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EMERGING PLAYERS IN AGING PROCESS: A REVIEW

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ABSTRACT

Aging is an inevitable phenomenon of the life of any organism. The rate of aging is significantly different among different populations and even within a single individual in different anatomical organs. All the molecules and organs appear to suffer from the deleterious effects of aging, including DNA, proteins, cells and organs. Proteins are the most affected molecules in all the biochemical changes of an organism as they are the structural unit of life. In recent years researches have been done on the proteins which are related aging and senescence. Superoxide dismutase, p^{53} and sirtuin proteins have been revealed to be involved in the aging and longevity. In this review then, we have discussed about the functioning of these three proteins in the aging process. Study of these proteins will help to pave the way for the new approaches for combating the aging related problems to increase longevity.

KEYWORDS: Aging, Proteins, p53, Sirtuins, Superoxide Dismutase

INTRODUCTION

Aging is a continuous and irreversible process of physical, psychological and social changes of an organism during its entire life span. It is the progressive accumulation of changes with time that are associated with or responsible for the ever-increasing susceptibility to disease and death which accompanies advancing age (1). The changes that occur in an organism or a part of an organism between maturity and death is called aging. The rate of aging is significantly different among different populations, even within a single individual in different anatomical organs. Aging is defined when two criteria are met, first, the probability of death at any point in time increases with the age of the organism and second, the characteristic changes in phenotype occur in all individuals over time due to the limiting processes. This statistical definition applies from yeast to mammals and reflects the nature of aging (2).

Aging and life span is related to each other because aging is a continuous process which goes throughout the life span of an organism. The life span of an organism therefore, is the sum of deleterious changes and counteracting repair and maintenance mechanisms that respond to the damage**.** The longest lived animal, the turtles, continue to grow throughout life and are credited with slow or negligible senescence. Turtles have special adaptations against oxygen deprivation, low metabolic rate and many antioxidant enzymes which protect from reactive oxygen species. Many theories are proposed to explain why aging exists and how it is caused but not all theories are satisfactory.

All the molecules appear to suffer from the deleterious effects of aging, including DNA, proteins, lipids, cells and organs. The instructions for aging process remain mainly in the chromosomes. In the recent era of studying aging process, scientists try to understand this phenomenon in the level of genes and proteins. Proteins are the main component of any organism's body system. They are the structural, functional component of a cell or an organ. In case of proteins, two major types of chemical reactions participate in the aging phenomena-

- Structural transformations induced by the addition of radicals by enzymatic or non enzymatic reactions,
- Proteolytic cleavages.

Among the reactions of structural transformation, non enzymatic glycation is the more generalized reaction. This glycation depends on the probabilities of encounters between circulating glucose molecules and free amino groups existing either at the N-terminal end of the polypeptide chains or on the lysyl side chains. These reactions are more frequent in the extracellular spaces and connective tissues. The binding of sugar residues to protein amino acid groups determines frequent modifications of the structure that make the molecule inactive.

Proteins are among the most affected macromolecules from the oxidative stress probably due to their amount in a cell. Various amino acid side chains and peptide bonds are the main targets of oxidation by the free radicals, which are the by-products of the aerobic metabolism (3-5). The free radical oxidation mainly results in loss of protein function (6, 7), which in turn can be threatening for the cell; especially in the case of proteins that are vital for the cell survival. The oxidant molecules (ROS) increase during aging process, so the destruction of the proteins is more in aging. The oxidative invasions on proteins lead chain fragmentation, aggregation or cross–linking, site specific amino acid modifications and alterations in protein surface properties (8, 9). Unless these modifications are repaired, they may lead to further cellular pathologies. Therefore, certain repair mechanisms have been evolved by the cells to prevent the alterations in cell functions. Degradation systems, like proteosomes and liposomal proteases, have been evolved to eliminate the irreversible changes on proteins (i.e. Protein carbonyls) and enzymatic repair systems, involving the thiredoxin and the glutaredoxin systems, etc., have been evolved for the repair the reversible changes (i.e. Formation of disulfide bridges, etc.). During aging process protein oxidation is increased in a wide variety of human and animal tissues. The quantification of oxidative damage to proteins has been studied almost exclusively by assessing the total carbonyl content. The impairment of protein repair mechanism can cause many problems during the aging process, as excessive oxidation of the proteins of repair system occurred at that time.

There are many proteins which are directly or indirectly related to the aging process and the longevity of the organism's life span. Actually, almost all the proteins are affected as an organism age. The superoxide dismutase, catalase, glutathione peroxidase are helpful in combating the oxidative stresses produce by reactive oxygen species(ROS).Some other proteins, such as p53, telomerase, collagen, elastin, sirtuin etc. are also related to aging process in different organisms. Different forms of these proteins are found in different species which influence the aging process positively or negatively. The damage of proteins during aging process is manifested by carboxylation and loss of sulflydryl group. In this review we will try to present some of these aging related proteins.

Superoxide Dismutase (SOD)

Superoxide dismutases are enzymes that catalyze the dismutation of superoxide (o_2^-), into oxygen and hydrogen peroxides. This enzyme was first isolated from bovine blood as a green copper protein whose biological function was believed to be copper storage (10). Over many years, the enzyme has been variably referred to as erythrocuprein, indophenols oxidase and tetrazolium oxidase. The catalytic function of the enzyme was discovered by Fridovich and McCord in 1969. The enzyme is ubiquitous, being widely distributed among o_2- consuming organisms, aero tolerant anaerobes and some obligate anaerobes (11). Several common forms of SOD exist and they are proteins co-factored with copper, zinc, manganese, iron or nickel.

Superoxiodes

One electron reduction of oxygen yields superoxide with a redox potential o_2/o_2^- of -160 mv at pH 7.0 under physiological conditions. It is now known that superoxide is extremely toxic (much more than H_2O_2) and that intracellular concentrations in the picomolar range are lethal. The dismutation reaction can be considered irreversible except in circumstances of very high levels of SOD and very low levels of superoxide. Superoxide, a by-product of aerobic metabolism, has been implicated in the production of oxidative DNA damage. Although superoxide is chemically incapable of damaging DNA directly it is thought to do so indirectly by participating in the production of hydroxyl radicals. A long-standing proposal is that it serves as a reduction of iron that is adventitiously bound to DNA.Subsequent oxidation of hydrogen peroxide generates the hydroxyl radical, a powerful oxidant that attacks the adjacent DNA.

$O_2^- + Fe3^+ \rightarrow O2 + Fe^{2+}$

$H_2O_2 + Fe2^+ \rightarrow OH^- + OH^{-+} Fe3+$ (Fenton reaction)

SOD Activity Related to Aging

The enzyme SOD has special significance for the free radical theory of aging. The general mechanism of superoxide dismutation can be summarized as follows,

O_2^-+SOD-M ⟶ O_2+SOD-M

O_2^-+2H^+ +SOD-M^- ⟶ H $_2O_2$+SOD-M

$O_2^- + O_2^- + 2H \longrightarrow H_2O_2 + O_2$. At P^H +7

Where M stands for metal ($Cu^{2+,}$ $Mn3^{+,}$ and $Zn2^+$), the superscripted minus sign (-) stands for negative electric charge, and the dots represent an unpaired electron.

Thus, SOD accelerates the destruction of superoxide by increasing the rate constant for spontaneous dismutation by more than 1,000 fold and making it more efficient at low concentration. As an enzyme cannot change the equilibrium position, but only the rate at which it is achieved, there is a lower limit of superoxide concentration below which the enzyme cannot reduce it.

Mitochondrial electron transport chain is an exceptionally strong source of superoxide, definitive proof come from the finding that mice lacking mitochondrial matrix MnSOD (SOD2) die several days after birth (12,13). The majority of studies which investigated total SOD activity in the brains of rats and mice, has found little change in the enzyme's activity with age (14). Skeletal muscle SOD, however, does increase with age in old rats compared to either young or middle aged rats (15-18).

Excessive expression of the SOD enzyme, such as in individuals with Down syndrome, lead to oxidative damage, premature aging and neurodegeneration (19). Experiments of Male Spragne-Dawley rats and their SOD activity in Cardiac and skeletal muscles during aging, shown that total SOD activity increased in most muscles between 3 and 23 months of age. There was no change in activity in liver between 3 and 19 months of age. Muscle tissues have an ability to regulate SOD during aging and these increases of SOD may be important in protecting against changes associated with aging (senescence). Lipid per oxidation products also accumulates in cardiac muscles during aging (20).

A decrease in SOD and a failure to induce SOD in the rat brain at 30 months of age in a study has been cited as a factor in cerebral senescence. This study demonstrated a compensatory increase in Mn SOD when Cu-Zn SOD decreased. Similarly, de Rosa et al. Observed that when Mn SOD in chicken liver decreased due to dietary Mn deficiency, Cu-Zn SOD activity increased (21). These studies suggest a regulatory relationship between the two forms of SOD and prompted researchers to examine Mn and Cu-Zn SOD in skeletal muscle to see if there were compensatory changes with aging. Marginal insufficiency of copper and manganese over a long period of time prevents the normal increase with aging in skeletal muscle SOD activity (22). Cell membranes and other cellular structures may be damaged resulting in the impaired muscle function. A study on mice has shown that enhanced expression of EC-SOD can protect synaptic plasticity and learning and memory against oxidative damage during the aging process. (23)

Studies suggest that the age dependant decrease of cytosol SOD activity detected in the cerebral cortex of the rat might lead to an impaired protection against the toxic effect of O_2^- , thus the major susceptibility to bio membranes to lipid per oxidation during aging may be related to the decrease of cytosol SOD activity. A protective effect of SOD on bio-membranes and linked enzymes have been reported by several groups. (24) The age dependant increase of mitochondrial SOD (Mn SOD) found in the cerebral cortex of rat may be due to an enhanced release of superoxide with age (25). Navarro et.al suggests that the activities of MnSOD decreased in aging. They observed increased Mn-SOD and Cu-Zn SOD activities in mouse brain and liver aging (26,27) using the classical xanthine oxidase. /cytochrome c assay (28) and decreased Mn-SOD and Cu-Zn-SOD in mouse, rat brain, liver in aging by the adrenochrome assay (29). Studies have shown that SOD acts as both an antioxidant and anti inflammatory in the body, neutralizing free radicals that can lead to aging.

P^{53} Protein

P^{53} protein was first identified by professor Sir David Lane in 1979 as a transformation- related- protein (30), a cellular protein which accumulates in the nuclei of cancer cells and binds tightly to simian virus 40 (SV40) large T antigen (31,32). The gene encoding P^{53} was initially found to have weak monogenic activity. P^{53} protein was observed to be over expressed in mouse and human tumour cells (33). The biological roles of P^{53} are cell-cycle regulation, induction of apoptosis, development, differentiation, gene amplification, DNA recombination, chromosomal segregation and cellular senescence. After some years of discovery of P^{53} protein, it was found that it has a role in regulating aging and longevity. p53 belongs to a unique protein family which includes three members: p53, p63 and p73. It is known as the 'Guardian of the Genome' referring to its role in preventing genome mutation. Mammalian p53 is a nuclear phosphoprotein of molecular weight 53 kDa encoded by a 20kb gene containing 11 exons and 10 introns (34) which is located on the small arm of chromosome 17 (35).

P^{53} and its Activities Related to Aging

P^{53} and Its Relation with Insulin /IGF-1 and TOR Pathways

P^{53} interact closely with insulin/IGF-1 and TOR pathways, two critical pathways that regulate aging and longevity. p^{53} can down regulate the activities of insulin/IGF-1 and TOR pathways by inducing the expression of a set of p53 target genes in these two pathways, including IGF-BP3, PTEN, TSC2, AMPK β1, sestrin 1 & 2 and REDDI. (36-41). p53 negatively regulates the insulin/IGF-1 and TOR signalling creating an inter pathway network, which leads to increases in the fidelity of the cell growth and division processes to keep away from error. But the decreased TOR/insulin/IGF-1 signalling extends life span.

p53 may regulate aging and longevity through its down regulation of the signalling of these two critical pathways.

P^{53} has been shown to decrease insulin/IGF-1 signalling in many different types of cells in both humans and mice. The decline of p53 activity with age may result in the activation of TOR and insulin/IGF-1 signalling and potentiate the aging process later in life. This protein has been shown to activate or inactivate autophagy (42, 43), a major outcome of TOR signalling.

P^{53} in Relation to Oxidative Stress and ROS

It has been seen that p53 function to inhibit and promote senescence and aging by controlling cell growth and metabolic stress through different pathways. The ability of p53 to exert both pro-oxidant and antioxidant function of cells, which could be a potential mechanism accounting for the dual activities of p53 in both promoting and preventing aging. It is known that under severe oxidative stress condition high levels of ROS can activate p53, which in turn leads to the p53 mediated apoptosis and senescence (44, 45). Activated p53 protein induces the expression of a set of pro-oxidant genes, including PIG 3, PIG 6, FDXR, Bax and Puma (46, 47).

The induction of these pro-oxidant genes can all increase intercellular ROS levels and further sensitize cells to oxidative stress to eliminate damaged cells through p53 mediated apoptosis and senescence. On the other hand, under condition of non stress or low stress p53 can exert its antioxidant function to reduce the ROS level in cells and prevent oxidative stress induced DNA damage and promote cell survival. P^{53} induce the expression of a set of antioxidant genes to lower ROS levels, including sestrins, TIGAR, GLS2, GPX1 and ALDH 4(48, 49). It might be possible that through the modulation of ROS level, p53 might contribute to both longevity and aging, depending on the stress conditions. Mild stress could lead to the p53 induced antioxidant function which decreases ROS and oxidative damage to promote longevity. But strong stress may lead to p53 induced pro-oxidant function to promote senescence stem or progenitor cells and thus aging. (Figure 1)

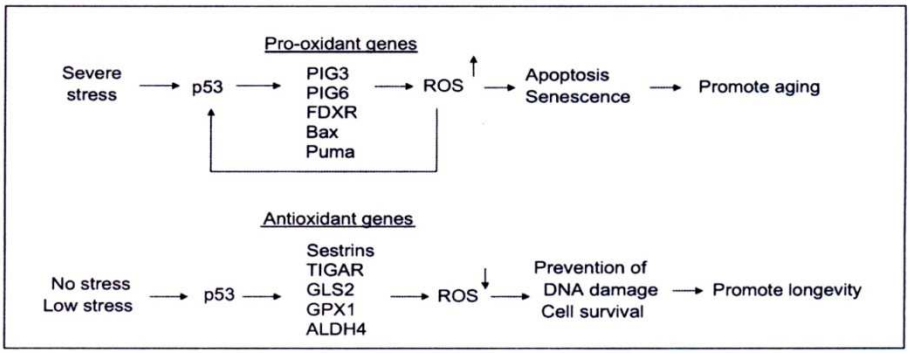

Figure 1: Regulation of Aging by P^{53} (Source: Feng, Z., Lin, M. and Wu, R., 2011. The Regulation of Aging and Longevity a New and Complex Role of p53. *Genes & Cancer*, *2*(4), pp.443-452)

p53 induces apoptosis or cell cycle arrest as a precondition to cellular senescence; both can damage the mobilization of stem cell and progenitor cell populations and by this it accelerates aging. Suppression of aging happen when p53 inhibits unregulated proliferation pathways that could directs to cellular aging and a senescence-associated secretory phenotype (SASP), which make a pro-inflammatory and degenerative environment (50).

P^{53} and its different isoforms play an important role in combating aging by promoting longevity. Studies have shown that in *Drosophila* reduction in p53 function in adult neurons has a beneficial effect on longevity. Some scientists

reported that the reduction of p53 activity in specific tissues mediated by dominant negative p53 could lead to the delayed aging and extended life span in flies. In *C. elegans*, the p53 ortholog Cep-1 lead to the increased life span. The mutation in the gld-1 protein which is a female germ line protein increases the activity of Cep-1 protein and extends life span in gld-1 mutants (51).

Different mouse models are used to study the potential role of p53 in regulating aging and longevity. The first direct link of p53 with aging mice was reported by Tyner and colleagues (52). They showed that $p53^{+}$/m mice were resistant to spontaneous cancers. The mice displayed a shortened life span (decreased by 20-30%) and premature aging phenotypes, including organ dystrophy, oesteoporosis and reduced tolerance to stresses. Maier *et.al.* Generated p53 hypermorphic mouse models expressing a 44 kDa truncated naturally occurring isoform of p53 (p44) which lacks the main transactivation domain of p53. The p44+/+ mice displayed enhanced activity of p53. P^{63} is the nearest ancestral member of p53 family during evolution. It has been shown that p63 also plays a role in the regulation of aging and longevity. Keyes and colleagues reported that p63+/- mice were not tumour prone but displayed features of accelerated aging and had a shortened life span. They also showed that cellular senescence and organismal aging were intimately linked and that these processes were mediated by the loss of p63 (53).

The impact of p53 on aging and longevity in human has been recently indicated by several epidemiological studies. The p53 gene contains a functional common coding single nucleotide polymorphism (SNP) that results in either an arginine (R72) or a proline (P72) residue at codon 72. The distribution of these polymorphisms in populations varies with racial groups. Naturally occurring SNPs of p53 and its signalling pathways, which affect the activities of p53 and its family members, including p63 and p73, provide a good opportunity for us to study their role in the regulation of aging and longevity in human(54).

Hence recent studies have demonstrated that p53 is emerging as an important but complex player in aging and longevity. But it is not well understood till today.

Sirtuin Proteins

Sirtuins are a conserved family of proteins found in all domains of life. They are a family of Nicotine adenine dinucleotide (NAD+) dependent deacetylases. These proteins are first discovered in Yeast (*Saccharomyces cerevisae).* In animals, sirtuins have been implicated in a wide variety of processes, including transcriptional silencing, metabolic regulation and aging (55).

The sirtuin reaction is thought to proceed in two stages: firstly the acetyl oxygen replaces nicotinamide either by an SN1 or more likely an SN2 mechanism to produce an O-alkyamidate intermediate and nicotinamide product; in the second step the acetyl group is transferred to ADP-ribose to form O-acetyl-ADP-ribose and deacetylated lysine. O-acetyl-ADP-ribose is a metabolite uniquely generated by sirtuin deacetlyases.

Sirtuin Family Protein Functions Related to Aging

The sirtuin's role in aging and longevity has not been resolved fully till today. Mammalian Sir2 homologs (SIRT1-7) have a highly conserved domain which is a NAD dependent sirtuin core domain. The regulation of oxidative stress, DNA damage and metabolism by mammalian sirtuins lead to control the aging process.

Nuclear Sirtuins

Nuclear sirtuins (SIRT1, SIRT6, and SIRT7) remove cellular oxidative stress, DNA damage and help in increasing the longevity.

SIRT1 mediates an oxidative stress response by directly deacetylating several transcription factors that regulate antioxidants and genes. It activates several members of the FOXO family of transcription factors which promote the expression of stress response gene including SOD2 (56, 57). SIRT1 also promotes mitochondrial biogenesis by activating peroxisome proliferator activated receptor co activator 1-α (PGC-1α). PGC-1α increases mitochondrial mass and up regulates the expression of oxidative stress genes, including glutathione peroxidise, catalase and MnSOD (58). The role of SIRT1 in antioxidant response leads to caloric restriction (CR) mediated life span extension studies. CR fails to increase the life span of SIRT1 knockout mice and these mice do not increase their physical activity, a phenotype typically associated with calorie restricted mice (59, 60).

SIRT1 deacetylase activity has also been implicated in the repair of DNA damage through its ability to deacetylate the tumour suppressor p53. This protein mediated deacetylation of p53 and suppresses the induction of apoptosis and prolongs cellular survival in response to DNA damage. So it helps in suppressing aging as well.

SIRT6 is a nuclear protein widely expressed in mouse tissue. SIRT6 knockout mice display premature aging symptoms, including loss of subcutaneous fat and decreased bone density and die within 4 weeks after birth. SIRT6 deficient cells display sensitivity to oxidative stress and reduced capacity for DNA repair (61). In support of the antiaging effects of SIRT6, male KO mice over expressing this protein have a significantly longer life span than their wild type counterparts. SIRT6 also stimulate DNA double strand break repair in response to oxidative stress by activating poly (ADP-ribose) polymerase-1 (PARP-1) (62, 63).

SIRT7 shows oxidative stress resistance in an investigation of cardiomyocytes from SIRT7 knockout mice. These cells are increasingly sensitive to both genotoxic and oxidative stress, such as H_2O_2, compared to wild type (64).

- **Cytoplasmic Sirtuins:** Only SIRT2 is reported to be localised in the cytoplasm but a fraction of SIRT2 is nuclear. In mammals SIRT2 have not so important role in the increase in longevity. But cell culture studies demonstrate SIRT2 may be important in regulating mammalian cell cycle. It regulates the asymmetric segregation of oxidatively damaged proteins from daughter cell to the mother cell during cell division and it implies the role of SIRT2 in lifespan extension.
- **Mitochondrial Sirtuins**: The mitochondrial localization of SIRT3-5 is important, as mitochondrial dysfunction is associated with mammalian aging. They regulate oxidative stress response and regulate metabolism.

SIRT3 appears to regulate mitochondrial functions, as its over expression increase respiration while decrease reactive oxygen species (ROS) production. SIRT3 deacetylates SOD2 at two important lysine residues to boost its catalytic activity and this activity of SOD2 is diminished when SIRT3 is deleted (65). Biochemical data supports requirement of SIRT3 in the protection of calorically restricted mice from age-associated hearing loss. It happens due to stimulation of one enzyme isocitrate dehydrogenase (IDH2) by SIRT3 (66, 67).

An example of the possible link between SIRT3 and aging was shown in a murine model of hearing loss, which is triggered by oxidative damage in the spiral ganglia neurons and sensory hear cells in the cochlea. Hearing loss and

oxidative damage were completely prevented by CR in these mice. But SIRT3 lacking mice were resistant to the protective effects of CR against hearing loss and oxidative damage. If this effect of SIRT3 extends to other neuronal types or more broadly to non-neuronal tissues, SIRT3 activators may be the simplest and most direct way to counteract aging itself by triggering mechanisms similar to those of calorie restriction. For example SIRT3 was shown to protect cardiovascular function by suppressing the production of ROS in endothelial and cardiac cells (68). SIRT3 is important for protecting the heart against aging-induced hypertrophy and fibrosis. This protein also related to maintainence of balance between cancer and aging by caretaking tumour suppressor agents. SIRT5 remains the least characterized sirtuin to date. No effect of SIRT5 in aging and longevity is known by researchers (69).

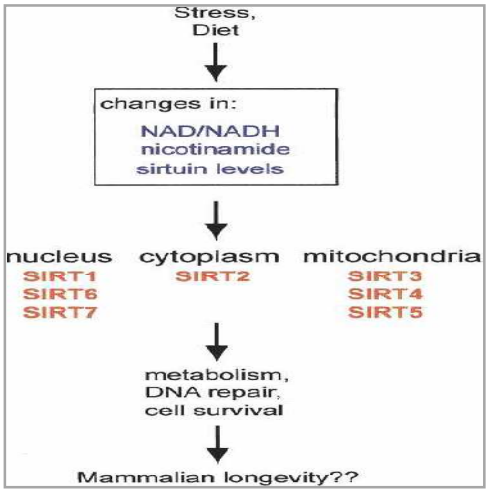

Figure 2: Model of Regulation of Aging by Different Sirtuins.
(**Source:** Haigis, M.C. and Guarente, L.P.,2006. Mammalian Sirtuins—Emerging Roles in Physiology, Aging, and Calorie Restriction *Genes & Development*, *20* (21), pp. 2913-2921)

So it is evident that most of the sirtuins have effect on metabolism, DNA repair, cell cycle, cell division etc. which may lead to control longevity response and aging process in organisms.

CONCLUSIONS

In a broad sense, aging reflects all the changes that occur over the course of life of an organism. The changes may be physical, psychological or other changes which lead to an ultimate fate of the aging process, death. Aging process varies in different organisms and even in different individuals. Many factors regulate the aging process. The factors may be genetic, environmental, some chemicals such as drugs etc. There is always an effort to increase the life span and decrease the aging related damages to the body.

Many proteins and enzymes are related to regulate aging negatively or positively. Proteins and enzymes are the molecules which interact with the metabolic and physiological processes and control the longevity responses. We know that the proteins are the main player in all body systems because they act as structural components, biological catalysts for normal body processes. So, it is important to study them to solve the mystery of aging process. Aging is the most complex phenomenon to understand may be solved by studying different aspects of proteins.

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