Retinal Aging and Sirtuins

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Key Words

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Abstract

The process of aging involves the accumulating changes in the microenvironment that lead to cell senescence or apoptosis, and subsequent tissue or organ dysfunction. Multiple extrinsic and intrinsic events that cause DNA instability are associated with aging. Cells containing unstable DNA are biologically vulnerable, and if the DNA damage is too great for the cell to repair, it becomes senescent or dies by apoptosis. Thus, the cell's capacity to repair its DNA determines the progress of aging, at least in part. Here, we focus on the sirtuins, the mammalian homologs of the yeast life-span-extending molecule, Sir2. Among the sirtuin family proteins in mammals, the one most similar to yeast Sir2 is SIRT1, which is involved in multiple pathways, including the repair of DNA double-strand breaks. Although the role of SIRT1 in mammalian longevity is not clear, it is expressed throughout the retina, where it may suppress aging. In fact, a mutant mouse model of retinal degeneration shows an abnormal subcellular localization of SIRT1 protein and accelerated retinal cell apoptosis. Further analyses are required to elucidate the mechanism of DNA damage and repair, including the contributions of the sirtuins, in the aged or diseased retinas, which will help us understand the mechanisms of retinal aging.

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Accumulating changes in the microenvironment promote the aging of tissues and organs, which can eventually lead to their malfunction. The underlying mechanisms have long been explored, and their molecular basis is beginning to emerge. In this section, we describe how aging is evaluated scientifically and why sirtuins are an important topic in aging science. We also discuss the possible role of sirtuins in retinal aging.

Aging of a Tissue/Organ and DNA Instability

Aging research attracts the attention of researchers across various biological fields. Not only the length of the individual life span, but also the duration of tissue or organ function is of interest. In skin biology, the mechanism of 'photoaging', through which skin aging is accelerated by light exposure, has been widely investigated [1]. UV irradiation results in DNA damage in skin cells, which triggers DNA damage responses, including DNA repair. However, when the damage exceeds the ability of the cell to repair it, a cell cycle checkpoint response is triggered that leads to growth arrest and premature senescence, or to apoptosis, in order to prevent the genomic abnormality and instability from being inherited by other cells. In this case, aging is induced through an extrinsic mechanism.

On the other hand, telomeres, protective DNA structures at the ends of the chromosomes, are progressively

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shortened during cell division, because the DNA replication machineries incompletely copy the 3' DNA ends, thereby inducing DNA loss [2]. Finally, this intrinsic mechanism causes the telomeres to shorten beyond a critical point that elicits a DNA damage response. This response causes irreversible cell cycle arrest (senescence), in cells that originally had normal genetic information.

Therefore, excessive DNA instability, induced by extrinsic or intrinsic events, to a level beyond its ability to be repaired, causes cell cycle arrest or apoptosis. As the number of senescent cells increases and that of normal-functioning cells decreases, the tissue/organ becomes compromised and aged.

The retina is part of the central nervous system, and mature retinal cells are thought never to divide under normal conditions. Thus, cell cycle withdrawal occurs physiologically. Most neuronal cells in the brain do not proliferate either. Thus, decreases in functioning cells in the central nervous system may more easily lead to tissue/organ dysfunction and aging than in other parts of the body [3, 4]. Abnormal DNA is a pathological finding associated with some age-related neurodegenerative diseases, consistent with our understanding of aging as a consequence of DNA instability.

Various cytokines may also play a role in the aging process, again as a result of DNA instability [2]. In senescent human fibroblasts and epithelial cells, more than 40 distinct molecules can be secreted in response to DNA damage. Thus, chronic, not transient, changes in the microenvironment, due to the secretion of cytokines such as interleukins 6 or 8, may be induced by DNA instability during tissue/organ aging.

DNA Instability and Sirtuins

Once DNA damage occurs, it triggers a series of downstream events, the purpose of which is to maintain and promote the survival of the tissue/organ as a whole [5, 6]. First, cell proliferation is interrupted to allow time for DNA repair before synthesis. Then, if the repair is unsuccessful, processes to eliminate the cells are initiated.

Among the types of DNA damage, which include DNA single-strand breaks and base damage, DNA double-strand breaks are the hardest to repair, since both alleles are damaged simultaneously, leaving no complete template for the repair. In one of the main repair systems for double-strand breaks, nonhomologous end-joining, a

Fig. 1. SIRT1 in the DNA damage response. DNA damage (double-strand breaks) induces ataxia telangiectasia mutated (ATM), which activates p53 to slow the cell cycle (cell cycle checkpoints) to gain time for DNA repair. ATM also activates SIRT1 through E2fs, which in turn activates Ku70 (a protein for double-strand break repair), for the purpose of DNA repair and cell survival, and suppresses p53, to inhibit apoptosis. SIRT1 can also associate with forkhead transcription factor (FOXO) to induce antiapoptotic factors, such as manganese superoxide dismutase and growth-arrest- and DNA-damage-inducible protein 45 (GADD45, a protein for DNA repair).

serine/threonine-specific protein kinase, ataxia telangiectasia mutated (ATM), is induced and activates p53 to stop the cell cycle at the G₁ phase, preventing it from entering the S phase (fig. 1). Such a pause in cell cycle progression is termed a 'cell cycle checkpoint'. ATM activates the E2f transcription factors, which upregulate SIRT1. Deacetylation by SIRT1 activates Ku70, a nonhomologous end-joining DNA repair protein, while SIRT1 activity inhibits excessive activation of p53 and apoptosis. Therefore, SIRT1 regulates DNA stability and promotes cell survival.

SIRT1 is a mammalian homolog of yeast silent information regulator (Sir) 2, which plays a role in the life span extension by caloric restriction in yeast [7, 8]. Sirtuins are class III histone deacetylases, which require nicotinamide adenine dinucleotide (NAD) as a cofactor. There are 7 mammalian Sir2 family members, designated SIRT1–SIRT7 [3, 9–12] (table 1), with SIRT1 being the most highly related to yeast Sir2 [3, 9, 13, 14]. The contribution of SIRT1/Sir2 to genome stability was shown in yeast cells and mouse embryonic stem cells [4]. SIRT1,

Table 1. Mammalian sirtuins

Human sirtuin	Subcellular localization	Enzyme activity	Biology
SIRT1	Nucleus (cytosol)	Deacetylase	DNA stability and cell proliferation (DNA double-strand break repair)
SIRT2	Cytosol	Deacetylase	Mitosis and cell differentiation (modulation of microtubule network)
SIRT3	Mitochondria	Deacetylase	Energy metabolism and responses to oxidative stress (ATP level)
SIRT4	Mitochondria	ADP-ribosyltransferase	Energy metabolism and responses to oxidative stress (insulin secretion)
SIRT5	Mitochondria	Deacetylase	Energy metabolism and responses to oxidative stress
SIRT6	Nucleus	ADP-ribosyltransferase, deacetylase	DNA stability and cell proliferation (DNA single-strand break repair)
SIRT7	Nucleus	Deacetylase	DNA stability and cell proliferation

like Sir2, modifies chromatin and silences the transcription of integrated reporter genes via histone deacetylation [15]. In *SIRT1*-deficient embryonic stem cells, oxidative-stress-induced chromosomal fusions, which generally result from aberrantly repaired DNA, are clearly upregulated [4]. On the other hand, SIRT1 overexpression inhibits irradiation-induced cancers, by increasing DNA stability [4]. This result is particularly interesting in view of possible cancer therapies. However, the potential roles of SIRT1 in life span regulation have not yet been documented.

A biological role in aging was reported for SIRT6 [16]. In *SIRT6*-deficient mammalian cells (mouse embryonic fibroblasts and embryonic stem cells), proliferation is reduced, and sensitivity to DNA-damaging agents is increased, as is DNA instability. These events are caused by defective base excision repair, a repair mechanism for DNA single-strand breaks. The *SIRT6*-deficient cells show chromosomal aberrations and genome instability, even in the absence of artificial stimuli. Moreover, *SIRT6*-deficient mice have a reduced life span. This may be due to direct impairment of the base excision repair system; however, these mice also show severely reduced insulin-like growth factor 1 and blood glucose levels, which might also promote DNA instability.

The deacetylase activity of SIRT1 has other substrates besides histones (fig. 1). It also deacetylates p53 (described above), nuclear factor κ B (NF- κ B) and forkhead transcription factor (FOXO) [6]. Deacetylated NF- κ B is easily degraded and has less activity, which inhibits apop-

toxis. FOXO, a downstream component of insulin signaling, can increase the transcription of both antiapoptotic factors, such as manganese superoxide dismutase (Mn-SOD), and a DNA repair factor, growth-arrest- and DNA-damage-inducible protein 45 (GADD45), and proapoptotic ones, such as B-cell-lymphoma-2-interacting mediator (BIM) and Fas ligand, depending on the context. These different effects can be explained, at least in part, by FOXO regulation by SIRT1. FOXO-induced apoptosis is decreased when SIRT1 is overexpressed in cerebellar neurons [17]. The deacetylation of FOXO by SIRT1 decreases the DNA-binding activity of FOXO and reduces the transcription of apoptotic factors [18]. On the other hand, the association of SIRT1 with FOXO upregulates the expression of antiapoptotic factors. Therefore, SIRT1 uses multiple pathways to modulate cellular resistance to genotoxic stress and apoptosis [13].

SIRT1 protein is mainly localized to the nucleus; however, it can be translocated to the cytoplasm in the presence of oxidative stress, leading to apoptosis [19]. This phenomenon is also observed in *Caenorhabditis elegans* [20]. Sir2.1 translocates from the nucleus to the cytoplasm as an early event of apoptosis, although the causal relationship and the kinetics of this translocation are still obscure.

Therefore, the sirtuins have critical roles in DNA stability and antiapoptotic processes, and they remain key molecules for aging research.

Sirtuins in the Retina

SIRT1 is expressed throughout the retina, including the retinal ganglion cells, inner retinal cells, photoreceptor cells and retinal pigment epithelial cells [21]. *SIRT1*-targeted mice show abnormal retinal development as early as embryonic day 12.5; these abnormalities include persistence of the optic fissure and disorganized retinal morphogenesis, with decreased cellularity in multiple retinal layers [22], although it is not yet known whether these embryonic retinal abnormalities are associated with an increase in p53 expression, as observed in *SIRT1*-targeted mouse embryonic fibroblasts. The deletion of the upstream inducers of SIRT1, E2fs, also cause retinal cell death with the hyperacetylation (i.e. activation) of p53 [23].

SIRT1 activates multiple pathways and has been reported to function in animal models of inflammatory disease. In a multiple sclerosis model of experimental autoimmune encephalomyelitis, the intravitreal injection of SIRT1 activators prevented the death of retinal ganglion cells [24]. This effect was obtained without suppressing the infiltration of inflammatory cells into the optic nerve sheath, suggesting that SIRT1 acted in the retinal ganglion cells themselves.

This successful treatment of ganglion cells is consistent with the result of an experiment using explant neurons of the dorsal root ganglion [25]. Ganglion cell death after axotomy, called wallerian degeneration, is avoided by introduction of nicotinamide mononucleotide adenylyltransferase 1 (Nmnat1), an enzyme in the NAD biosynthesis pathway, which leads to an increase in nuclear NAD. SIRT1 activity induced by a polyphenol compound, resveratrol, has a similar effect. These results indicated that SIRT1, which is NAD dependent, is the effector of the ganglion cell protection conferred by the increased Nmnat1 activity and NAD. Moreover, Nmnat1 is normally expressed in retinal ganglion cells and their axons [26], which should enable SIRT1 activation in these cells.

A decrease in the deacetylation activity of SIRT1 during retinal inflammation was reported in the endotoxin-induced uveitis model [27]. In this mouse model, 8-oxo-2'-deoxyguanosine (8-OHdG), an oxidized form of deoxyguanosine, a component of DNA, is upregulated, and resveratrol suppresses this upregulation. It would be interesting to examine whether this reduction in DNA oxidation is related to an effect of SIRT1 on DNA and discuss the possible involvement of inflammation in aging.

Role of Sirtuins in the Aging of the Retina

Age-related DNA damage is reported in the rodent retina. An increase in oxidative DNA damage in the 2-year-old retina compared to the 5-month-old retina was shown by 8-OHdG labeling [28]. The authors concluded that this increase in labeling reflected mitochondrial DNA damage resulting from decreased levels of DNA repair enzymes. However, the role of sirtuins in this phenomenon is not addressed.

Abnormal SIRT1 expression is observed in a mutant mouse model of retinal degeneration (*rd* mice) that progresses with age; these *rd* mice have a point mutation in the β -subunit of rod photoreceptor cGMP phosphodiesterase [21]. In the normal mouse retinal ganglion cells, inner nuclear layer cells and a subset of photoreceptors, thought to be cone cells, SIRT1 immunoreactivity appears as granular staining in the nuclei. In the rod photoreceptor cells, it appears as weak peripheral nuclear staining (but is still within the nucleus). However, in the *rd* mice, SIRT1 in the rod photoreceptor cells translocates to the cytoplasm around postnatal day 15, when apoptotic markers (caspase 12 or apoptosis-inducing factor (AIF) as well as transferase-mediated uridine nick end-labeling (TUNEL) staining) become apparent. This finding is consistent with another report that the translocation of SIRT1 protein from the nucleus to the cytoplasm precedes apoptosis in *C. elegans* [20]. The *rd* mutants lose their photoreceptor cells precociously, which is considered an aging phenotype. A similar theory has been proposed to explain brain diseases, such as Parkinson's disease and Alzheimer's disease, in which brain cells degenerate earlier than in the normal life course. Further analyses are required to determine whether the translocation of SIRT1 from the nucleus leads to DNA instability in *rd* mice and in other systems.

In summary, the aging of tissues/organs, including the retina, is caused by accumulated changes that lead to their dysfunction. The fundamental cause is believed to involve DNA instability, which may be induced by extrinsic stimuli, such as light exposure or inflammation, or by intrinsic factors, such as gene abnormalities. The ability of sirtuins to stabilize DNA may, at least in part, determine the progress of aging in the retina.

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