One New Hypothesis about the Ageing Process of Man

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Abstract

Ageing, the natural, complex, purposeful, polysyllabic 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. Life is essentially a conglomeration of more or less complex physiological and biochemical processes that are constantly taking place, and whose alteration or shutdown leads to its termination. An accompanying and inevitable phenomenon related to a large part of these processes is the generation of the so-called reactive oxygen species (ROS), molecular structures that are characterized by two crucial properties, aggression and destruction of molecular body structures, and signaling effects, as secondary messengers, on the mentioned processes. Among these, the oxidative destruction of exposed molecular structures, according to some of the latest findings, have special importance for the process of programmed aging. Among these structures, two large transmembrane multiligand receptors, low-density lipoprotein receptor-related protein 1 (LRP1) and receptor for advanced glycation end products (RAGE), and three enzymes deoxyribonucleic acid cytosine methyltransferases (DNMTs), and two Sp1 and Sp3 transcription factors (Sp1 and Sp3 proteins) otherwise abundantly present in tissues and cells, are specially affected. ROS elements lead to a strong activation, transcription and expression of those two factors, Sp1 and Sp3, resulting in their strong effect on DNMTs promoters with pronounced transcription and formation of DNMTs proteins. What is the function of DNMTs proteins? As components of crucial importance in the systems of epigenetics, these proteins condition the methylation processes of DNA molecules (adding the methyl group -CH3 to the molecules), generate the formation of 5-methyl cytosine (5mC) on the template strands of DNA, and the decrease in the transcription of methylated genes while shutting down their expression. Due to the specific conditions related to LRP1 and RAGE receptors, their promoters have different reactions to DNMTs-induced methylation. In LRP1 promoter methylation is fast and intense, while in RAGE promoter methylation is extremely slowed down and reduced. Thus, the final effects of those two genes, or their receptors, are extremely different. Biochemical and physiological processes related to LRP1 gradually slow down and dampen, and processes related to RAGE become more and more expressive. Another group of processes related to epigenetics and programmed aging includes oxidative demethylation of 5mC DNA segments via ten-eleven translocases (TET), thymine DNA glycosylase (TDG) and base excision repair enzymes (BER). Everything indicates that this second group of events is less efficient than the first group, and methylation clearly dominates. If nature hadn't programmed it that way, the aging process would be unpredictable. The increasing penetration of thought analyzes into the essence of the aging process, and the experimental results, increasingly point to the crucial importance of the process of transcription of the genes shown earlier. Regenerating transcriptions under the strong control of Sp proteins, and their programs located in the genome, influence the maximum possible life span of individuals of a species. The aim of this study is to provide additional explanations of the role of the mentioned receptors in the programmed aging of living beings. An additional goal of this study is the presentation of the latest findings on the

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specific blockade of, in old age, increased expression of the RAGE receptor, and on the targeted gene therapy, also in old age, of the muted expression of the LRP1 receptor.

Keywords: Aging process, epigenetics, methylation and demethylation, LRP1 and RAGE receptors

Sažetak

Jedna nova hipoteza o procesu starenja čovjeka

Proces starenja, u biti prirodan, složen, svrsishodan, višeprofilan, i neminovan proces u životnom ciklusu živih bića, prema tome i čovjeka, po današnjim saznanjima, najvjerojatnije se bazira na programu lociranom u genomu. To ne umanjuje važnost podjele tog procesa u normalno ili fiziološko starenje, i ubrzano ili patološko starenje vezano uz bolesti.

Život je u biti konglomerat više ili manje složenih fizioloških i biokemijskih procesa koji se permanentno odvijaju, i čija alteracija ili gašenje dovodi do njegovog prestanka. Popratna i neminovna pojava vezana uz veliki dio tih procesa je generiranje tzv. reaktivnih metabolita kisika (ROS), molekularnih struktura koje se odlikuju sa dva krucijalna svojstva, agresijom i destrukcijom molekularnih tjelesnih struktura, te signalnim učincima, kao secundar messangers, na spomenute procese. Među tim, oksidativnom destrukcijom izloženih molekularnih struktura, po nekim najnovijim saznanjima, posebnu važnost za proces programiranog starenja, imaju dva velika transmembranska multiligandska receptora, low-density lipoprotein receptor-related protein 1 (LRP1) i receptor for advanced glycation end products (RAGE), te tri enzymes deoxyribonucleic acid citosine methyltransferases (DNMTs), i dva Sp1 i Sp3 transkripciona faktora (Sp1 and Sp3 proteins) inače obilno prisutna u tkivima i stanicama. ROS elementi dovode do snažne aktivacije, transkripcije i ekspresije ta dva faktora, Sp1 i Sp3, Rezultira njihov snažan učinak na DNMTs promotore uz izraženu njihovu transkripciju i stvaranje DNMTs proteina. Koja je funkcija DNMTs proteina? Kao komponente krucijalnog značaja u sustavima epigenetike ti proteini uvjetuju procese metilacije DNA molekula (adding the methyl group -CH3 to the molecules), generiraju nastanak 5-methyl cytosina (5mC) na template lancima DNA, te pad transkripcije metiliranih gena uz gašenje njihovih ekspresija. Zbog specifičnih uvjeta vezanih uz LRP1 i RAGE receptore, njihovi promotori imaju različite reakcije na DNMTs uvjetovanu metilaciju. Kod LRP1 metilacija promotora je brza i intenzivna, a kod RAGE promotora metilacija je izrazito usporena i smanjena. Time su i konačni učinci ta dva gena, odnosno njihovih receptora, izrazito različiti. Biokemijski i fiziološki procesi vezani uz LRP1 se postepeno usporavaju i prigušuju, a procesi vezani uz RAGE postaju sve više ekspresivniji. Druga grupa procesa vezana uz epigenetiku i programirano starenje obuhvaća oksidativnu demetilaciju 5mC segmenata DNA putem ten-eleven translocases (TET), thymine DNA glicosylase (TDG) i base excision repair enzymes (BER). Sve ukazuje kako je ova druga grupa zbivanja manje efikasna od prve grupe, te metilacija očito dominira. Da to priroda nije tako programirala, proces starenja bio bi nepredvidiv. Sve veći prodor misaonih analiza u bit procesa starenja, te eksperimentalni rezultati, sve više upućuju na presudnu važnost procesa transkripcije ranije prikazanih gena. Obnavljajuće transkripcije pod snažnom kontrolom Sp proteina, i njihovih u genomu lociranih programa, utiču na maksimalno moguće trajanje života individua neke vrste. Cilj ove studije je dati dodatna objašnjenja uloge spomenutih receptora u programiranom starenju živih bića. Dodatni cilj ove studije je prikaz najnovijih saznanja o specifičnoj blokadi, u staroj dobi, povećane ekspresije RAGE receptora, i ciljane genske terapije, također u staroj dobi, prigušene ekspresije LRP1 receptora.

Ključne riječi: Proces starenja, epigenetika, metilacija i demetilacija, LRP1 i RAGE receptori

Introduction

The aging process, essentially a natural, complex, inevitable, expedient and multi-profile process in the life cycle of living beings, therefore also of humans, according to today's knowledge, most likely takes place on the basis of a program located in the genome. Looking at the human being, this does not diminish the importance of dividing that process into normal or physiological aging and accelerated or pathological aging related to diseases. Programmed aging, according to the author of this study, is based on the assumption of the effects and control of two large transmembrane receptors, LRP1 (gene position 12q13.3) and RAGE (gene position 6p21.32), and three DNMTs (DNMT3A gene position 2p23.3, DNMT1 gene position 19p13.2, DNMT3B gene position 20q11.21) and two Sp proteins (Sp1, specificity protein 1, gene position 12q13.13, Sp3, specificity protein 3, gene position 2q31.1) becomes clearer after the involvement of epigenetics and reactive oxygen species (ROS – O_2^{*-} superoxide radical, H_2O_2 hydrogen peroxide, *OH hydroxyl radical) in this process[1-5].

In the presented study, a new hypothesis was put forward about the crucial role of the two large receptors, LRP1 and RAGE, in the process of programmed aging. Science has been dealing with the problem of programmed aging for many years. A detailed study of the theories known to this day about that process (a whole set of current theories try to explain the essence of this process. In addition to different variations of the programmed aging theory, on the other hand, actual are the theory of reactive oxygen species (ROS) [6], cross-linking theory of long protein molecules [7], mutation theory [8], autoimmune theory [9], free radical Harman's theory [10], microglial aging theory [11], mitochondrial theory of aging [12], non-enzymatic glycation theory that is the result of the effects of advanced glycation end products - AGEs compounds[13], and chronographic aging theory[14]) indicate that a solution has not yet been found. By analyzing the pathophysiology of Alzheimer's disease, which has been extensively written about, where there is an evident connection between the disease and the aging process, it was observed that in this disease, and even without it, the expression of LRP1 decreases with aging, while the expression of RAGE tends to increase [15,16]. This led the author of this study to think about the possible role of these two large transmembrane receptors in the process of natural programmed aging of living beings. Some examples of events in the LRP1 expression decrease during aging can be given: drop of proteases degradation, drop of lysosomal enzymes activity, drop of endocytosis, transcytosis and exocytosis, drop of the activity of cell signaling; drop of the phagocytosis of myelin debris and phagocytosis of apoptotic cells; the resulting drop of tissue cleaning from toxic and dangerous compounds and their accumulation in the cells. Drop in the prevention of cancer invasion. Rise in the possibility of cancer invasion. Something related to the RAGE receptor and its gene: age related decline of methylated cytosines in RAGE promoter, strong 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-kB cascade (rise of PDGF, VCAM-1, ICAM-1, e-SELECTIN, MCP-1, M-CSF, COX-2, MMP-2 TNFα, IL-8, IL-6, IL-1), evident signs of atherogenesis, reinforced astrocyte dysfunction. RAGE dependent microglial activation, signs of chronic inflammation. These are just some of the moments related to the decrease in LRP1 expression and the increase in RAGE expression **(Table 1 and 2)**[17-19].

Further analysis of these events on the BBB related to AD and aging is presented by Doreen Osgood et al.[20]. At this site, Aβ-transport receptors, in fact specific proteins, LRP1 and RAGE, show significant alterations during aging. The expression of the efflux transporters LRP-1 and P-gp decreases, and the expression of the influx transporter RAGE increases. These receptors otherwise have important roles in maintaining biochemical homeostasis of the brain. They are also highly conserved throughout mammalian evolution. The authors of the paper admit that the current knowledge about how and why their expression in the BBB region is significantly altered during aging and in AD is very insufficient. The data suggest that gene transcription is altered with aging by some upstream events rather than a post-transcriptional, translational or direct effect on these cell surface receptors. Transcriptional alterations of gene expression may occur via modification of the gene promoter. One hundred male F344/BN hybrid rats with similar characteristics were used in this study. Analysis of graphs obtained from experiments on these mice, which show the correlation between the relationship between receptors and their mRNAs, indicates a close match between the descending curves for LRP1/age and the ascending curves for RAGE/age. Again, it is important to emphasize the modifications of gene promoters.

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 connective tissue growth factor (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-kB signaling (NF-kB cascade);
High sensibility of LRP1 promoter to methylation;	Strong rise of LRP1 promoter methylation by ageing;

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 o range of important parameters linked with RAGE during ageing and age

Parameter	characteristics and functions
Methylated cytosines in the 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 Protein kinase C (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;
AGEs binding with RAGE, AGE-induced RAGE overexpression;	⟶ RAGE dependent microglial activation, Signes 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, increased generation of oxygen radicals, activation of nuclear factor-kappa B, increased expression and release of pro-inflammatory cytokines; onset or accelerated course of atherosclerosis, coronary artery disease, hypertension, cerebral vascular disease, hyperthyroidism, Alzheimer's disease, end-stage renal disease, and diabetes mellitus.

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-kB, 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 α;

Reactive oxygen species (ROS) and advanced glycation end products (AGEs)

The creation of reactive oxygen species (ROS) in the human body and the occurrence of oxidative stress is largely related to the formation and accumulation of so-called advanced glycation end products (AGEs), complex molecules resulting from the covalent bonding, of a sugar molecule, such as glucose or fructose, to a protein or lipid molecule (glycation or nonenzymatic glycosylation). This binding takes place slowly and without enzyme control. The creation of these endogenous compounds takes place slowly and permanently throughout life (Maillard reaction). Binding of AGE compounds to RAGE leads to activation of protein kinase C (PKC) and NADPH oxidase, with strong generation of ROS. Due to the effects of AGEs on mitochondria and the mitochondrial electronic transport chain, there are frequent damages to that chain with the escape of electrons (e^-), their joining with molecular oxygen (O_2), and the formation of ROS. On the other hand, with the intake of unhealthy food (food processing by dry heat, highly processed food, high lipid and high protein food-meat, cheese, eggs, high temperatures in food processing, grilling, broiling roasting) and exposure to various adverse external influences, for example: ultraviolet (UV) radiation, cigarette smoking, alcohol consumption, exogenous AGEs enter the body with subsequent harmful effects. In addition to harmful effects on the body, ROS, on the other hand, play an important role in intermolecular signaling and especially in the control of transcription factors [1,16].

Activation of Sp1 and Sp3 proteins

It has already been emphasized that Sp1 and Sp3 proteins are abundantly present in tissues and cells. Local ROS elements lead to strong activation, transcription and expression of these two factors (Sp1 and Sp3). The result is their strong effect on DNMTs promoters with pronounced gene transcription and formation of DNMTs proteins (DNMT1, DNMT3A, DNMT3B) (***Fig.1***)[20]. Liwei Wang, et al.[21], 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 NH2 terminal kinase-related signaling pathway, both of which may be responsible for the Sp1 transcriptor overactivation. This leads to the consequent expression of multiple Sp1 downstream genes (for example DNMTs genes).

It is obvious 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 (***Fig.1***).

Guang Jia, et al.[22], reveal that c-Jun NH2-terminal kinase is strongly activated by a variety of stressful cellular environments, such as chemotherapy and oxidative stress. And this is where protein phosphorylation is important.

Kathryn Z Guyton, et al.[23], examining the influence of ROS on ERK1(p42) and ERK2 (p44), found that in both kinases, ROS leads to their strong activation, with direct phosphorylation (addition of phosphoryl-PO3-group to a molecule) of the Sp1 protein on its threonine 453 and 273. Thus activated Sp1 strongly activates many downstream genes (the human genome contains about 20,000 genes used to code proteins).

Julia Milanini, et al.[24], indicate that p42/p44 MAPK directly phosphorylates Sp1 on threonine 453 and 739 both in vitro and in vivo. Mutation of the mentioned positions halves the strength of transcriptional activity of Sp1.

Jin Wu, et al.[25], present the finding that oxidative stress, through activation of c-Jun NH2 terminal kinase, contributes to the proinflammatory phenotype of diabetic mesangial cells.

Linda Weis, et al.[26], indicate that c-Jun NH2-terminal kinases (JNKs) are a group of mitogen-activated protein kinases (MAP) that participate in signal transduction events important for specific cellular functions. Cellular stress and cytokines lead to the phosphorylation of JNK and its subsequent activation. Phosphorylation takes place on threonine and tyrosine residues (dual phosphorylation) within protein kinase subdomain VIII. The activation of JNK is conditioned by the activation of a wide range of stimulators, among them some growth factors, cytokines, UV-irradiation, heat shock, ceramide, and certain inhibitors of protein synthesis.

It is important to point out here that the human genome contains about 28 million CpG sites, of which about 60% are methylated at position 5 of cytosine (5mC) [27].

As can be seen from the previous presentation. two transcription factors, Sp1 and Sp3, are strongly activated by ROS with their enhanced expression and transcription. Increased concentration of Sp protein leads to strong transcription and expression of DNMTs genes, and generation, after translation in ER, of their transcripts, DNMTs protein. The basic function of DNMTs protein is the methylation of CpG sequences (adding a -CH3 or methyl group) in the promoters of a number of genes in the genome, among them LRP1 and RAGE genes. The methylation processes of RAGE and LRP1 differ significantly.

AGE/RAGE/cytokines molecular net

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, and methylation is weak. This is additionally contributed by the increasing

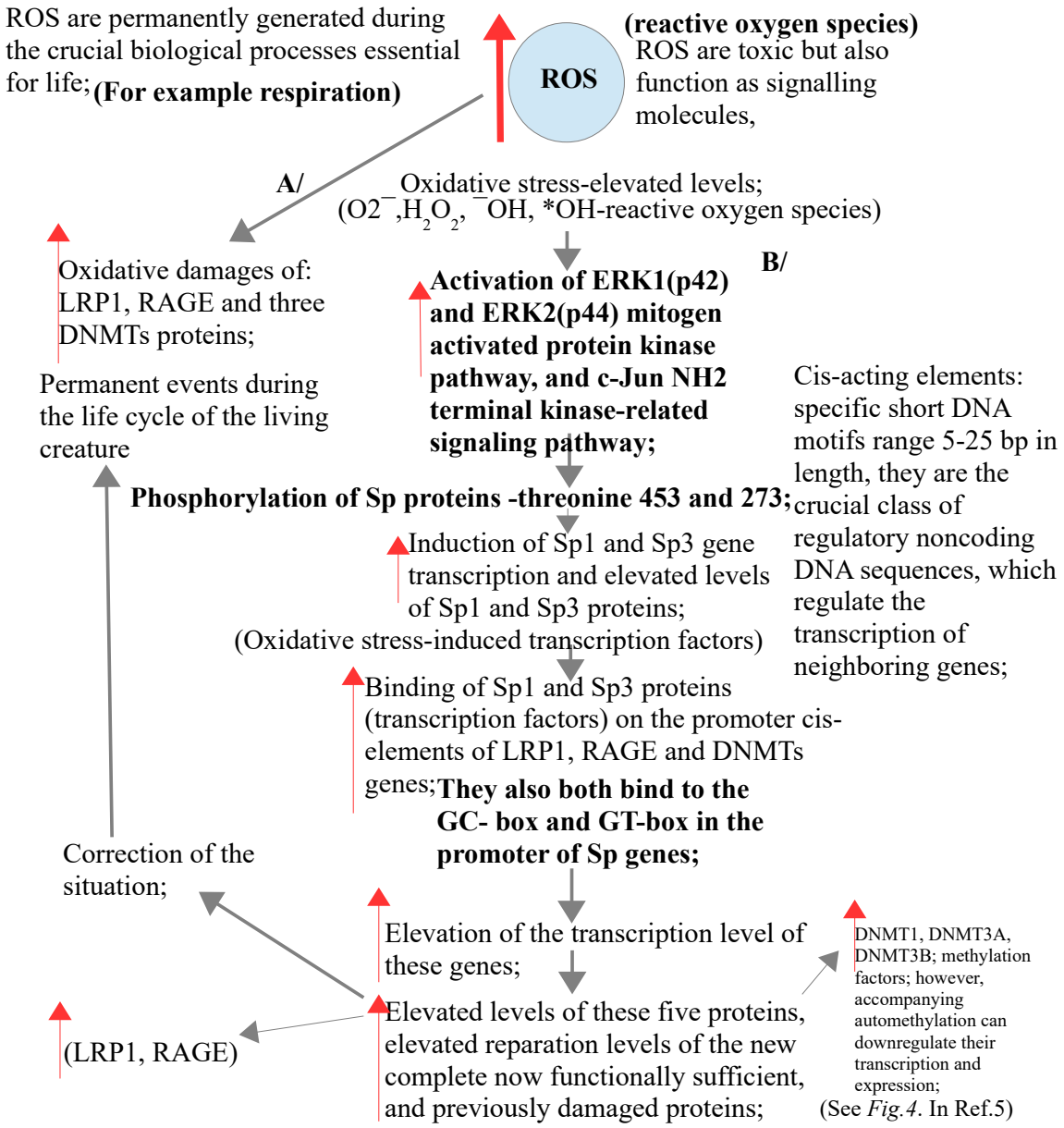

Fig.1. **Schematic presentation of the protective role of Sp1 and Sp3**

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; $O2^{-}$, superoxide, diatomic oxygen, an inorganic radical anion, a member of ROS; H_2O_2, hydrogen peroxide.

attraction of AGEs to RAGE (active form-there is not necessary the furin action in ER) during the life cycle, and their increasing accumulation around the RAGE promoter, especially in old age. Between AGEs and RAGE (active form) 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 [15] Vital processes related to the expression of LRP1 (LRP1 protein obtained by mRNA LRP1 translation become active by the furin action in ER) 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 and 2**)[6,13-18].

It is also necessary to pay attention to the following values of the mentioned proteins: DNMT1-size 1632 aa, molec. mass 185 kDa; DNMT3A-size 912 aa, molec. mass 102 kDa; DNMT3B-size 853 aa, molec. mass 95 kDa; LRP1-size 4544 aa, molec. mass 600 kDa; RAGE-size 320 aa, molec. mass 50-55 kDa; Sp1 protein-size 785 aa, molec. mass 81 kDa; Sp3-size 781 aa, molec. mass 82 kDa. Sp1 and Sp3 play an important role in cell growth, differentiation, apoptosis and carcinogenesis.

Sterile chronic inflammation and ageing

Experimental research and theoretical analyses clearly indicate that the process of programmed human aging is essentially related to signs of sterile chronic inflammation. What is inflammation? Inflammation is part of the biological response of body tissues to harmful stimuli such as pathogens, damaged cells and various irritants. Infection is the invasion and growth of microorganisms in the body. These can be bacteria, viruses or fungi. Inflammation can be due to infection (septic inflammation) or non-infectious agents. The process of programmed aging is essentially an aseptic non-infectious process. From the previous text it is evident that Sp1 and Sp3 are strongly involved in the pathophysiology of aseptic non-infectious inflammation.

Mengzhou Zhou, et al.[28], provides an exhaustive analysis of the so-called AGEs-RAGE axis and its implication in inflammatory pathologies. This axis has a decisive influence on the occurrence and development of chronic aseptic inflammation, crucial for the course of a whole series of chronic non-infectious diseases such as diabetes mellitus, atherasclerosis, cancer, but also programmed aging. AGEs and their receptor RAGE are key factors in these chronic events. AGE formation and accumulation are the result of the so-called Maillard reactions that occur by non-enzymatic interaction of reducing sugars (glucose, fructose) with the amino group of proteins, lipids or nucleic acids, the resulting Schiff base, Amadori rearrangement and oxidative modification generate the formation ofAGEs. AGE activates RAGE.

Activation of RAGE leads to biochemical events that, on a genetic basis, lead to Alzheimer's disease (AD) or determine the aging process. Activated PKC (electron donor), NADPH (NOX-2), and generated ROS participate here. All this results in the progression of the NF-kB cascade with the formation of PDGF, VCAM-1, ICAM-1, e-SELECTIN, MCP-1, M-CSF, COX-2, MMP-2, TNFα, IL-8, IL-6 and IL-1. The aforementioned platelet-derived growth factor (PDGF), adhesive molecules (VCAM-1, ICAM-1), enzymes and cytokines promote atherogenesis. The resulting ROS enhances the activation and transcription of both Sp genes (***Fig.2***).

The totality of physiological and biochemical processes essential for sustaining life, but also the simultaneous generation of ROS products is given (***Fig. 3***).

Fang Fang, et al.[29], in their research on the behavior of RAGE receptors on the membranes of neurons and microglia, found in these cells increased production of IL-1β and TNF-α, increased tissue infiltration of astrocytes and microglia, accumulation of Aβ, and reduced acetylcholine esterase (AchE) activity. Activated microglia leads to neuronal damage and neuroinflammation.

Aino Soro Paavonen, et al.[30], state that RAGE is a key mediator in the development of atherogenesis in the diabetic vasculature. They indicate the important role of RAGE activation in the development of diabetic nephropathy, neuropathy and impaired angiogenesis. RAGE also plays an important role in supporting proinflammatory and prothrombotic mechanisms. It also plays an important role in increasing the expression of NADPH oxidase, mitochondrial oxidative activity and suppressing endogenous antioxidant activity.

Ravichandran Ramasamy, et al.[31], indicate that the RAGE cytoplasmic tail binds to the formin Diaphanous 1 (DIAPH 1) and conditions AGE signaling. DIAPH1 is a protein member of the protein group called formin and has an important role in cytoskeletal rearrangement by nucleation of actin filaments. It is necessary for the formation of stress fibers, endocytosis in endosomes, and stabilization of microtubules and cellular migration.

Qing Yue, Yu Song, Zi Liu, et al. [32], claim that RAGE is a critical molecule in the onset and support of the inflammatory response. By binding to RAGE, its ligands trigger an intracellular signal cascade, act on intracellular signal transduction, stimulate cytokine secretion, and play a key role in the occurrence and development of immune-related diseases (eg Lupus erythematodes, rheumatoid arthritis, Alzheimer's disease). They are also active in inflammation, apoptosis, autophagy, and endoplasmic reticulum stress.

Laura M Senatus and Ann Marie Schmidt [33], already at the beginning of their presentation, say that the process of advanced glycation leads to the creation and accumulation of a heterogeneous group of molecules called advanced glycation end products, or AGEs. They are rapidly created in disorders such as diabetes,

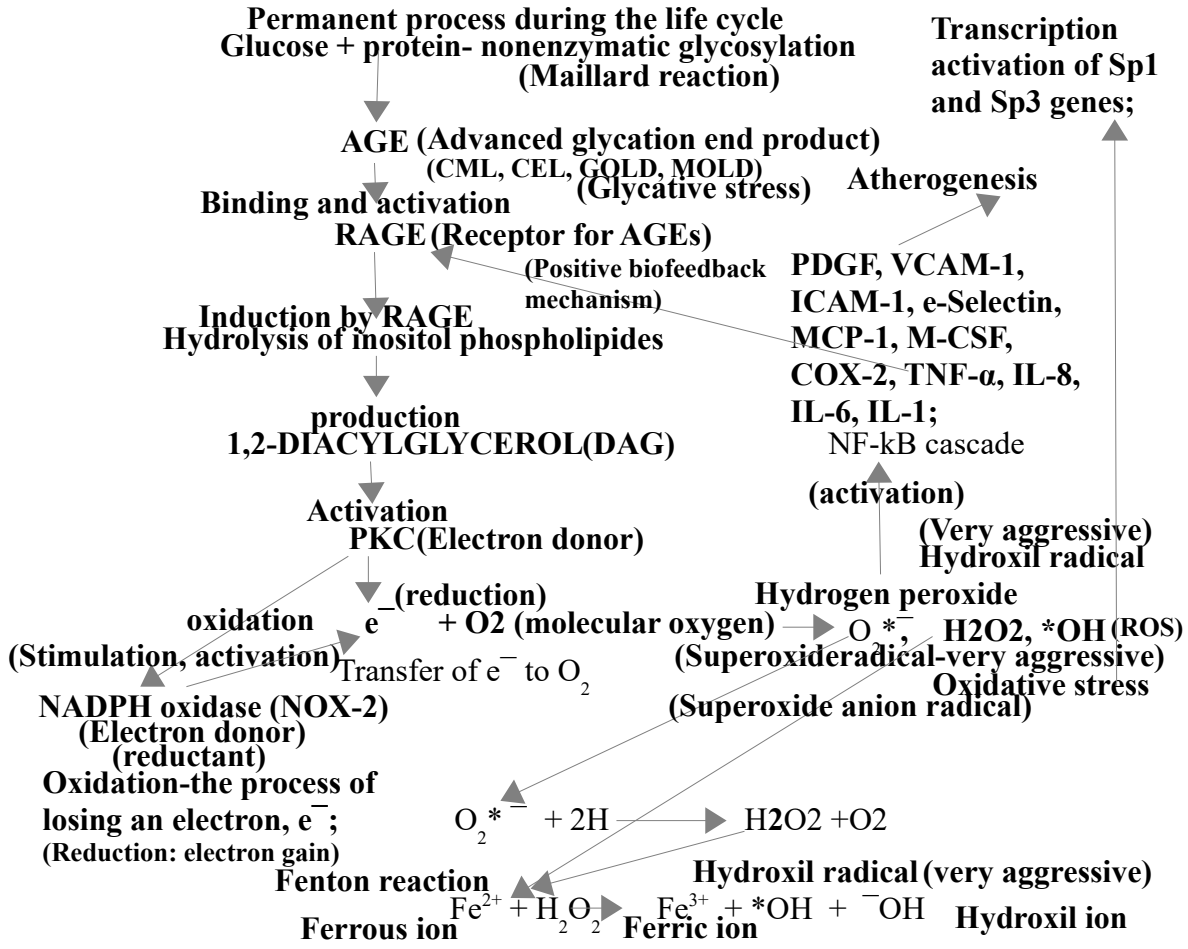

Fig. 2. Activation of NADPH oxidase and induction of oxidative stress

NADPH oxidase a family of enzymes whose function is to catalize 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 molecule. One electron reduction of O_2 gives rise to superoxide anion radical (O2*-), which then undergones 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 reaction, including AGE formation and accumulation, glycation-induced dysfunction of proteins, cellular signaling, inflammation, oxidation and tissue damage; *Nε*-(carboxymethyl)-lysine, CML; *Nε*-(carboxyethyl)-lysine, CEL; glyoxal-lysine dimer, GOLD; methyl-glyoxal-lysine dimer, MOLD.

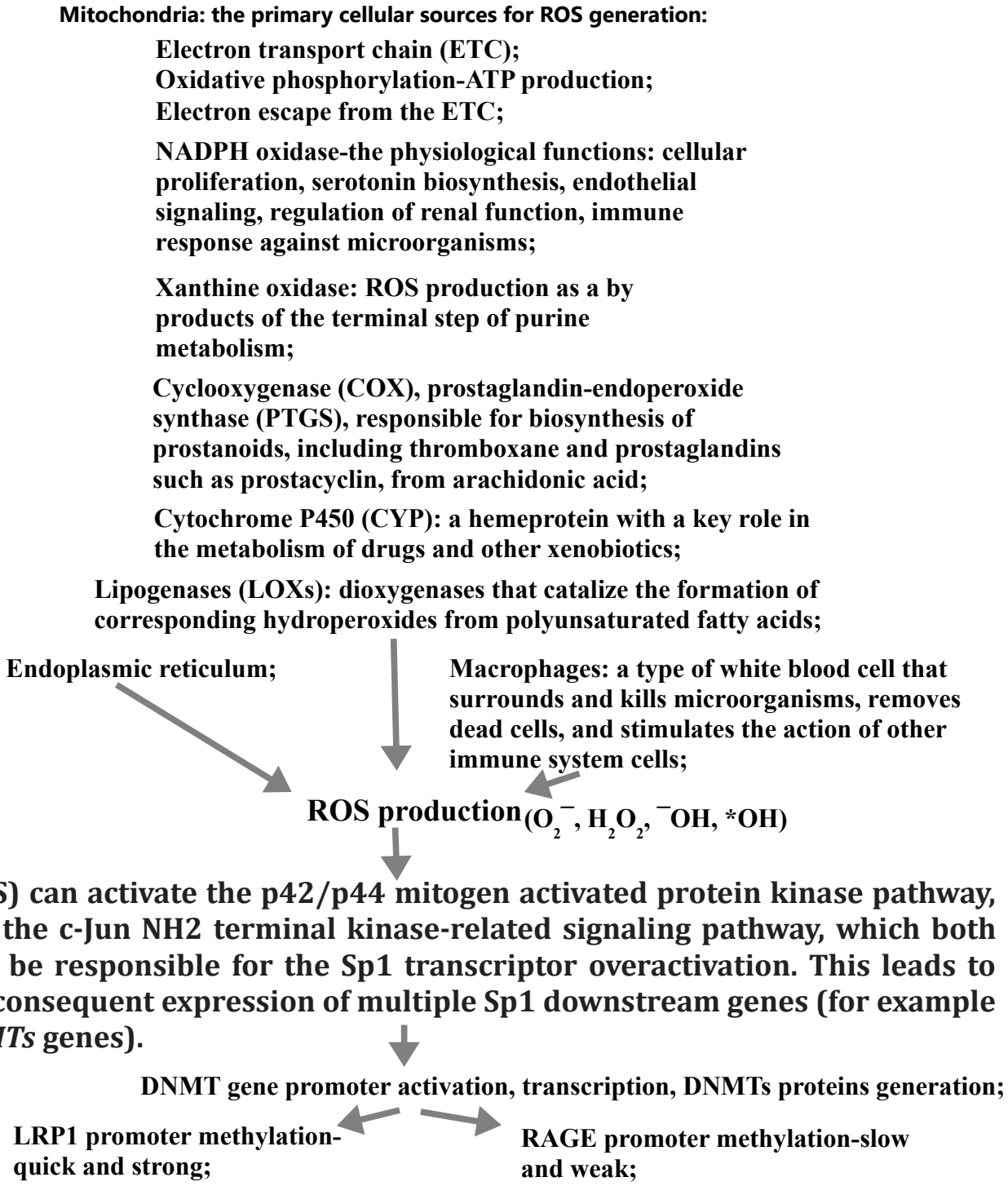

Fig. 3. **Endogenous ROS generation, Sp transcriptors overactivation, and LRP1 and RAGE methylation**

$O2^-$, superoxide , diatomic oxygen, an inorganic radical anion; H_2O_2, hydrogen peroxide; OH^-, hidroxil ion; OH*, hidroxil radical; ROS, reactive oxygen species; Sp1 and Sp3, transcription factors; DNMT, DNA methyltransferase.

renal disorders, inflammation, neurodegeneration, and aging (this refers to endogenous production). They are also found in certain foods and tobacco products (exogenous consumption). In the body, AGEs lead to cross-linking of long-lived molecules, such as collagen, thereby causing stiffening of vessels with hyperpermeability and loss of structural integrity. By binding to RAGE, AGE compounds stimulate a cascade of events that results in the loss of vascular and tissue homeostasis, which favors the development of cardiovascular disease.

Katrin Kierdorf, et al.[34], believe that RAGE is the key molecule that initiates and supports the inflammatory response. These authors think that RAGE forms the link between the innate and adaptive immune systems. RAGE is expressed on activated endothelium, where it mediates leukocyte adhesion and transmigration.

Another process closely related to oxidative stress and its harmful effects is the process of glycation or non-enzymatic glycosylation. In essence, it consists of covalent bonding of sugar molecules, such as glucose or fructose, with protein or lipid molecules. This crucially important biological process takes place in the organism of a living being slowly and continuously throughout the entire life cycle. No enzymes are needed for its development. In addition to the endogenous process that takes place in the structures of a living organism (endogenous glycation), the same process takes place outside the body (exogenous glycation). In the body, glycation leads to a relatively stable intermediate of the reaction. dysfunction of biomolecules and it does not need ATP for that. The glycation process begins with the joining of the amino group (NH_2) of the protein and the carbonyl group of the sugar molecule (C=O). The glycation process continues spontaneously and via Schiff base and enaminol conditions the formation of the Amadori product, an early glycation product. Oxidation of Amadori products produces 3-deoxyglucosone (3DG), glyoxal (GO), methylglyoxal (MGO), and further lipid peroxidation Nε-carboxymethyl-lysine (CML), Nε-carboxyethyl-lysine (CEL), glyoxal-lysine dimer (GOLD) and methyl-glyoxal-lysine dimer (MOLD). CML, CEL, GOLD and MOLD are typical AGE compounds. The Amadori product by oxidation gives 2,3-enediol, which turns into dicarbonyl, and this by oxidative cleavage (by ROS) gives CML and erythronic acid. CML binds to RAGE with its strong activation (***FIG.4***)[,34]. Katarina Zgutka, Marta Tkacz, et al. [35], already at the beginning of their work, they point out that aging is a complex process that includes numerous changes at the cellular, tissue, organic and whole-body levels. AGEs compounds are products of non-enzymatic reactions between reducing sugars and proteins, lipids or nucleic acids. They are created in physiological and pathological conditions. Their accumulation leads to severe cellular and tissue damage in various organ systems. An important harmful effect is their mediation in the creation of reactive oxygen species (ROS), cross-linking of extracellular matrix proteins, and pro-inflammatory cytokines. In addition to hyperglycemia, a number of exogenous factors, such as cigarette smoke, high levels of refined and simple carbohydrates in the diet, hypercaloric diets, foods cooked at high temperature, and sedentary lifestyle, induce AGE production. The authors point out two basic ways of harmful effects of AGEs compounds in the occurrence of various diseases and disorders. One way is through the binding of AGEs to the RAGE receptor, and the other through cross-linking ECM proteins and lipids. AGEs activate mitogen-activated protein kinase (MAPK), nuclear factor kappa B (NF-kB) and signal transducer and transcriptional activator (STAT) pathways. All this results in increased production of pro-inflammatory molecules, such as cytokines, chemokines, and acute-phase proteins (inhibitors or mediators of the inflammatory processes): C-reactive protein, α1-acid glicoprotein, haptoglobulin, fibrinogen, α1-antitrypsin, complement components C3 and C4. They play an important role in the occurrence of diabetes mellitus, cardiovascular diseases, atherosclerosis, neurodegenerative diseases (Alzheimer's disease), and some types of cancer. In human body AGEs accumulate during normal aging and age-related diseases.

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

Based on the necessity of developing efficient high-affinity Aβ/RAGE blockers that are safe and non-toxic, Deane R, et al.[36], performed a screening of a small molecular library and discovered new tertiary amides that blocked the Aβ/RAGE interaction with strong binding affinity. The subsequent synthesis of a second-generation library with the identification of a high-affinity RAGE-specific inhibitor FPS-ZM1, that binds to the V domain of RAGE, easily passes through the BBB, and inhibits the Aβ-induced cellular stress in RAGE expressing cells in vitro and in vivo. Bounding with RAGE (experiments with mice) in the brain, FPS-ZM1 inhibits β-secretase activity, Aβ production, microglia activity and the neuroinflammatory response (15-17 month-old APPsw/mice). There were no signs of toxicity or increased rate of infection.

Therapeutic dose was 1 mg/kg ip.

Huang J, et al.[37], have found that FPS-ZM1 is a promising therapeutic agent in human inflammatory diseases caused by oral bacteria.

Yan Hong, et al.[38], in experiments with APPsw/0 transgenic mice, applied the intrahippocampal injections of AGEs, and after this administration they found signs of inflammation, oxidative stress, and increased escape latency of rats in the Morris water test. All of these are significantly reduced by FPS-ZM1 treatment.

Lan Wang, et al.[39], after intraperitoneal (i.p.) application of LPS 5mg/kg they have found in the hippocampus of C57BL/ mice overproduction of microglial pro-inflammatory cytokines IL-1β and TNF-α. It was found that FPS-ZM1 downregulated LPS-mediated increases in the phosphorylation levels of JAK/STAT-both in vivo and in vitro.

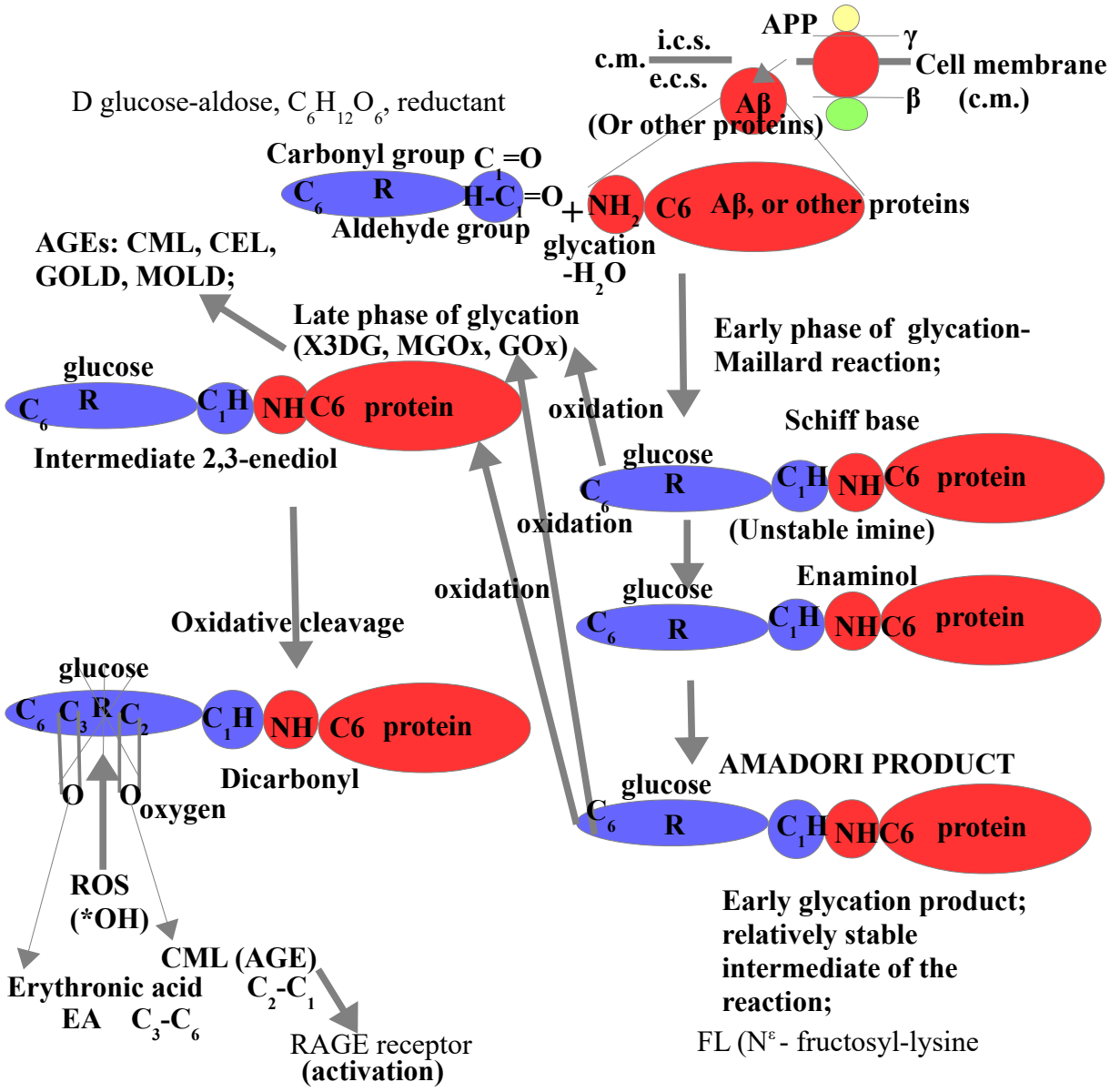

Fig. 4. **Schematic diagram of the glycation (nonenzymatic glycosylation) of proteins**

Glycation, sometimes called non-enzymatic glycosylation is the result of the covalent bonding of a sugar molecule, such as glucose or fructose ,to aprotein or lipid molecule, without the controlling action of an enzyme. It may occur either inside the body (endogenous glycation) or outside the body (exogenous glycation). It is haphazard process that impairs the functionining ofbiomolecules and does not require the expenditure of ATP. Protein glycation starts with a none nzymatic reactiobetween a sugar aldehyde or ketone, with a free amino group of amino acid. It leads, connected with the activated RAGE receptor to PKC activation, NADPH oxidase activation, ROS production, and NF-kB cascade, X3DG, 3-deoxyglucosone; GOx, glyoxal; MGOx, methylglyoxal; AGEs, advanced glycation end products; CML, Nε-(carboxymethyl)-lysine;CEL Nε -(carboxyethylethyl)-lysine; GOLD, glyoxal-lysine dimer; MOLD, methyl-glyoxal-lysine-dimer;

Yanian Kong, et al.[40], already at the beginning of their study, pointed out that strong expression of RAGE receptors is extremely harmful to the human body. By causing a cascade of biochemical events, this expression leads to the appearance of Alzheimer's disease (AD) and its accelerated development. The trigger for these adverse events is the fusion of RAGE and AGEs molecules. The aforementioned cascade leads to strong activation of PKC and NADPH. This is followed by strong oxidative stress, NF-kB cascade and dysfunction of astrocytes. On the other hand, hyperphosphorylation of tau protein and the development of neurofibrillary tangles (NFT) occur. RAGE inhibitors, among them FPS-ZM1 in particular, strongly block the binding of ligands to RAGE and thereby prevent the aforementioned cascade and its consequences. The authors of this study believe that FPS-ZM1 can be used as a disease modifying agent for AD.

Slowing down the aging process by *LRP1* gene therapy

Sagare, et al.[41], 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 that has a crucial role. Effective gene transfer to 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. So, it is necessary to identify molecular signature epitopes in the cerebral endothelial cells of the aging brain, and present these epitopes by 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.

Angeliki Maria Nikolakopoulou, et al.[17], 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 (abundant intracellular pro-inflammatory cytokine) 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.[42], 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**.**

Shaza S Issa, et al.[43], 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**.**

Michael F Naso, et al. [44], declare that the Adeno-associated virus (AAV) is a non-enveloped virus that can be engineered to deliver DNA to target cells. Today, it is possible to generate in the laboratory the recombinant AAV viral particles, without original AAV genome, but with the incorporated DNA (cDNA) sequences of interest for various therapeutic applications. Consequently, the recombinant DNA (cDNA) is a form of DNA which is generated by a artificial way.

Berislav V Zlokovic, et al.[45], already point out at the beginning that low-density lipoprotein receptor-related protein-1 (LRP1), a member of the large low-density lipoprotein receptor family, is strongly involved in cellular transport of cholesterol, endocytosis of numerous ligands, transcytosis of ligands across the blood brain barrier (BBB), and transmembrane and nuclear signaling. LRP1 has been found to regulate brain and systemic clearance of Alzheimer's disease (AD) amyloid β-peptide. The authors point out that gene therapy strategies aimed at increasing LRP1 levels in the cerebrovascular system and in the BBB may have the potential to alleviate initial vascular damage by reducing Aβ accumulation during hit 1 and hit 2 states of AD pathogenesis.

Steffen E Storck, et al.[46], point out that brain endothelial LRP1

ablation results in protease-mediated tight junction degradation, P-glycoprotein (P-gp) reduction and a loss of BBB integrity. Deletion of LRP1 increases levels of cyclophilin A (CyPA, a ubiquitously distributed immunophilin protein), which increases metalloproteinase-9-mediated tight junction protein degradation and paracellular brain penetration of blood proteins, as well as neuronal damage. LRP1 gene therapy targeting the BBB partially corrects vascular leakage, neuronal damage, and behavioral deficits in mice.

Some interesting facts about the physiology and biochemistry of AAV-2 virus are presented in the paper by Jalish M Riyad and Thomas Weber,[47].

Conclusion

Aging is a natural, complex, multifaceted, inevitable and irreversible process. Its cause is still unknown. Aging essentially consists of two mutually closely related components: physiological or normal aging and accelerated or pathological aging related to diseases. Physiological aging, according to a series of indications, occurs due to a certain program in the genome. Several prominent proteins play a crucial role in this: LRP1, RAGE, three DNMTs and two Sp. Due to the different methylation conditions of their promoters, LRP1 is methylated faster and more strongly, while RAGE is methylated more slowly and weakly. This causes a faster weakening of LRP1 expression and the entire complex of physiological and biochemical events related to this receptor during life, and especially in the periods of aging and old age. Conversely, RAGE expression increases during aging, and thus a series of pro-atherogenic events accelerates, all of which results in the acceleration of programmed aging.

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