysiology. The most recent results of these investigations indicate a close connection between oxidative stress and epigenetics, and show that there is hope for a successful solution of a number of problems related to the prevention and therapy of this disease.

CONFLICT OF INTEREST STATEMENT

The author states that the performance of this review entailed no issues representing a conflict of interest.

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