Glycative Stress Research

Online edition : ISSN 2188-3610
Print edition : ISSN 2188-3602
Received : November 11, 2024
Accepted : December 16, 2024
Published online : March 31, 2025
doi:10.24659/gsr.12.1_7

Original article

The crucial role of three enzymes DNMTs cytosine methyltransferases with low-density lipoprotein receptor-related protein 1 (LRP1) and receptor for advanced glycation end products (RAGE) in programmed human aging

Nikola Barić [1], Yoshikazu Yonei [2]

1) Private Family Medicine Practice Labin, Croatia
2) Anti-Aging Medical Research Center and Glycative Stress Research Center, Faculty of Life and Medical Sciences , Doshisha University, Kyoto, Japan

Abstract

Aging, the natural, complex and inevitable process in the life cycle of living beings, therefore also of man, according to today's knowledge, most likely takes place on the basis of a program located in the genome. This does not reduce the importance of dividing this process into normal or physiological aging and accelerated or pathological aging related to diseases. A whole set of current theories try to explain the essence of this process. In addition to different variations of the programmed aging theory, the following theories are also relevant: the theory of reactive oxygen species (ROS), cross-linking theory of long protein molecules, mutation theory, autoimmune theory, free radical Harman's theory, microglial aging theory, and the non-enzymatic glycation theory that is the result of the effects of advanced glycation end products (AGE compounds). According to the authors of this study, today's understanding of this inevitable, complex, one-way and irreversible event connected to living nature, with the crucial role of the five proteins mentioned in the title of this paper, is primarily based on the strong involvement of epigenetics in this complex process. Two basic processes related to epigenetics and essential for explaining the aging process, are deoxyribonucleic acids (DNA) methylation and DNA demethylation of CpG (cytosine/phosphate/guanine) sequences in the promoters of the *LRP1* and *RAGE* genes. The first process, determined by deoxyribonucleic acid methyltransferases proteins (DNMTs), involves the insertion of methyl groups ($-CH_3$) at the C5 position of cytosine in the template strand of DNA, where these genes are located (formation of 5mC). The second process is the oxidative demethylation of 5mC via TET (ten-eleven translocates), TDG (thymine DNA glycosylase) and BER enzymes (base excision repair enzymes). The transcription processes of the five genes involved (*LRP1, RAGE, DNMT1, DNMT3A, DNMT3B*) have been taking place since the very beginning of life. In addition to their role in controlling growth and development, the crucial function of these processes is to repair the damage caused by a set of adverse events in complex macromolecular structures, mostly caused by the action of oxidation and glycation stress. In essence, this means the creation of new "healthy" receptors as mentioned. Here it is important to emphasize that genomic damage is otherwise subject to the effects of other reparation systems, but their analysis is not the subject of this study. The aforementioned "restorative" transcriptions, under the strong influence of transcription factors (TFs: Sp1, specificity protein 1; Sp3, specificity protein 3), take place under the control of their programs located in the genome. In order to maintain life in the harsh selection struggle typical for each species, there is a precisely determined maximum possible lifespan for its individuals. Here, the processes of DNA methylation and demethylation play an important role. They also have their own precisely defined programs, and the aim of this study is to try to provide answers to the question of the aforementioned interactions. An additional goal of this study is to present the latest findings on the specific blockade of, in old age, the increased expression of the RAGE receptor, and on targeted gene therapy aimed at the muted expression of the LRP1 receptor in old age.

KEY WORDS: aging process, DNA methylation and demethylation, LRP1 and RAGE receptors, therapy of LRP1 and RAGE disordered expression

Correspondence to: Nikola Barić MDPhD
Private Family Medicine Practice
Presika 153, 52220 Labin, Croatia
TEL & FAX: +385-052-852-008
e-mail: nikolabaric100@gmail.com

Introduction

Due to the effects of even mildly increased levels of oxidative and glycative stress, permanently, during the life cycle of an individual, inevitable structural and functional damage occurs to a whole series of macromolecules, particularly cellular receptors (in this study, this refers to LRP1, low density lipoprotein receptor related protein 1, and the receptor for advanced glycation end products, RAGE). The necessary elimination or successful repair of these damages, outside of gene structures, by a series of biological mechanisms known today, often requires their permanent replacement, and this is achieved primarily by the transcription process of receptor genes. Therefore, the transcription of receptor genes, i.e. the creation of their mRNA, is a fundamental event without which there is no life. In addition to the aforementioned damage to receptors, damage also occurs to their genes in the promoters and gene coding regions. Adequate systems of reparation (at the gene level - currently in the phase of intensive research) try to repair these damages [1-3]. Based on the well-founded hypothesis about the crucial role of LRP1 (gene position 12q13.3) and RAGE (gene position 6p21.32) receptors in the programmed aging of living beings, including humans, the course of transcription of their genes, which is subject to a complex of epigenetic influences, becomes important. Among these influences, the processes of methylation and demethylation of cytosine-phosphate-guanine (CpG) sequences on the template chain of the DNA molecule and in the promoters of these genes are increasingly becoming the subject of intensive research. Crucial factors of the methylation process, which today is considered as the basic process of epigenetics, are three enzymes, DNA cytosine methyltransferases: DNMT3A, DNMT1 and DNMT3B, located at gene positions 2p23.3, 19p13.2 and 20q11.21[4-6]. According to the essential program outlined by evolution, these three genes encode, by the transcription of mRNA molecules, three enzymes, DNMT3A, DNMT1 and DNMT3B in their gene coding regions. The mentioned mRNAs leave the nucleus via nuclear tubules, and upon reaching the endoplasmic reticulum (ER), are converted by translation on the ribosomes into enzymes. In the case of the *LRP1* gene, the mRNA must be transformed into an active form by Furin (serine protease, a proteolytic enzyme resident in the Golgi) in the Golgi apparatus. The *RAGE* gene mRNA is converted directly into the active form of the enzyme in the ER[5]. As with DNMTs, the transcription of RAGE and LRP1 also occurs continuously, following a defined biological program. The methylation processes of RAGE and LRP1 differ significantly, which has implications for their crucial role in the process of programmed aging. The LRP1 promoter is not protected by the AGE/RAGE/cytokines molecular network. Large molecules of DNMTs (the largest enzymes in humans, 1620 aa) easily approach the LRP1 promoter and perform intense methylation. In the case of the RAGE promoter, this approach is extremely difficult due to the comprehensive network of AGE/RAGE/cytokines, resulting in weak methylation. This is further influenced by the increasing attraction of AGEs to RAGE during the life cycle, and their increasing accumulation around the RAGE promoter, especially in old age. Between AGEs and RAGE there is normally a strong mutual attraction and binding, which is not the case with LRP1. In addition, AGEs lead to a strong expression of RAGE through a positive feedback mechanism. As a result, the expression of LRP1 decreases with age, and the expression of RAGE increases. Vital processes related to the expression of LRP1 become more and more muted over time, and processes related to RAGE become more expressive. LRP1-induced processes are essential for vitality and longevity, and RAGE-related processes are pro-atherogenic in nature, blocking vitality and the possibility of longevity (***Table 1, 2***)[5,7-13].

The function of LRP1 and RAGE receptors in the life cycle of an individual

As previously pointed out, the process of programmed aging is under the strong control of five genes, three *DNMTs*, and one each of *LRP1* and *RAGE*. These genes have existed in the genome of living beings since the beginning of life. Closely related to the mechanisms of natural selection determined by evolution, these genes are crucial in the program of limiting the maximum possible lifespan of individuals of a species (about 120 years in humans), giving the potential opportunity for new genomes (mutants) to better adapt and survive. Transcription, along with the processes of methylation and demethylation, plays a crucial role in the aforementioned limiting of the maximum possible length of life. A better understanding of these three processes requires a good knowledge of the mentioned genes` genomes, especially the structure and function of their promoters, and the causes of weaker or stronger methylation in the CpG sequences located within them. What is methylation? DNMTs enzymes, using the abundantly locally present molecule S-adenosyl-L-methionine (SAM, AdoMet, methyl donor, cofactor), take the SAM's methyl group ($-CH_3$) and by a complex mechanism insert it into the DNMT pocket at the position of the C5 cytosine that was earlier everted from the DNA chain. The DNMT enzyme previously approached the DNA molecule and captured its template chain. This leads to the methylation of cytosine and to the strong blocking of LRP1 transcription, and to **extremely weak RAGE methylation**. The specificity of the structure of these two receptor promoters, after methylation, results in a change in the whole set of vital functions that they control. With LRP1 there is a gradual weakening and shutdown of these functions, while with RAGE there is a typical increased expression of pro-atherogenic events[6,14-20]. ***Table 1*** gives a summary of the decline in the values of a number of parameters related to the LRP1 receptor, typical of aging. It is necessary to immediately emphasize that LRP1 is a large multifunctional receptor for more than 100 ligands. In the brain, its predominant expression is on the abluminal side of the endothelial cells of the blood-brain barrier (BBB)[5]. ***Table 2*** shows changes in the values of a number of parameters related to the RAGE receptor typical for aging and old age. It should also be emphasized that RAGE is a multifunctional receptor with many ligands, especially advanced glycation end products, or AGEs. Its predominant position in the brain is on the luminal side of the endothelial cells of the blood-brain barrier (BBB). Ligand binding primarily occurs on the extracellular electropositive V domain (23-116 aa). The short cytoplasmic region (364-404 aa) is crucial for signalization. The RAGE receptor has two more extracellular domains, C1 (124-221 aa) and C2 (227-317 aa) (***Fig. 1***)[5].

Table 1. Drop of the values (activity) of a range of important parameters linked with the LRP1 during ageing and age.

Parameter	Characteristics and functions
Drop of proteases degradation; Drop of lysosomal enzymes activity	Lysosomal enzymes (acid hydrolases) are responsible for breaking down complex chemicals within a cell. They contain about 40 types of hydrollytic enzymes including proteases, nucleases, glycosidases, lipases, sulfatases, phospholipases, and phosphatases;
Drop of endocytosis Drop of transcytosis Drop of exocytosis Drop of the activity of cell signaling Drop of the phagocytosis of myelin debris Drop of the phagocytosis of apoptotic cells	Drop of tissue cleaning from toxic and dangerous compounds and their accumulation in the cells.
Drop in tumor invasion	One of the most important LRP1 function is in clearing proteases such as plasmin, urokinase-type plasminogen activator, and metalloproteinases, which contributes to prevention of cancer invasion. LRP1 absance increases possibility of cancer invasion.
LRP1 deficiency in neurons	Drop in insulin signaling, reduced levels of glucose transporters GLUT3 and GLUT4; drop in glucose uptake; rise in glucose intolerance; Disbalance in lipid homeostasis; Decline in cholesterol transport in the brain; Disbalance in regulation of cell proliferation, migration, apoptosis, and contraction of vascular cells, drop in maintaining the vascular homeostasis.
Smooth muscle cells alteration	Excess matrix deposition into the arterial wall, (smLRP1$^{-/-}$) mice; medial thickening of the arterial vessels, aortic dilatation with disorganized and degraded elastic lamina; smLRP1$^{-/-}$ mice contain a 4-fold increase in protein levels of high-temperature requirement factor A1 (HtrA1) which degrades matrix components and impairs elastogenesis with fragmentation of elastic fibers. LRP1$^{-/-}$ mice in their vassel walls also have excessive accumulation of CTGF which is a key mediator of fibrosis.
Drop of LRP1 receptor level located on the microglial cell membranes	Microglia expresses the increassed pro-inflammatory signaling (pro-inflammatory cytokines); activation of both JNK and NF-κB signaling (NF-κB cascade)

Endocytosis, biological process by which the extracellular materials are transported into intracellular compartmenta; transcytosis, transport of different biological material across the cell; exocytosis, biological process in which a cell transports different materials out of a cell; phagocytosis, process by which a special cells phagocytes ingest or engulf other cells or particles; myelin debris, material compossed of inflammatory and neurotoxic factors; apoptosis, the process of programmed cell death; urokinase-type plasminogen activator, uPA, serine protease; GLUT 3 and GLUT 4, proteins responsible for transport of glucose across the plasma membranes; HtrA1, high-temperature requirement factor A1, serine protease, tumor suppressor.

Table 2. Moving of the values of orange of important parameters linked with RAGE during ageing and age.

Parameter	Characteristics and functions
Methylated cytosines inthe RAGE promoter region	It is found a significant age-related decline of methylated cytosine in the RAGE promoter region in the human parietal cortex (superior parietal lobule or supramarginal gyrus-APP promoter region). This reduction in the number of methylcytosines (5mC) at transcription factor binding sites increases the expression of RAGE, which may in turn play a role in the ageing of the brain.
Rise in PKC activation	Stronger activation of nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidase); rise of oxidative stress (accumulation of ROS ($O_2{*}^-$, H_2O_2, *OH); activation of NF-κB cascade; elevation of PDGF, VCAM1, ICAM1, E-selectin, MCP-1, M-CSF, COX-2, MMP, TNF-α, Evident signs of atherogenesis. Reinforced astrocyte dysfunction, Consequence of the stronger oxidative stress; dangerous compounds and their accumulation in the cells.
AGEs binding with RAGE AGE-induced RAGE overexpression	RAGE dependent microglial activation, Signs of atherogenesis elevation, RAGE binding with damage-associated molecular pattern molecules (DAMPs): S100s, AGEs, HMGB1, and DNA; support of conditions of chronic inflammation, elevated RAGE signaling and induction of diabetic vascular complications, cardiovascular disease (CVD), cancer, Alzheimer's disease, and a range of inflammatory diseases.

AGE, advanced glycation end products; HMGB1, high mobility group box 1 protein, one of most important chromatin protein, mediator of inflammation and immune response; S100s, protein, included in signal transduction, cell differentation, transcription and cell cycle progression; DNA, deoxyribonucleic acid, molecular carrier of genetic informations; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells, transcription factor protein complex; VCAM-1, vascular cell adhesion protein 1, protein; PDGF, platelet derived growth factor,protein; ICAM-1, intercellular adhesion molecule1, protein; E-selectin, endothelial-leukocyte adhesion molecule 1; MCP-1, monocyte chemoatractant protein-1; M-CSF, macrophage colony-stimulating factor; COX-2, cyclooxygenase-2; TNF-α, tumor necrosis factor alpha.

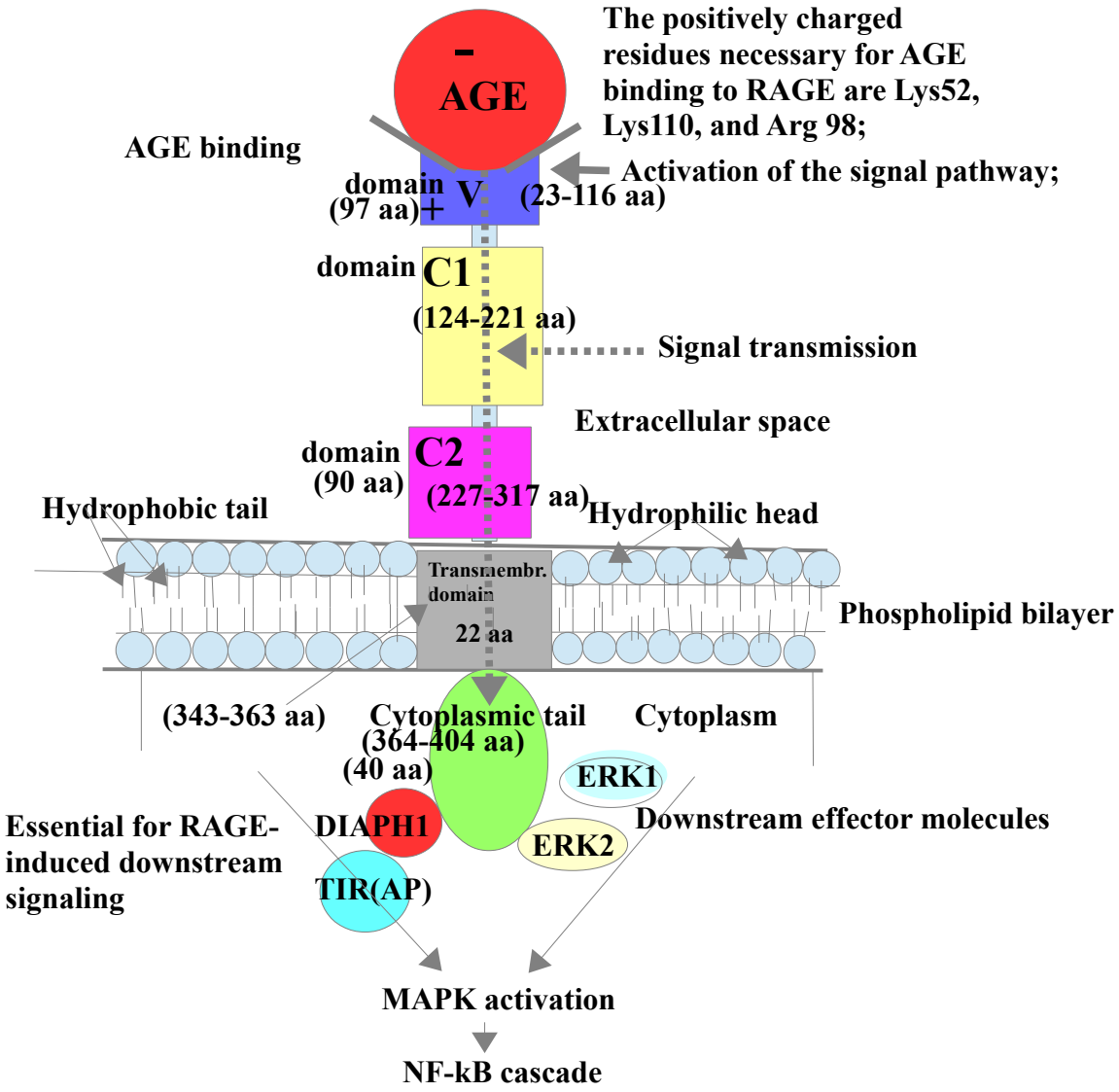

Fig. 1. **Presentation of the RAGE receptor with attached AGE.**

AGEs, advanced glycation end products; RAGE, receptor for advanced glycation end products; phospholipid bilayer; DIAPH1, diaphanous-related formin 1, essential for RAGE -induced downstream signaling, scaffold protein for MAPRE1 and APC to stabilize microtubule and promote cell migration; MAPRE1, microtubule-associated protein, regulate assembly and stability of microtubules; APC protein, adenomatous polyposis coli protein, tumor suppressor, important for cell adhesion; TOLL-interleukin 1 receptor domain containing adaptor protein (TIRAP); ERK1/2, extracellular signal-regulated kinase 1 and 2; MAPK, mitogen activated protein kinase, a type of serine threonine-specific protein kinase, is involved in signal transduction pathways; MAPK pathways message, amplify and integrate signals from a diverse range of stimuli and induce adequate physiological responses including cellular proliferation, differentation, developement, inflammatory responses, and apoptosis; NF-ϰB, a family of transcription factor complexes; NF-ϰB induces the expression of various pro-inflammatory genes as those encoding cytokines and chemokines; oxidative stress can activate NF-ϰB pathway.

DNA transcription process as the crucial event in the aging process

In the previous text, it was emphasized that the processes of continuous gene transcription are crucial for repairing the damage of their final products caused by oxidative and glycative stress. Both types of stress occur simultaneously and permanently, at varying speeds, during the life cycle of individuals of a species. In humans, damage to the five mentioned macromolecules (three DNMTs, one LRP1 and one RAGE each) is particularly important. The aforementioned macromolecules are complex proteins. Their reparative biosynthesis begins with transcription and ends with translation. Transcription takes place in gene coding regions and translation takes place in the endoplasmic reticulum (ER) on ribosomes[21,22]. The process of DNA methylation of LRP1 and RAGE promoter sequences, as well as the methylation or automethylation of DNMT1, DNMT3A, and DNMT3B promoter sequences, seems to be at the very core of programmed aging. Let us pay attention to the key aspects of the DNA transcription process. In doing so, it is necessary to remember the basic threadlike appearance of the gene with pronounced alternating structures of introns and exons. Genes are built from the DNA molecules which are composed of two nucleotide chains that wind about each other in the form of a helix. Stretched out end to end, one DNA molecule extends to about 1.5 cm. The human *LRP1* gene has 89 exons, and the human *RAGE* gene has 11. During splicing, the introns (non-coding regions within a gene) are removed from the pre-mRNA, and the exons (coding sections of an RNA transcript, or the DNA encoding the transcript) are stuck together to form a mature mRNA that does not contain the intron sequences. During transcription, the template strand of a gene`s DNA serves as a model for base-pairing, and an enzyme called RNA polymerase II catalyzes the formation of an mRNA precursor (pre-mRNA) molecule that is then extensively cotranscriptionally processed (5'-end capping, splicing, and 3'-end cleavage and polyadenylation) with consequent formation of mature mRNA. Only the exons of a gene encode a protein. Maturation of most pre-mRNAs requires the attachment of a 5'-7-methylguanosine cap (which is important for the binding of mRNA to ribosome during the initiation of translation, and also promotes splicing and polyadenylation) to the 5' end, and to intron excision, together with exon ligation, and the formation of a 3' end by cleavage and the addition of a non-templated poly (A) tail[23].

Kishikawa S *et al.*[24], show the effects of two transcription factors, Sp1 (specificity protein 1, gene location 12q13.13) and Sp3 (specificity protein 3, gene location 2q31.1) zinc finger proteins on the transcription of the *DNMT1* gene. The effect is caused by the interaction between the GC-rich binding site (GGGCGG) in the promoter of the *DNMT1* gene and the C-terminal zinc finger motifs in the Sp1 and Sp3 proteins. The authors state that the DNA GC-rich cis-element (nucleotides -161-147, essentially an enhancer) in the promoter of the *DNMT1* gene (as shown in experiments with mice) is crucial for the binding of transcription factors Sp1 and Sp3. These two factors are found in a wide range of mammalian cells, and are not part of the coding sequences of the gene they regulate, but are still bound close to it. They activate (activators) and enhance (enhancers) DNMT1 transcriptional activity. The promoter of this gene is located upstream of its transcribed region. The gene enhancer segment, the DNA sequence regulator, by binding to specific transcription factors, enhances gene transcription. It can be located closer to or farther from the gene, either upstream or downstream. The transcription start site is essentially the place between the promoter and the transcribed region, where the first DNA nucleotide is transcribed into mRNA. The Sp1 protein has three zinc finger protein motifs at its C-terminus, with which it binds directly to DNA and enables Sp1 to interact with other transcriptional regulators.

Jinawath A *et al.*[25], in their study, present the transcriptional regulation of the human DNA methyltransferase *DNMT3A* and *DNMT3B* genes by the Sp3 and Sp1 zinc finger proteins. The promoters of these two genes do not have typical TATA sequences close to their transcription start sites, but have several Sp1 binding sites. Inhibition (blockade) of the Sp1 binding sites by specific inhibitors disables the binding of Sp1 to its site and decreases promoter activities and the mRNA expression of DNMT3A and DNMT3B. On the other hand, the overexpression of Sp1 and Sp3 increases the promoter activities of these two genes. The binding of Sp1 and Sp3 to DNMT3A and DNMT3B promoters was confirmed by a gel shift assay. Once again, the authors emphasize the great functional importance of Sp proteins, particularly Sp3, in the regulation of *DNMT3A* and *DNMT3B* gene expression. It is important to emphasize that Sp1 and Sp3 proteins are abundantly expressed in a wide variety of mammalian tissues and cells[24,25]. Their expression and function are crucial in understanding the DNMTs gene transcription program and its role in the aging process.

Ryu H *et al,*[26], based on their experiments, have demonstrated that oxidative stress significantly elevates Sp1 and Sp3 protein levels (transcription) and their DNA binding in neurons. They have found that the elevated expression of these two transcriptors is neuroprotective. Both factors are induced by stimuli that lead to oxidative stress *in vivo*. Their overexpression can prevent neuronal damage and death connected with oxidative stress and other established apoptosis inducers. Both proteins are sufficient components of the protective homeostatic response to oxidative stress.

Wang L *et al.*[27] emphasize that Sp1 activity can be elevated by the fact that these stress factors (ROS) can activate the p42/p44 mitogen activated protein kinase pathway, and the c-Jun NH_2 terminal kinase-related signaling pathway, which both may be responsible for the Sp1 transcriptor overactivation. This leads to the changed expression of multiple Sp1 downstream genes (for example *DNMTs* genes). It is clear that in the case of oxidative stress, the increased amount of ROS, along with the damage to receptors (LRP1 and RAGE) and to DNMTs proteins, also acts as a signaling pathway for the activation of Sp1 and Sp3 transcriptors. This is followed by the activation of *LRP1, RAGE* and *DNMTs* genes and their transcription. This is in fact a protective mechanism for creating new correct DNMTs proteins.

By analyzing a number of transcription factors and their binding to the *LRP1* gene promoter, Lu K *et al.*[28] prove the strong attraction and interaction between the C-terminal zinc

finger motifs of the Sp1 protein and the GC-rich binding site of DNA. This is the beginning of an important signaling pathway for the activation of a number of biological factors and processes, both in normal physiological situations and in pathology.

Kohlgrüber S *et al.*[29] emphasize the central role of transcription factors in intracellular signaling. Besides their role as pathogen destructors, ROS function as redox messengers (second messengers). ROS-mediated redox messenger activity is thought to be greater than the ROS-mediated macromolecular damage activity.

Checa J *et al.*[30] also point out that low-level local ROS play an important role, both as redox-signaling molecules in a wide spectrum of pathways involved in the maintenance of cellular homeostasis, and as regulating key transcription factors.

The analysis of the seven papers mentioned (Ref.24-30) nicely shows how Sp1 and Sp3 bind to cis-regulatory elements located in the proximal promoter region of the respective genes, determining the basal activity of the promoters. These elements form short DNA segments 5-25 base pairs (bp) long. They have no coding function. One cis-element can bind one or several transcription factors. Otherwise, the gene promoter may have several cis-elements. In the promoter, they are located upstream of the TATA-box (T/A rich sequence located about 25-30 bp upstream of the transcription start site). Their importance in programmed aging can be inferred from the aforementioned. In Reference[26], the importance of oxidative stress is especially emphasized. Reactive oxygen species (ROS) are permanently generated during vital physiological processes. Oxidative stress occurs as a result of an imbalance between the generation of ROS and the strength of antioxidant defense systems. ROS have dual effects, toxic and signaling. Among the signaling effects, those related to the increased level of ROS, lead to a strong expression of Sp1 and Sp3 with their binding to the cis-elements of the DNMTs gene and the amplification of their transcription. Knowing the protective importance of these genes in programmed aging, the role of ROS in this program becomes clear. Along with DNMTs-induced methylation, i.e. automethylation, the methylation processes of demethylation factors, TET1, TET2, TET3, TDG and BER, and their role in programmed aging also become clear. The issue of the effects of Sp1 and Sp3 transcription factors and their connection with oxidative stress are presented graphically. Their strong neuroprotective action is evident (***Fig. 2***). Here, it is necessary to point out the close connection between oxidative and glycative stress, and therefore also the causes of the strong expression of the aforementioned Sp1 and Sp3 proteins. Glycation, or the Maillard reaction, is a non-enzymatic reaction between reducing sugars and amino acids (proteins). This reaction takes place permanently, at varying rates, in the body during a lifetime. Its final result is the accumulation of advanced glycation end products (AGEs: CML, N^{ε}-(carboxymethyl)-lysine); CEL, Nε-(carboxyethyl)-lysine; GOLD, glyoxal-lysine dimer; MOLD, methyl-glyoxal-lysine dimer). The reaction of these products with the RAGE receptor activates, under certain unfavorable conditions, a strong production of proinflammatory cytokines and ROS. This is followed by oxidative stress and the NF-kB cascade (strong secretion of PDGF, VCAM-1, ICAM-1, e-SELECTIN, MCP-1, M-CSF, COX-2, MMP-2, TNF-α, IL-8, IL-6, IL-1). ROS strongly activates the production of Sp1 and Sp3 proteins. The effects of the latter are analyzed in the previous text (***Fig. 3***)[3-5,9]. It is necessary to point out again that Sp1 and Sp3 proteins are permanently created by the transcription of their genes in gene promoters. There are two Sp1/Sp3 transcription factor binding sites in both promoters. Transcribed Sp1 and Sp3 proteins, besides binding to other gene promoters, also bind to their own promoters at Sp1/Sp3 binding sites, i.e. to cis-regulatory elements. Adequate transcription follows. Binding and transcription are both stimulated by oxidative stress.

Priya Dharshini LC *et al.*[31] give a detailed analysis of the occurrence of oxidative stress and its effects. The primary cause of oxidative stress lies in the imbalance between the formation of reactive oxygen species (ROS) and the antioxidant defense system, along with oxidative tissue damage. Changes in tissue structure and function, caused by the effects of ROS, lead to the establishment of various signaling pathways, which either activate or suppress numerous transcription factors. Among these factors related to the aging process, Sp1 and Sp3 proteins are particularly important. Oxidative stress increases their levels and DNA binding in cortical neurons, *in vitro* and *in vivo*, and significantly increases neuronal survival. In conditions without significant stress, the basal value of binding to DNA of both of these proteins is extremely low, but in conditions of high stress, their generation and binding to DNA is significantly increased. Sp proteins normally bind to GC box DNA sequences. The GC box is upstream of the TATA box, and approximately 110 bases upstream from the transcription initiation site. A TATA box is a DNA sequence that indicates where a genetic sequence can be read and decoded. It is evident that the processes of methylation and demethylation also affects the gene promoters of these two proteins.

The translation process

The mature mRNAs go out of the nucleus across the nuclear pores, enter the ER, and join ribosomes in the ribosome-abundant ER. A ribosome is structured from two separate RNA-protein complexes, one large and one small. During the translation process these units assemble together on the strand of mRNA, and attract the transfer RNA (tRNAs, which are typically composed of ≈ 76 nucleotides) that carry specific amino acids. The mRNA sequence is made up of 300-400 nucleotides. Codons are sequences of three immediately adjacent nucleotides, and certain combinations of them correspond to amino acids attached to tRNA. For example, the codon that is formed on the mRNA chain of adenine (A), guanine (G), and thymine (T) is attracted to tRNA attached to cytosine. The ribosome cleaves the incoming amino acid, the cytosine mentioned here, from the tRNA and inserts it at the beginning of the developing chain of the coded protein (LRP1, RAGE and three DNMTs). When the sliding ribosome reaches a certain termination codon, the translation stops, and the resulting protein in a certain vesicle is transported to the Golgi apparatus. However, before leaving the ER, the protein is still subject to the folding process, which gives it its final form

and function. In the Golgi apparatus, Furin, by structure a protease (proteolytic enzyme), cleaves and removes certain non-functional parts of the inactive LRP1 precursor, and thus LRP1 becomes active. In the corresponding vesicles, LRP1 and RAGE are transported to their destinations on the cell membranes. The three formed DNMTs enzymes remain mainly in the nucleus near the chromosomes. In the brain, the final destination for the LRP1 receptor is primarily on the abluminal side of the BBB endothelial membrane, and for RAGE it is the luminal side of the same membrane (contact with the blood stream). Here, it is important to emphasize that the lumen of the ER space is extremely prone to protein folding (wrapping)[32-35].

Turi Z *et al.*[36] point out that ribosome biosynthesis is a complex process that requires a coordinated activity of a whole series of factors and a high consumption of cellular energy. Ribosomes are crucial factors in protein production. Their biogenesis is initiated in the nuclear nucleolus. Disruptions in the biogenesis of these structures leads to cell cycle arrest, to aging or to apoptosis. The ribosome structure consists of 80 ribosomal proteins that are transcribed in the nucleus by RNA polymerase II and translated into the cytoplasm. Related to their damage are neurodegenerative diseases such as Alzheimer's, Parkinson's, Huntington's, and other progressive age-dependent diseases.

The process of DNA demethylation

In this study it is also necessary to analyze the process of active DNA demethylation that originates through the interplay of DNA oxidative reactions and DNA repair mechanisms.

According to Bayraktar G *et al.*[15], the first step of active demethylation is 5mC oxidation induced by the ten-eleven translocation (TET) family of dioxygenases with the generation of 5-hydroxymethylcytosine (5hmC). The second step is further hydroxylation of 5hmC by TET enzymes. The result is 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC). By Thymine DNA glycosylase (TDG) continues the 5fC and 5caC excision of the glycosidic bond, which results in an apyrimidinic (AP) site. The AP site can be repaired by Base Excision Repair (BER) enzymes with the generation of mark-free cytosine. It is important to emphasize that the level of DNA demethylation is continuously lower than the level of DNA methylation. Three mammalian TET proteins, TET1 (gene location 10q21.3), TET2 (gene location 4q24) and TET3 (gene location 2p13) successively oxidize 5mC to 5hmC. TET proteins maintain the consistency of gene transcription[4].

Bernstein C *et al.*[16] have found that in mice, within 6 hours of fertilization, the paternal chromosomes are close to 100% actively demethylated through TET3 and repair activity. In distinction from the situation with paternal chromosomes, methyl groups on maternal DNA passively become highly diluted over the next four days.

Gkountela S *et al.*[37] emphasize that the purposefulness of the global DNA demethylation in the preimplantation phase of mammals, including humans, lies in the strong prevention of the inherited transmission of abnormal cytosine methylations from parents to children.

Guo H *et al.*[38] stress that during the embryonic and fetal periods of life, there is an intensive demethylation process of the methylated paternal and maternal genes. Demethylation of the paternal genome is much faster than that of the maternal genome. It has already been emphasized that programmed aging is closely related to the methylation, or automethylation, of CpG sequences in the promoter regions of three *DNMTs* genes. Through continuous transcription regulated by the binding of transcription factors to their promoters (TFs, activators or repressors - here activators) and coactivators of mediators, these genes create mRNA of specific enzymes (DNMT1, DNMT3A, DNMT3B), which pass from the nucleus to the ER (rough endoplasmic reticulum) with accompanying translation into an effective enzyme on ribosomes. From the ER, via transport vesicles, these enzymes go to the Golgi apparatus, and then again via nuclear pores, return to the promoters of other genes where they perform the methylation of cytosines (among them LRP1 and RAGE). Part of the DNMTs enzymes return through the nuclear pores back to the nucleus and promoters of the DNMTs gene (bidirectional transport of macromolecules between the nucleus and the cytoplasm), There they automethylate the corresponding cytosines[5,34]. This automethylation progresses throughout life, especially during aging, and the accompanying demethylation still fails to annul its effects. Over time, the described automethylation leads to a progressive decline in the production of DNMTs enzymes, with a concomitant decrease in DNA methylation in the general genome. All of this is reflected in the constant suppression of the biological functions related to the LRP1 receptor, and the increased expression of the RAGE receptor and its pro-atherogenic functions. In the opinion of the authors of this study, these events lie in the essence of the programmed aging of living beings, including humans.

The causes of the TFs activation, and thus the beginning of the transcription process, are extensively analyzed in the study by Boija A *etal.*[39] TF essentially consists of two systems: DNA binding domains (DBDs) and activating domains (ADs). Diverse AD form phase-separated condensates with the Mediator coactivator. The ability to activate genes depends on the same amino acid residues. What is a Mediator? A Mediator is a multiprotein complex that functions as a transcriptional coactivator in all eukaryotes. At the same time it interacts with TF, RNA polymerase II, and DNA. It transmits signals between these three complexes. It has a crucial role in the transcription process. So, it is evident that the transcription of three DNMTs is a very complicated process which requires even more fundamental investigations. It is obvious that DNMTs proteins, among other things, also methylate TET1 (gene loc. 10Q21.3), TET2 (gene loc. 4Q24), TET3 (gene loc. 2P13), TDG (gene loc. 12Q24.1), and BER encoding genes. The consequent decrease in their demethylation activity suppresses the expression of the *LRP1* gene (its strong methylation is relevant), and thus favors the accelerated aging program. A detailed graphic presentation of the DNA demethylation process can be found in Barić N. Ref.[4], and Bayraktar Getal.Ref.[15]. Attempts to slow down the aging process in humans by RAGE blockers are presented in (***Fig. 4***)[40-48], and by LRP1 gene therapy in (***Fig. 5***)[5,49-51].

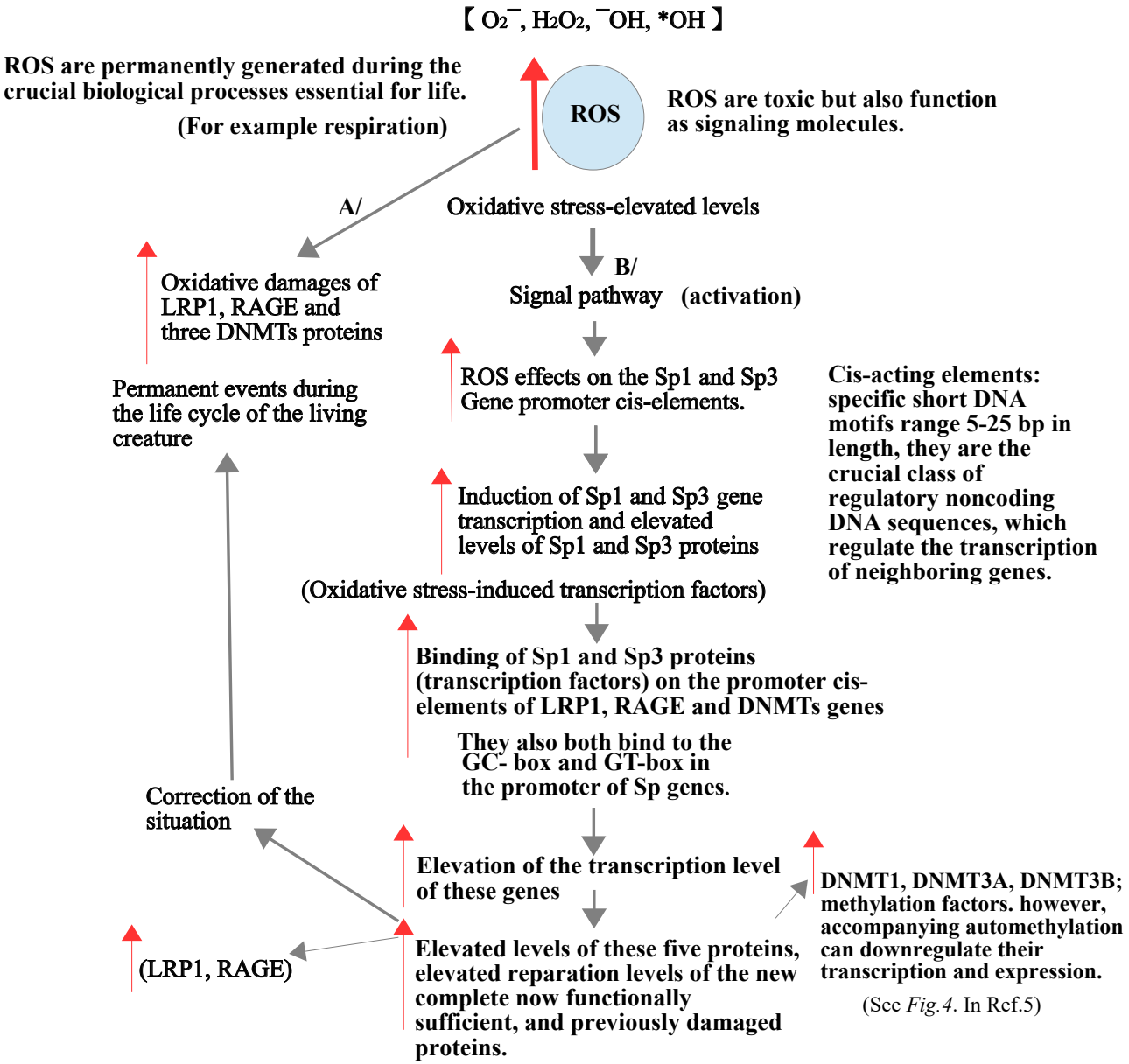

Fig. 2. **Schematic presentation of the protective role of Sp1 and Sp3 transcription factors against the ROS induced protein damages.**

H_2O_2, hydrogen peroxide; OH^- , hidroxil ion; OH*, hidroxil radical; LRP1, low density lipoprotein receptor related protein 1; AGEs, advanced glycation end products; RAGE, receptor for AGEs; DNMT, DNA methyltransferase; oxidative stress, an imbalance of reactive oxygen species and antioxidants in the body that leads to cell damage; signal pathway, the binding of signaling molecules (ligands) to receptors that trigger biological events inside the cell; transcription, the composite biological process by which a cell makes an RNA copy of a piece of DNA; Sp1 and Sp3 transcription factors are zinc finger DNA-binding domain proteins that recognize the specific DNA-binding motifs GC-box (GGGCGGG) and GT-box (GGTGTGGGG), they are essential for the expression of DNMTs proteins; gene promoter cis-elements, specific short DNA motifs range from 5 to 25 bp in length, regions of non-coding DNA, regulators of the neighboring genes transcription; O_2^-, superoxide, diatomic oxygen, an inorganic radical anion, a member of ROS,

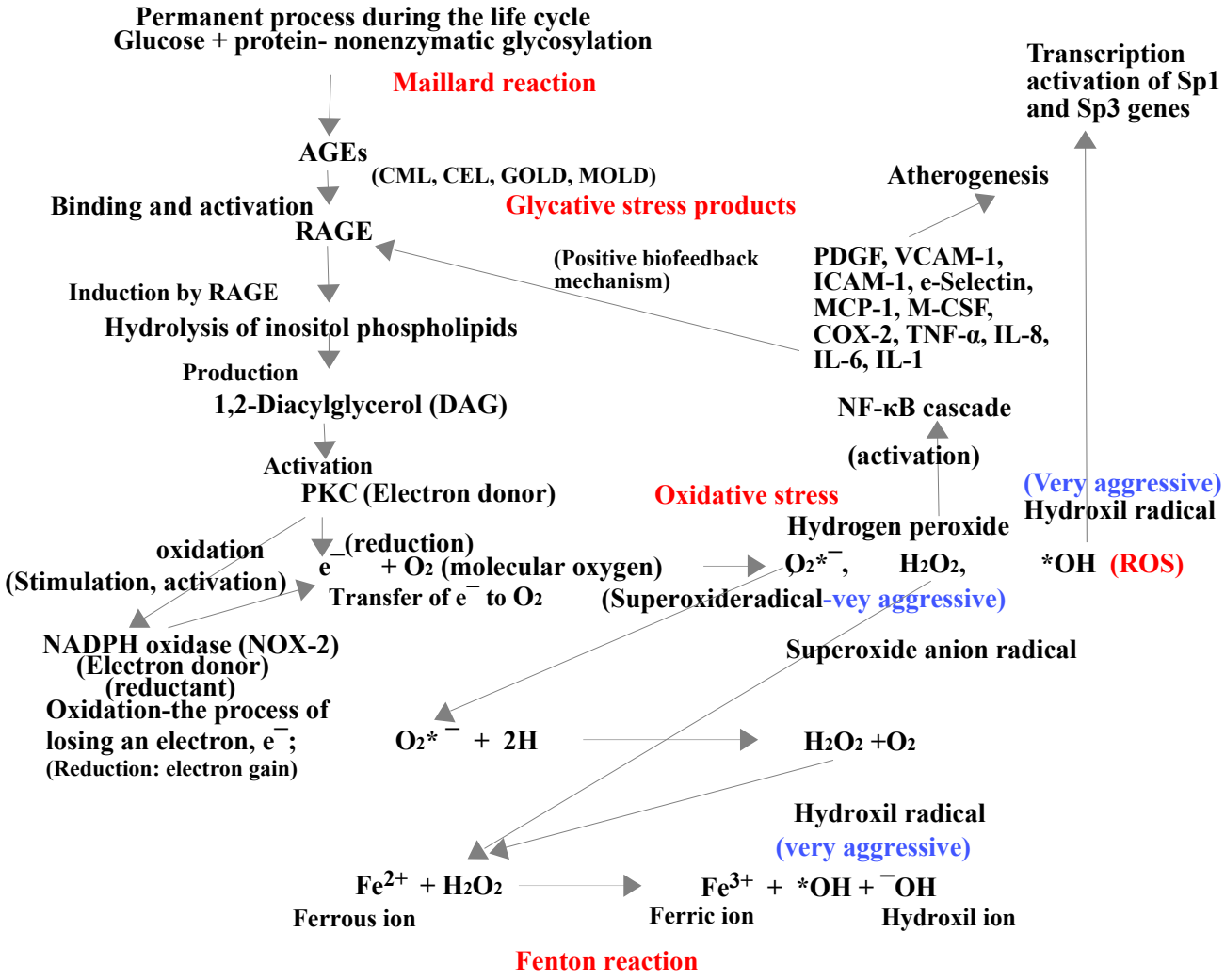

Fig. 3. **Activation of NADPH oxidase and induction of oxidative stress.**

NADPH oxidase: a family of enzymes whose function is to catalyze the transfer of electrons to O_2 generating superoxide or H_2O_2 using NADPH as an electron donor. It is reductant.The 1,2-diacylglycerol (DAG) activates protein kinase C (PKC) . Phosphatidylinositol (PI), also known as inositol phospholipid, is a lipid composed of a phosphate group, two fatty acid chains, and one inositol molecule. One electron reduction of O_2 gives rise to superoxide anion radical (O_2▪−), which then undergoes another one electron reduction to yield hydrogen peroxide (H_2O_2). One electron reduction of hydrogen peroxide generates hydroxyl radical (OH▪), which can then be reduced by one electron to form water. AGEs, via RAGE stimulate endothelial cells to generate ROS and to activate cellular signaling pathways; Glycative stress, biological stress induced by non-enzymatic glycation reactions, including AGE formation and accumulation, glycation-induced dysfunction of proteins, cellular signaling, inflammation, oxidation and tissue damage; Nε-(carboxymethyl)-lysine, CML; N^{ε}-(carboxyethyl)-lysine, CEL; glyoxal-lysine dimer, GOLD; methyl-glyoxal-lysine dimer, MOLD;

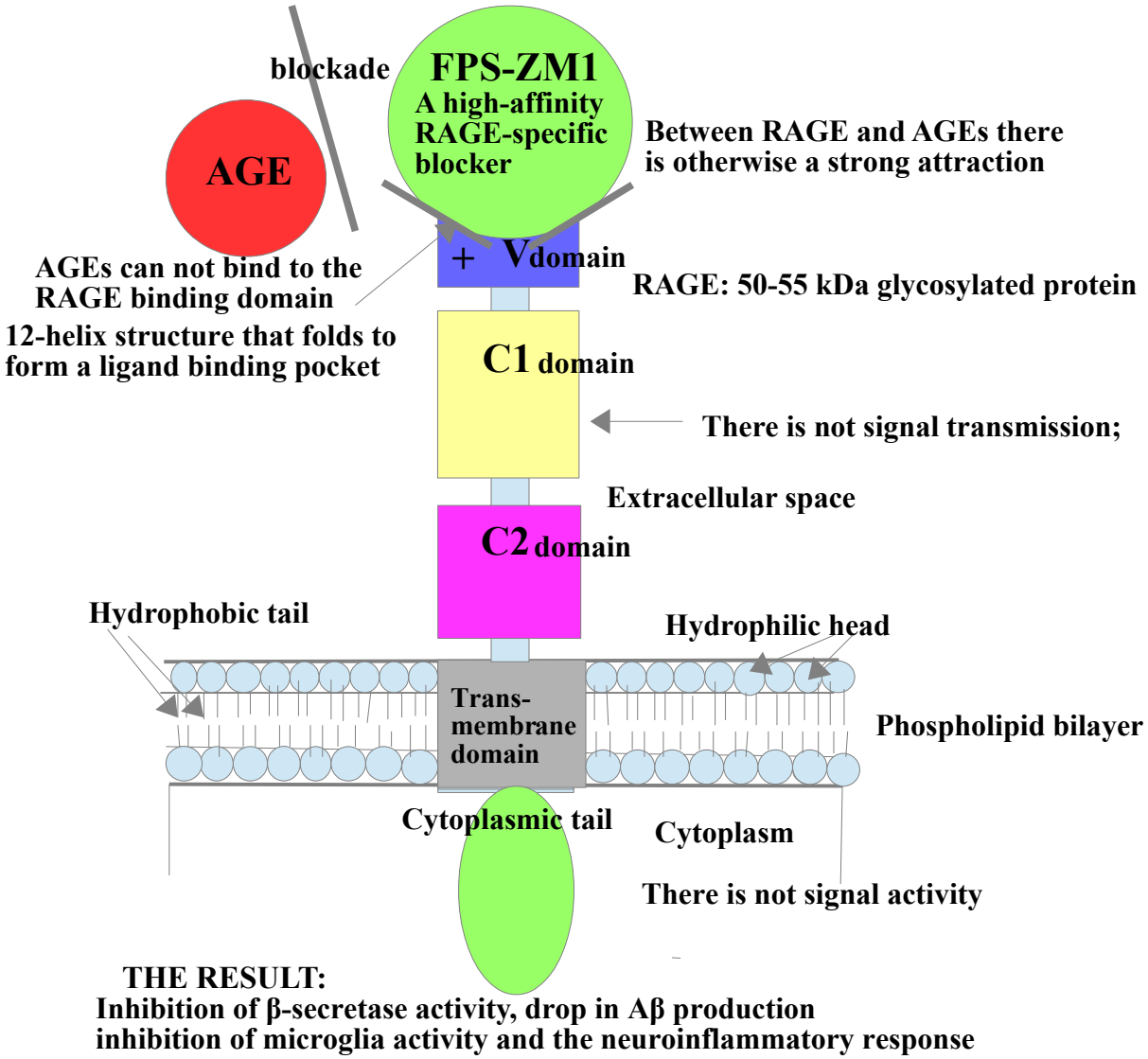

Fig. 4. **Schematic presentation of RAGE receptor, AGEs, and attached FPS- ZM1 blocker.**

After RAGE blocking by FPS-ZM1 there is not any receptor activation and signaling; All pro-atherogenic and pro-inflammatory RAGE functions are suppressed. Binding of FPS-ZM1 with RAGE, in experiments with mice, according to Yanian Kong, et al.[37], result in restoration of cerebral blood flow, inhibition of neurotoxicity, drop of microglial activity, drop of neuroinflammation, and improved cognitive behaviour; ***Table 2***. in the text in details presents these results. FPS-ZM1, formula $C_{20}H_{22}ClNO$; FPS-ZM1 chemical name, 4-chloro-N-cyclohexyl-N-(phenylmethyl)-benzamide; FPS-ZM1 MWt 327.85;

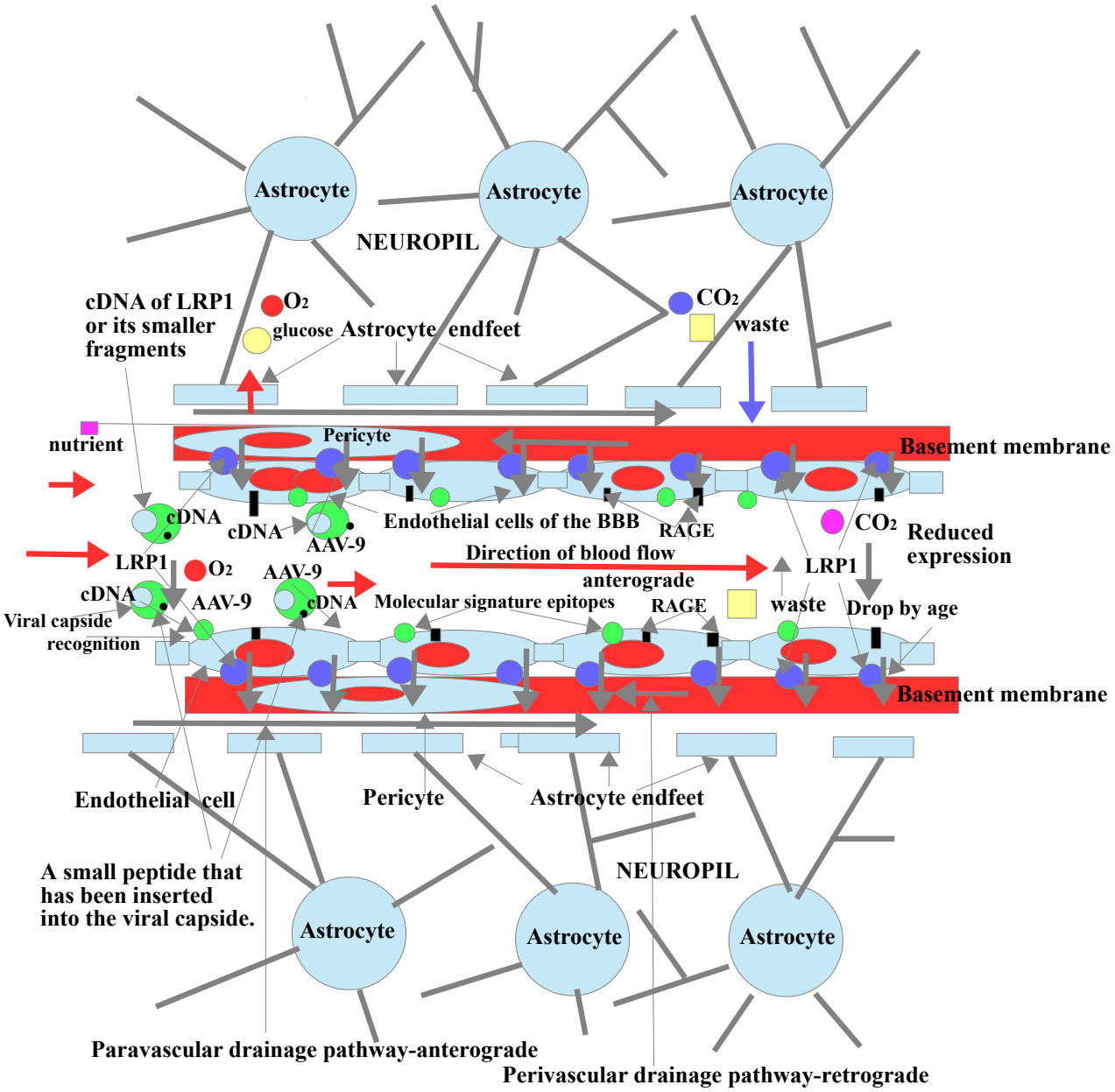

Fig. 5. **Presentation of AA-9 based targeting vectors with deliverance of LRP1 whole cDNA or a part of its domain with the aim to restore reduced LRP1 expression at the BBB vascular endothelial cells.**

Bloodbrain barrier, BBB; AAV-9 serotype; in genetics, complementary DNA (cDNA) is DNA that was reverse transcribed via reverse transcriptase from an RNA. It exists in both single and double stranded form; capside, the protein shellof a virus surrounding its nucleic acid; Every virus has its own capside; ., small intra capside inserted protein important for the recognition of endothelial cell membrane (BBB) molecular signature epitopes;LRP1 expression declines with age; RAGE expression rises with age;

Slowing down the aging process by FPS-ZM1 RAGE receptor blockade.

Deane R *et al.*[40] point out that Alzheimer's disease (AD) is a neurodegenerative disorder related to the accumulation of amyloid β-peptide (Aβ) in brain structures. Both the amyloid and vascular hypotheses about the causes of AD agree that the breakdown of the blood-brain barrier (BBB), leads to the accumulation of a series of vasculotoxic and neurotoxic macromolecules in the brain, along with the reduction of cerebral blood flow (CBF) and hypoxia, and causes functional and structural changes in neurons even before the amyloid deposition. In these events, the RAGE receptor that mediates Aβ-induced perturbations in cerebral vessels, neurons, and microglia, plays a key role. Based on these findings and on the necessity of developing new, efficient high-affinity Aβ/RAGE blockers that are safe and non-toxic, these authors performed a screening of a small molecule library and identified new tertiary amides that blocked the Aβ/RAGE interaction with strong binding affinity. This was followed by the synthesis of a second-generation library leading to the identification of FPS-ZM1, a high-affinity RAGE-specific inhibitor that binds to the V domain of RAGE, easily passes through the BBB, and inhibits Aβ-induced cellular stress in RAGE-expressing cells *in vitro* and *in vivo*. In these experiments it was found that FPS-ZM1 was not toxic to cells and to mice. Bounding with RAGE in the brain, FPS-ZM1 inhibits β-secretase activity, Aβ production, microglia activity and the neuroinflammatory response. An increased rate of infection in 15-17 month-old APPsw/0 mice treated with FPS-ZM1 was not observed. Mice treated with high doses of FPS-ZM1 (*i.e.*, 500 mg/kg i.p.) had normal biochemical laboratory values, with no histological changes in organs normally expressing RAGE (*e.g.*, lung, liver, kidney). The normal therapeutic dose in experimental APPsw/0 mice was 1 mg/kg i.p.

Hong J *et al.*[41] show the blocking effect of FPS-ZM1 on lipoprotein (LPS)-induced increase in the expression of high mobility group protein B1 (HMGB1) and interleukin-6 (IL-6) in the human gingival fibroblast (HGF) culture. LPS is a large molecule made of lipids and polysaccharides located in the outer membrane of gram-negative bacteria. It causes a strong immune response. By binding to RAGE and activating it, LPS triggers the NF-kB signaling pathway through the RAGE tail leading to the expression of HMGB1 and interleukin-6 (IL-6). The HGF culture was obtained using the enzymatic digestion-tissue explants method. LPS increased RAGE expression in HGF. Concurrently, stronger mRNA and protein expression of HMGB1 and IL-6 occurred in HGFs. However, pretreatment with FPS-ZM1 significantly reduced these effects. Pretreatment with FPS-ZM1 (250, 500 nM) also strongly blocked the LPS-induced NF-kB activity. The results of the experiment indicate that FPS-ZM1 is considered a promising therapeutic agent in inflammatory diseases caused by oral bacteria. It is important to explain the interaction of NF-kB with IkB proteins. Because of the aforementioned interaction, NF-kB dimers located in the cytosol of unstimulated cells, are retained in an inactive form. Otherwise, IkB proteins are strong NF-kB inhibitors. After RAGE stimulation, phosphorylation and degradation of IkB proteins occur in its tail region (mediated by the locally present IkB kinase IKK complex). This leads to NF-kB (strong transcription factor) activation and its nuclear translocation. Now occurs the induction of the target genes transcription (genes for cytokines, adhesion molecules, and RAGE itself with positive feedback loop)[9, 10, 42].

Wang L *et al.*[43] investigated, both *in vivo* and *in vitro*, the lipopolysaccharide (LPS)-mediated microglial inflammation that is the result of the LPS induced overproduction of interleukin-1 beta (IL-1β), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and of cyclooxygenase 2 (COX-2), in both, BV-2 cells and primary microglial cells. After the intraperitoneal (i.p.) application of LPS 5 mg/kg, they found, in the hippocampus of C57BL/ mice, the overproduction of microglial pro-inflammatory cytokines IL-1β and TNF-α. RNA-Sequencing (RNA-Seq) analysis showed the involvement of Janus kinase (JAK)-signal transducers, and activators of the transcription (STAT) signaling pathway in the regulation of FPS-ZM1 on LPS-induced microglial inflammation. It was found that FPS-ZM1 downregulated LPS-mediated increases in the phosphorylation levels of JAK/STAT, both *in vivo* and *in vitro*. The nuclear translocation of transcription factors STAT1/3/5 in BV-2 cells was also suppressed. Although the interaction between LPS and RAGE is not mentioned here, it is clear that it exists.

Nair M *et al.*[44] give an account of their research that provides strong confirmation of the binding of lipopolysaccharides (LPS) to the RAGE receptor. In their experiments they used thoracic dorsal root ganglia (tDRG) and tracheas from three groups of mice: wild types (WT) (the first group in the experiment), RAGE knock-out (RAGE-KO) (the second group in the experiment), and mice with the RAGE antagonist FPS-ZM1 (the third group in the experiment). They exposed each group of mice to lipopolysaccharides (LPS). In WT neurons (1. gr.), LPS increased the capsaicin (CAP)-evoked currents and the action potential (AP) generation, and LPS also caused the submucosal gland hypersecretion in tracheas from WT mice (1. gr.). In contrast to the first group, there were no reactions in the other two groups (the second group has no RAGE, and in the third group RAGE is blocked). It was evident that LPS upregulated full-length RAGE (encoded by Tv1-RAGE). These findings revealed that in response to LPS treatment, the full-length membrane-associated RAGE expression encoded by Tv1-RAGE is upregulated and is required for the sensitization of tDRG neurons.

Kong Y *et al.*[45] point out that the strong expression of the RAGE receptor is extremely harmful to the human body. By conditioning a cascade of biochemical events, this expression leads to the onset of Alzheimer's disease (AD) and its accelerated development. The trigger for the strong expression of this large multiligand transmembrane receptor is primarily its binding with AGE macromolecules. The mentioned cascade leads to a strong activation of protein kinase (PKC) and of nicotinamide adenine dinucleotide phosphate-oxidase (NADPH oxidase). This is followed by strong oxidative stress (ROS, reactive oxygen species = O^{*-}, H_2O_2,, *O) along with the NF-kB cascade (increased expression of PDGF, VCAM-1, ICAM-1, e-Selectin, MCP-1, M-CSF, COX-2, MMP-2, TNF-α, IL-8, IL-6, IL-1), and astrocyte dysfunction. On the other hand, hyperphosphorylation of tau protein and the formation of neurofibrillary tangles (NFT) occur. In addition to AGEs,

strong expression of RAGE is also caused by the binding of Aβ molecules to it. RAGE inhibitors, particularly FPS-ZM1, strongly block the binding of ligands to RAGE and thereby prevent the aforementioned cascade and its consequences. The authors of the study suppose that FPS-ZM1 can be used as a disease-modifying agent for AD. However, they do not mention its effects as a possible gero-prophylactic agent [9].

Teissier T *et al.* [46] emphasize that the concentrations of AGEs increase in conditions such as diabetes, as well as during aging. The interaction between the receptor for advanced glycation end-products (RAGE) and its numerous ligands, mainly results in a pro-inflammatory response favoring mitochondrial dysfunction or cellular senescence. RAGE is a strong regulator of innate immunity that has a central role in inflammaging, the chronic low-grade and sterile inflammation that increases with age and is an important contributory factor in aging. Pro-longevity effects seen upon blocking RAGE, or upon its deletion, are thus the result of reduced inflammaging. It has to be stressed that inflammation is an age-related increase in the level of pro-inflammatory markers in blood and tissues. The same authors present identical results with WT and RAGE-/- mice in Ref.12 of this study.

Bongarzone S *et al.* [47] present a comprehensive analysis of all aspects of RAGE targeting. A graphic presentation of the exposed material is given on (***Fig. 1*** and ***Fig. 4***).

Slowing down the aging process by LRP1 gene therapy

Sagare AP *et al.* [48] point out that the LRP1 expression at the BBB is reduced during normal aging. This reduction can be corrected by lifestyle changes, pharmacological agents, and gene therapy which plays a crucial role. Effective gene transfer to the BBB can be achieved by non-viral and viral systems. Viral based systems are more effective in mediating cell entry and transfer of genes to endothelial BBB cells. Adeno-associated virus (AAV) is optimal for achieving long-term gene expression in these cells. It is unable to replicate autonomously, which excludes pathogenicity. In direct targeting to the BBB endothelial cell genes, the important function has a small peptide (7-9 aa long) that has been inserted into the viral capsid sequence (protective coat surrounding the viral genome) to modify viral tropism (***Fig. 5***). So, it is necessary to identify molecular signature epitopes in the cerebral endothelial cells of the aging brain, and present these epitopes using the mentioned analogous small peptides on the capsid of AAV to enhance site-specific distribution after intravenous injections. In this way, it would be possible to use AAV-2 carrying the cDNA of LRP1 (complementary DNA-contains only coding sequences), or LRP1 smaller fragments to restore reduced LRP1 expression in vascular endothelial cells in the aging brain (***Fig. 5***).

Nikolakopoulou AM *et al.* [20] in their study present a number of interesting facts related to the endothelial LRP1 protective role against neurodegeneration by inhibiting the proinflammatory cyclophilin A-matrix metalloprotenase-9 pathway at the BBB. They emphasize that endothelial LRP1 gene replacement therapy, in the presence of endothelial LRP1 loss, can prevent or reverse the development of neurodegeneration. Experiments with mice show that LRP1 endothelial knockout, with CypA activation, can be restored with brain endothelial-specific *in vivo* LRP1 gene therapy. It is important to note that cyclophilins have a crucial function in protein folding, signaling, nucleic acid interaction, protein degradation, apoptosis, and in response to different stress stimuli. Overexpression of CypA is also linked with aging. It is a potential atherogenic cytokine as well as a potential promoter of cardiac hypertrophy.

Ramanathan A *et al.* [49], in a detailed study on the problem of reduced expression of the LRP1 receptor in AD and old age, express their opinion on the selective targeting of this receptor through the delivery of gene transfer vectors. Viral mediated gene transfer methods, particularly by the adeno-associated viral (AAV) system, have been proven effective in a number of peripheral cellular types, as well as in the CNS. The LRP1 receptor is particularly suitable for intravenously used targeted gene therapy due to its favorable location on the abluminal endothelial membrane of BBB cells. The animal models used were successfully tested by the AAV-2 vector system, using peptides with a strong affinity for cerebral vasculature. Recently, serotype AAV-9 has been shown to be particularly effective in the endothelial transduction at the BBB. These latest techniques used LRP1 whole cDNAs (complementary DNA, in genetics cDNA is DNA that is reversely transcribed from an RNA), or parts of their domains. Very good results have been obtained. The use of *LRP1* gene therapy on liver hepatocytes has also been shown to be effective.

Issa SS *et al.* [50] emphasize that advances in genetic engineering have enabled the development of effective gene therapy methods for a number of diseases based on adeno-associated viruses (AAVs). In their study they present a review of AAV discovery, properties, different serotypes, tropism, and uses in gene therapy of different diseases. However, they point out that today, there is a small number of approved AAV-based gene therapy medications. For example, the US Food and Drug Administration (FDA) has recently approved Etranacogene dezaparvovec, sold under the brand name "Hemgenix" (etranacogene dezaparvovec), a drug for the treatment of hemophilia type B (congenital deficiency of factor IX). The gene for this disease in the therapy carries an AAV serotype 5 (AAV5). The treatment is obtained with a one-time infusion that offers elevated factor IX levels for years. Roctavian (valoctocogene roxaparvovec-rvox), also recently approved by the FDA, uses AAV5 as a carrier of the deficient factor VIII gene in hemophilia A. It is also a one-time intravenous infusion gene therapy for adults with severe hemophilia A, without active factor VIII inhibitors. "Zolgensma" (onasemnogene abeparvovec) is a therapeutic for spinal muscular atrophy (SMA). This disease is characterized by the degeneration of the anterior horn motor neurons in the spinal cord. Zolgensma is an AAVserotype-9 (AAV9)-based vector. It is used by one-time intravenous administration in children less than 2 years old. (***Fig. 5***) gives a complete explanation of this problem.

Chen J *et al.* [51] present a detailed review of the role of the LRP1 receptor in the pathogenesis of atherosclerosis. LRP1 is normally widely distributed in body tissues. Its deletion leads to embryonic death, indicating its strong role in embryogenesis and development. It has been proven that it plays a crucial role in a number of physiological and pathological processes. These include homeostasis of plasma lipoproteins, atherosclerosis, tumor evolution, fibrinolysis, neuronal regeneration, and survival. Its enhanced expression

can be achieved with gene therapy. Using adeno-associated virus-2 (AAV-2) carrying LRP1-cDNA or smaller DNA segments, LRP1 downregulated expression can be upregulated and atherogenesis slowed. In experiments with AD mice, evidently favorable therapeutic results were achieved with the aforementioned gene therapy. It should be noted that the cDNA (copy DNA, complementary DNA) is synthetic DNA. It is transcribed from a specific RNA using the enzyme reverse transcriptase. cDNA contains only coding sequences.

Although it is not the main aim of this study, for a better understanding of the aging process, the authors present the basic characteristics related to the difference between glycative and oxidative stress in (*Table 3, Fig. 6, 7*)[52,53].

Yonei Y argues that the difference between oxidation and glycation is easy to understand when considering the difference in the biological defense mechanisms. Organisms have independent enzyme groups that deal with reactive oxygen species (superoxide dismutase [SOD], catalase, peroxidase) and aldehydes (ALDH, GAPDH, glyoxalase [GLO]). In the course of evolution, organisms have been fighting the threats of oxidative stress, which is an excess of ROS, and glycative stress, which is an excess of aldehydes. Glucose oxidation

Table 3. The Difference between Glycation and Oxidation in vivo.

	Glycative stress	Oxidative stress
Cause	Aldehydes	Free radicals / ROS
Lifespan (estimation)	10 ～100 minutes	micro ～milli second order
Defense enzyme	ALDH 1 ～17, GAPDH, GLO	SOD, peroxidase, catalase
【Reaction Products】		
Protein	AGEs	Protein oxides
Lipid	Amadori PE, FA-derived aldehydes	Lipid oxides, peroxides, FA-derived aldehydes
DNA	Carbonylated cytosine	Oxidative damage

ALDH, aldehyde-dehydrogenase; GAPDGH, glyceraldehyde-3-phosphate dehydrogenase; GLO, glyoxalase; , these enzymes are ricn in liver, kidney, atem cells amd prognetor cells; ROS, reactive oxygen species; SOD, superoxide dysmutase, AGEs, advance glycation endproducts; PE, pPhosphatidylethanolamine; FA, fatty acid.

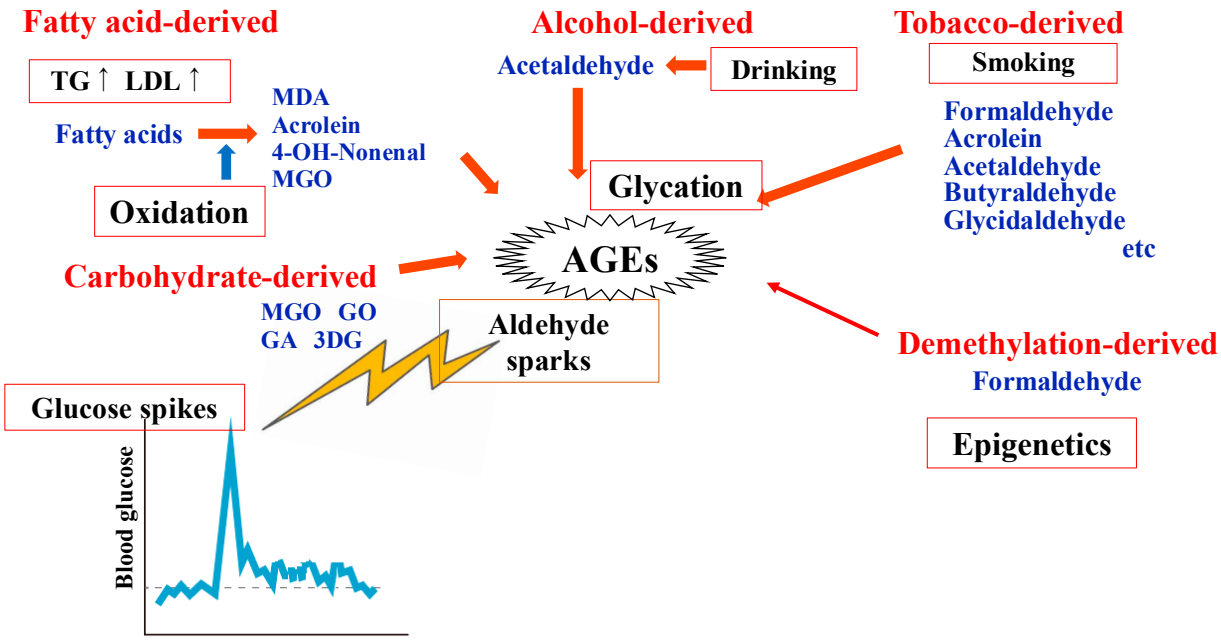

Fig. 6. **Origin of aldehydes *in vivo*.**

Aldehydes produced in the body are mainly derived from carbohydrates and fatty acids. Trace amounts of formaldehyde are producewith the demethylation of DNA and histone proteins. Exogenous aldehydes come from drinking alcohol and smoking. Cells that are actively dividing, proliferating, and differentiating generate a large amount of formaldehyde due to demethylation associated with epigenetics and DNA repair. HFD, high fat diet; TG, triglyceride; LDL, low-density lipoprotein; AGEs, advanced glycation endproducts. MDA, ; MGO, methylglyoxal; GO. glyoxal; 3DG, 3-deoxyglucosone; GA, glyceraldehyde.

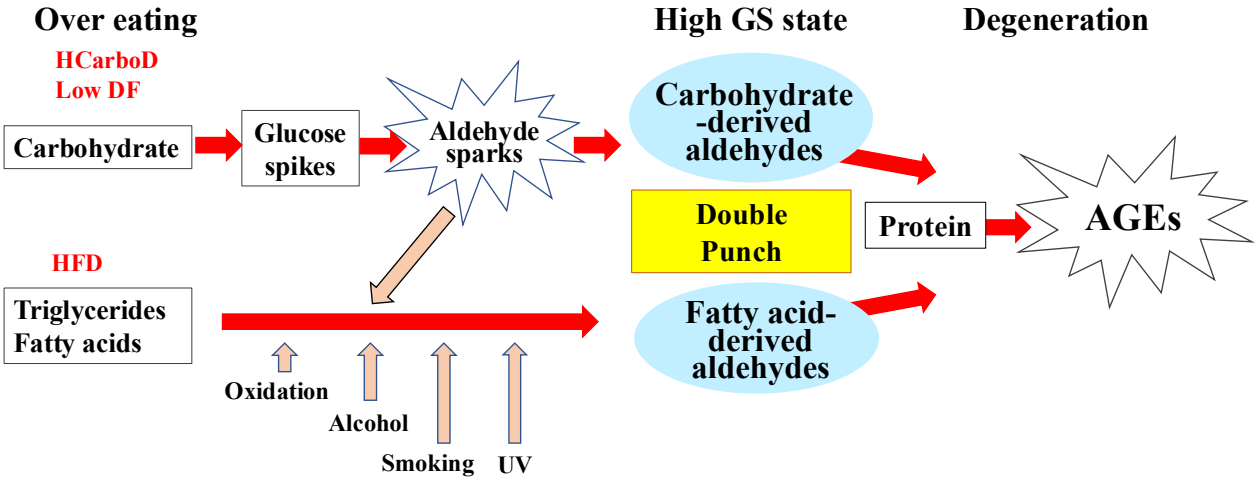

Fig. 7. **Double Punch by carbohydrate- and fatty acid-derived aldehydes.**
High carbohydrate diet (HCarboD) and low dietary fiber (DF) diet cause overproduction of aldehydes derived from carbohydrates, and HFD causes the production of aldehydes derived from fatty acids. Sugar and fat attack our bodies with a double punch. AGEs, advanced glycation endproducts; UV, ultra violet exposure.

produces carbohydrate-derived aldehydes (glyoxal [GO], methylglyoxal [MGO], 3-deoxyglucosone [3DG]), while fatty acid oxidation produces fatty acid-derived aldehydes (MGO, acrolein, malondialdehyde). GO, MGO, and 3DG are highly reactive aldehyde-type dicarbonyl compounds. MGO is an aldehyde produced from both carbohydrates and fatty acids, and is a threat to the organism. To combat this, cells produce GLO. In particular, in organs with a high lipid ratio such as the brain, both of these attack important proteins and DNA (cytosine) with a double punch.

Fig. 3 shows "glucose + protein - nonenzymatic glycation → AGE (Maillard reaction)," but since this reaction is caused by the reaction between the exposed aldehyde group (-CHO) of ring-open glucose and the amino residue (-NH_2) of protein, it is essentially a carbonylation reaction. In the future, it will be necessary to add not only the pathway via carbohydrate-derived aldehydes, but also the pathway via fatty acid-derived aldehydes.

In the section on **"The process of DNA demethylation"**, it has been pointed out that the frequency of DNA demethylation increases significantly during the early developmental stages of fertilized eggs, the formation of germ cells, and cell differentiation[37, 38]. The problem is that demethylation increases the production of formaldehyde derived from free methyl groups. For this reason, aldehyde-degrading enzymes are expressed in large quantities in stem cells and progenitor cells.

Since fetuses undergo rapid cell division, proliferation, and differentiation, formaldehyde has a significant effect on fetal development. Drinking alcohol and smoking during pregnancy are known to be risks for low birth weight babies, and these are likely caused by an increase in aldehyde load. Yonei et al. have been practicing glycative stress care for pregnant women and babies with the aim of reducing low birth weight babies[54]. The care is expected to have a positive effect on the development of the fetal central nervous system.

Conclusion

The aging process is a chronic, complex, progressive, unstoppable and irreversible biological process. It is obviously composed of two closely intertwined components: normal or physiological type of aging without the signs of chronic non-infectious diseases, and accelerated, pathological aging with obvious effects of chronic non-infectious diseases. The causes of this process, typical for all living beings, are essentially still unknown today. Nevertheless, the analysis of the effects of two large transmembrane receptors, LRP1 and RAGE, point to a justified presumption that they, along with programmed genetic and epigenetic changes in the body, especially changes in the DNMT receptor system, can be a key link in programmed aging. The observed changes in the expression of these two receptors related to aging, the decrease in LRP1 expression and the increasingly strong expression of the RAGE receptor, provide justified hope for slowing down the aging process using gene therapy with the *LRP1* gene and RAGE blockers, along with a whole set of additional measures.

Conflict of interest declaration

Not applicable.

Funding

No competitive funding was obtained.

References

1) Barić N. The role of the LRP1 receptor in the draining of amyloid beta (Aβ) peptide across the blood brain barrier (BBB). *RAD*. 2021; 548: 70-83.
2) Egawa T, Hayashi T. Association of glycative stress with motor and muscle function. *Front Physiol*. 2022; 13: 8553 58.
3) Barić N. 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. *RAD*. 2022; 553: 100-114.
4) Barić N. Effects of DNA methylation and demethylation on LRP1 and RAGE genes as crucial factors in Alzheimer's disease pathophysiology. *Glycative Stress Res*. 2023; 10: 79-88.
5) Barić N. The role of the low density lipoprotein receptor related protein 1 (LRP1) and the receptor for advanced glycation end products (RAGE) in the ageing process. *Glycative Stress Res*. 2024; 11: 7-20.
6) Yang J, Lior-Hoffman L, Wang S, et al. DNA cytosine methylation: Structural and thermodynamic characterization of the epigenetic marking mechanism. *Biochemistri*. 2013; 52: 2828-2838.
7) Xing P, Liao Z, Ren Z, et al. Roles of low-density lipoprotein receptor-related protein 1 in tumors. *Chin J Cancer*. 2016; 35: 6.
8) Silverberg GD, Miller MC, Messier AA, et al. Amyloid deposition and influx transporter expression at the blood-brain barrier increase in normal aging. *J Neuropathol Exp Neurol*. 2010; 69: 98-108.
9) Barić N. Role of advanced glycation end products in Alzheimer's disease. *Glycative Stress Res*. 2014; 1: 68-83.
10) Shen C, Ma Y, Zeng Z, et al. RAGE-specific inhibitor FPS-ZM1 attenuates AGE-induced neuroinflammation and oxidative stress in rat primary microglia. *Neurochem Res*. 2017; 42: 2902-2911.
11) Singh H, Agrawal DK.. Therapeutic potential of targeting the receptor for advanced glycation end products (RAGE) by small molecule inhibitors. *Drug Dev Res*. 2022; 83: 1257-1269.
12) Teissier T, Quersin V, Gnemmi V, et al. Knockout of receptor for advanced glycation end-products attenuates age-related renal lesions. *Aging Cell*. 2019; 18: e12850.
13) Hudson BL, Lippman ME. Targeting RAGE signaling in inflammatory disease. *Annu Rev Med*. 2018; 69: 349-364.
14) Slieker RC, Relton CL, Gaunt TR, et al. Age-related DNA methylation changes are tissue-specific with ELOVL2 promoter methylation as exception. *Epigenetics Chromatin*. 2018; 11: 25.
15) Bayraktar G, Kreutz MR. The role of activity-dependent DNA demethylation in the adult brain and in neurological disorders. *Front Mol Neurosci*. 2018; 11: 169.
16) Bernstein C, Bernstein H. Demethylation in early embrionic development and memory. In: DNA Methylation Mechanism, ed. Budak M, Yildiz M, 2020, Tucson, AZ, USA. https://www.intechopen.com/chapters/70316
17) Liu C-C, Hu J, Tsai C-W, et al. Neuronal LRP1 regulates glucose metabolism and insulin signaling in the brain. *J Neurosci*. 2015; 35: 5851-5859.
18) Muratoglu SC, Belgrave S, Hampton B, et al. LRP1 protects the vasculature by regulating levels of connective tissue growth Ffactor and HtrA1. *Arterioscler Thromb Vasc Biol*. 2013; 33: 2137-2146.
19) Liu Q, Trotter J, Zhang J, et al. Neuronal LRP1 knockout in adult mice leads to impaired brain lipid metabolism and progressive, age-dependent synapse loss and neurodegeneration. *J Neurosci*. 2010; 30: 17068-17078.
20) Nikolakopoulou AM, Wang Y, Ma Q, et al. Endothelial LRP1 protects against neurodegeneration by blocking cyclophilin A. *J Exp Med*. 2021; 218: e20202207.
21) Auderset L, Landowski LM, Foa L, et al. Low density lipoprotein receptor related protein as regulators of neural stem and progenitor cell function. *Stem Cells Intl*. 2016; 2016: 2108495.
22) Kierdorf K, Fritz G. RAGE regulation and signaling in inflammation and beyond. *J Leukoc Biol*.2013; 94: 55-68.
23) Cowling VH. Regulation of mRNA cap methylation. *Biochem*. 2010; 425: 295-302.
24) Kishikawa S, Murata T, Kimura H. Regulation of transcription of the DNMT1 gene by Sp1 and Sp3 zinc finger proteins. *Eur J Biochem*. 2002; 269: 2961-2970.
25) Jinawath A, Miyake S, Yanagisawa Y, et al. Transcriptional regulation of the human DNA methyltransferase 3A and 3B genes by Sp3 and Sp1 zinc finger proteins. *Biochem J*. 2005; 385: 557-564.
26) Ryu H, Lee J, Zaman K, et al. Sp1 and Sp3 are oxidative stress inducible, antideath transcription factors in cortical neurons. *J Neurosci*. 2003; 23: 3597-3606.
27) Wang L, Wei D, Huang S, et al. Transcription factor Sp1 expression is a significant predictor of survival in human gastric cancer. *Clin Cancer Res*. 2003; 9: 6371-6380.
28) Lu K, Wang Q, Hao L, et al. miR-204 ameliorates osteoarthritis pain by inhibiting SP1-LRP1 signaling and blocking neuro-cartilage interaction. *Bioact Mater*. 2023; 26: 425-436.
29) Kohlgrüber S, Upadhye A, Dyballa-Rukes N, et al. Regulation of transcription factors by reactive oxygen species and nitric oxide in vascular physiology and pathology. *Antioxid Redox Signal*. 2017; 26: 679-699.
30) Checa J, Aran JM. Reactive oxygen species: Drivers of physiological and pathological processes. *J Inflamm Res*. 2020; 13: 1057-1073.
31) Priya Dharshini LC, Vishnupriya S, Sakthivel KM, et al. Oxidative stress responsive transcription factors in cellular signalling transduction mechanisms. *Cell Signal*. 2020; 72: 109670.
32) Braakman I, Bulleid NJ. Protein folding and modification in the mammalian endoplasmic reticulum. *Annu Rev Biochem*. 2011; 80: 71-99
33) Kettel P, Karagöz GE. Endoplasmic reticulum: Monitoring and maintaining protein and membrane homeostasis in the endoplasmic reticulum by the unfolded protein response. *Int J Biochem Cell Biol*. 2024; 172: 106598.
34) Zhang Yi, Gao X, Bai X, et al. The emerging role of furin in neurodegeneration and neuropsychiatric diseases. *Transl Neurodegener*. 2022; 11: 39.
35) Paola De Magistris. The great escape: mRNA export through the nuclear pore complex. *Int J Mol Sci*. 2021; 22: 11767.
36) Turi Z, Lacey M, Mistrik M, et al. Impaired ribosome biogenesis: Mechanisms and relevance to cancer and aging. *Aging (Albany NY)*. 2019; 11: 2512-2540.

37) Gkountela S, Zhang KX, Shafig TA, et al. DNA demethylation dynamics in the human prenatal germline. *Cell*. 2015; 161: 1425-1436.
38) Guo H, Zhu P, Yan L, etal. The DNA methylation landscape of human early embryos. *Nature*. 2014; 511(7511): 606-610.
39) Boija A, Klein IA, Sabari BR, et al. Transcription factors activate genes through the phase-separation capacity of their activation domains. *Cell*. 2018; 175: 1842-1855.
40) Deane R, Singh I, Sagare AP, et al. A multimodal RAGE-specific inhibitor reduces amyloid β-mediated brain disorder in a mouse model of Alzheimer's disease. *J Clin Invest*. 2012; 122: 1377-1392.
41) Hong Y, Shen C, Yin Q, et al. Effects of RAGE-Specific inhibitor FPS-ZM1 on amyloid -β metabolism and AGEs-induced inflammation and oxidative stress in rat hyppocampus. *Neurochem Res*. 2016; 41: 1192-1199.
42) Giridharan S, Srinivasan M. Mechanism of NF-kB p65 and strategies for therapeutic manipulation. *J Inflamm Res*. 2018; 11: 407-419.
43) Wang L, Zhao D, Wang H, et al. FPS-ZM1 inhibits LPS-induced microglial inflammation by suppressing JAK/STAT signaling pathway. *Int Immunopharmacol*. 2021; 100: 108117.
44) Nair M, Jagadeeshan S, Katselis G, et al. Lipopolysaccharides induce a RAGE-mediated sensitization of sensory neurons and fluid hypersecretion in the upper airways. *Sci Rep*. 2021; 11: 8336.
45) Kong Y, Liu C, Zhou Y, et al. Progress of RAGE molecular imaging in Alzheimer's disease. *Front Aging Neurosci*. 2020; 12: 227.
46) Teissier T, Boulanger E. The receptor for advanced glycation end-products (RAGE) is an important pattern recognition receptor (PRR) for inflammaging. *Biogerontology*. 2019; 20: 279-301.
47) Bongarzone S, Savickas V, Luzi F, et al. Targeting the receptor for advanced glycation endproducts (RAGE): A medicinal chemistry perspective. *J Med Chem*. 2017; 60: 7213-7232.
48) Sagare AP, Deane R, Zlokovic BV. Low-density lipoprotein receptor-related protein 1: A physiological Aβ homeostatic mechanism with multiple therapeutic opportunities. *Pharmacol Ther*. 2012; 136: 94-105.
49) Ramanathan A, Nelson AR, Sagare A, 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.
50) Issa SS, Shaimardanova AA, Solovyeva VV, et al. Various AAV serotypes and their applications in gene therapy: An overview. *Cells*. 2023; 12: 785.
51) Chen J, Su Y, Pi S, et al. The dual role of low-density lipoprotein receptor-related protein 1 in atherosclerosis. *Front Cardiovasc Med*. 2021; 8: 682389.
52) Yonei Y, Yagi M, Sato K, et al. Glycative stress: Molecular impacts and defense mechanism. *Glycative Stress Res*. 2023; 10: 145-158.
53) Yonei Y, Saito Y, Yagi M, et al. From fatty liver to steatohepatitis: Involvement of aldehydes. *Glycative Stress Res*. 2024; 11: 79-93.
54) Ukezono M, Minamide K, Saika K, et al. Report from Izumiotsu Maternity Support Project: Next Generation Aid. *Glycative Stress Res*. 2024; 11: 204-211.