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Molecular and cellular parameters of ageing: an overview

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Abstract: Ageing is characterized by a progressive loss of physiological integrity, leading to impaired function and increased propensity to death. Ageing is an actively regulated metabolic process. This wear and tear is the primary risk factor for major human pathologies including diabetes, cancer, cardiovascular disorders, and neurodegenerative diseases. Approximately worldwide 1 lakh people die each day of age related causes. Ageing, which we define as the time-dependent functional decline that affects most living organisms, has attracted inquisitiveness and excited imagination throughout the history of mankind. Nowadays, ageing is subjected to scientific analysis based on the ever-expanding familiarity of the molecular and cellular basis of life and disease as specific genes have been recognized that regulate ageing. The time-dependent accretion of cellular damage is widely considered the general cause of ageing. In this study we tried to exemplify few parameters which regulates ageing and some pathways to increase life span or protecting mutation of cells into cancerous growth.

Keywords: Ageing, Apoptosis, Autophagy, Reactive Oxygen Species (ROS).

Introduction

"Ageing is not merely getting old, wrinkles, tumbling feet, diseases but it's an achievement of a lifetime innocence, exuberance, reverence and perseverance".

Ageing is accretion of changes in a person over the time [1]. Approximately worldwide 1 lakh people die each day of age related causes [2]. The time-dependent accretion of cellular damage is widely considered the reason of ageing [3-4]. People are living longer life in comparison with their ancestors. The life expectancy as per Census of India in 2011 is 65 years. Only 5.27% of our population is over the age of 65 years as per World Bank. In other parts of the world with much better admittance to medicine and healthcare, these numbers are significantly higher. In Japan, for example, nearly 17% of the population is aged over 65 years. And the average Japanese, with a life-expectancy of 80 years, lives fully a third longer than the average Indian. Evolutionary processes have shaped the homeodynamic space in accordance with the need of the genre for fulfilling the biological purpose of life. This evolutionary lifespan is called 'essential lifespan' (ELS) [5-6], for which an efficient homeodynamic space is the basic requirement. ELS is generally much shorter than the maximum lifespan potential of a species or the average lifespan of organism within the species. For example, ELS for rats and mice in nature is less than 1 year, but in the highly protected laboratory conditions, these animals can live for 2 to 3 years and genetically engineered mice can live up to 5 years. Similarly, ELS for humans is about 40 years, but in modern societies with protected environments and good nutritional and healthcare access, human populations can expect to live more than double ELS [7-8]. Ageing leads decline in physical and cognitive functions (not in semantic memory) as there is cell loss in the limbic system (hippocampus, parahipocampal and cingulate gyri) is of special interest in regard of memory, age dependent loss of lean muscle mass (sarcopenia), various diseases as body's immune system go feeble.

There are various deleterious determinants of ageing such reactive oxygen species (ROS) and free radicals, dys-functioning of telomere, epigenetic alterations, impaired protein homeostasis (proteostasis), autophagy, apoptosis and cellular senescence. In contrast there are few emerging parameters which

increases the longevity of life such as caloric restriction, hormesis, gene silencing, polyamines such as spermidine, spermine and clinical trials of few drugs are in progress and these things proves the statement given by the two pioneer in biogerontology “leonard Hayflick” and “Robin Holiday” that ageing is no longer an inexplicable problem in biology.

So this current review aims to summarize our present understanding of ageing, it’s positive and negative parameters and the latest advancements that lead to decline in ageing or longevity in physiological aspects of life. This review was done by way of searching various articles and websites associated with the ageing. The terms which were used for searching the data were; "Ageing and its parameters", "Various markers of Ageing". Based on only published data, from pubmed, US national library and various websites, this article was constructed.

Deleterious Determinants

Reactive Oxygen Species And Free Radicals: Reactive oxygen species (ROS) are those chemically active, highly reactive species or molecules containing atoms with unpaired electron in its outer orbit [9] and involving oxygen molecule in it and in contrast with it ‘free radicals’ do not contain oxygen. They are required for cell signaling and maintenance of normal cell structure or may damage cell structures leading to oxidative stress [10].

The source of ROS and free radicals exogenously are smoking, herbicides, pesticides, fried foods, etc and endogenously as By-product of cellular respiration through electron transport system-often oxygen is the terminal electron acceptor in the cell mitochondria (mitochondria is the major source). Free radical theory of ageing also states that ageing is due to damage to cells by mitochondrial derived ROS. The damaging effects of ROS are seen when the antioxidant production is depleted [11].

There is another family of reactive chemical body i.e reactive nitrogen species (RNS) derived from nitric oxide and superoxide. Both RNS and ROS on excessive production and limited antioxidant production leads nitrosative stress response [12]. The ROS work in primary condition as homeostatic agent in an harmonized fashion as signaling pathway as the age advances, cellular damage causing increase in ROS. Beyond certain threshold level this ROS leads damage to the cells. As an example ROS increases leads to prolongation of lifespan of yeast and *Caenorhabditis elegans* [13-14] where this ROS in genetic manipulation in mice does not cause increase in life span of mice [15]. So this conflicts regarding prolongation of ageing or may have damaging effects.

Epigenetics Alteration: Epigenetics in biology is study of changes which are prolonged or long term alterations or which are heritable that does not code for DNA and observed generally during differentiation [16]. Epigenetics inheritance involves DNA methylation, Histone acetylation, Histone phosphorylation and Histone ubiquitination are all influencing the gene expression [17]. Different types epigenetics alterations leads accumulation of cell damage or various diseases throughout life [18].

Greer et al studied that specific epigenetic alterations may show sign of longevity. He found that deletion of component of lifespan regulators of nematodes, flies and worms (H3K4 & H3K27) show increase in longevity [19-20]. DNA methylation changes with environmental influence [21]. It has been seen that there is loss methylation as age increases [22] but it shows hypermethylated changes also. Epigenetics alterations are biomarkers of ageing [23]. These epigenetic alterations are reversible and can be protective if supplements such as Methionine, Folate, Vitamin B-12 are taken [24].

Proteostasis: Proteostasis means combination of protein and homeostasis or we can say maintenance of trafficking, accumulation of damaged and folding-unfolding of protein, regeneration or degeneration or regulation of biosynthesis within the cell or outside it, contributed by protein in the prolongation of age related diseases such as Alzheimer’s [25]. It is important to maintain proteonome so that the functionality of cellular system, its ability and adaptability in different environments remains preserved. Rate of protein synthesis and accordingly anabolic signals by enhancing

pressure on endoplasmic reticulum affects protein folding-unfolding, trafficking and secretion potential [26-27]. This enhanced pressure on pancreatic beta cells to increase pro-insulin synthesis by 10 times. This leads oxidative stress that disrupts folding in endoplasmic reticulum, which in turn damage mitochondrial function to reduce oxidative phosphorylation. This later transmitted across all the cell membrane. Proteostasis network (PN) do have a signaling arm that acts cooperatively to return affected compartment containing folded-unfolded protein to its homeostasis. Damaged or misfolded protein in cells accumulate during ageing which has been shown in classical organism such as *Drosophila melanogaster* and *Caenorhabditis elegans* [28-29].

It has been seen that regulation of protein and chaperone mediated proteins is at most important for homeostasis, cellular stress response and longevity [30-31]. The potential cause for the late onset of diseases is interaction between hsf-1 and ILS (Insulin like signaling) pathway which on down regulation suppresses longevity of mutants [32-33]. There has been an increasing progress in identification of novel small molecules that they have arm-specific unfolding protein response activator [34] and also drugs to protect accumulation of damaged protein. Future aspects will address several age related diseases on basis of changes in protein quality with respect to ageing.

Autophagy: Autophagy is a self-deteriorating process derived from Greeks ‘auto’ means ‘self’ and ‘phagein’ means ‘eating’. It is first given by Belgian biochemist Christian De Duve in 1963 [35]. It is the primary intracellular cannibalism mechanism for degrading and recycling altered proteins and organelles. Autophagic cargo is sequestered with double-membrane structures called autophagosomes that fuse with lysosomes to degrade their contents in bulk. Autophagosomes can also fuse with endosomes to form amphisomes which then deliver their content to lysosomes. The resulting macromolecules are released through permeases and recycled in cytosol [36]. Autophagy is not self destructive process [37]. Autophagy provide energy by degrading part of cytosol and provide amino-acids and free fatty acids which are than utilize to maintain energetic balance in the cell. By eliminating altered or damaged protein and organelles, it play a role in cellular defense. The cells which are not able to maintain normal physiological process are than taken from cytosol and delivered the cytoplasmic substrates to lysosomes for degradation. It is done by different physiological pathways of autophagy which includes macroautophagy, microautophagy and chaperone mediated autophagy (CPA). Massey et al. 2007, found LC-II as a marker to be used in macroautophagy to detect the pathway of autophagy. There are various example such as in *C. elegans* [38] if autophagy is blocked it may lead to decrease in lifespan, whereas in *D. melanogaster* [39-40] over expression of autophagy leads to increase in lifespan. In the autophagy target of rapamycin i.e. TOR is playing a role of protein kinase. TOR regulates two functions i.e. autophagy and synthesis of protein. In mammalian it is called as mTOR and on interaction with other protein it forms two complexes i.e. mTORC1 and mTORC2 [41].

Telomere Dysfunction: Telomeres are distinctive DNA protein having a region of repetitive nucleotide sequences i.e. non-coding repeats TTAGGG which protects the chromosomes from damage [42]. Due to repetitive cell division there is shortening of telomeres. This shortening of telomeres depends on the telomerase enzyme [43]. Telomerase is an enzyme or type of DNA polymerase which specializes in replication and protect the deterioration of chromosomes. Those mammals who does not comprise of telomerase enzymes will undergo early damage and loss of telomere properties to replicate [44]. This shortening of telomere is also observed in mice and humans [45].

Deficiency of telomerase enzyme will lead to development of early diseases which involves loss of capacity of tissues to regenerate i.e. aplastic anemia, pulmonary fibrosis [46]. It has been formulated that telomeres undergone dysfunction are able to induce repeated fusion/breakage cycles that leads to instability of chromosomes [47] and also may lead to formation of cancer with ageing prolongs. It has been seen in humans having increase in the number of DNA damage foci in blood

cells (lymphocytes) [48] and in the cells of intestinal epithelium with the patients having a chronic inflammatory disease i.e. colitis ulcerosa with enhanced telomere shortening [49]. There has been establishment of genetically-modified animal models which shows the links to the loss of telomere, cellular senescence and organismal ageing, thus mice exhibit increase or decrease in lifespan having lengthened or shortened telomeres [50-51]. It has been seen that genetically reactivation of telomerase enzyme will enhance lifespan and longevity [52] and no increase in the incidence of cancer was seen [53]. Thus this shows that telomere dysfunction or enlightening the activity of telomerase enzyme is the most important part of ageing.

Apoptosis: Apoptosis is a process of programmed cell death by activation of intrinsic suicidal pathway or cellular process i.e. differentiation and proliferation [54]. It acts as a protective mechanism during pathological condition, as it cleanses injured and unfit cells without leading to inflammation [55]. Apoptosis works by two pathways, Intrinsic and Extrinsic [56]. During extrinsic pathway receptors that activate death genes or receptors are CD95 (Fas/Apo), TNFR1 and TNFR2 which in turn activates cytoplasmic receptors such as Fas associated death domain(FADD) and TNFR-associated death domain(TRADD) and these protein molecules activates caspases (caspase3,7) inducing death and also facilitating activation of intrinsic pathway [57].

During intrinsic pathway receptors such as cytochrome c and Smac/DIABLO triggering formation of apoptosome and activating caspases (caspase9,3,7) and leading to apoptosis. Ageing alters gene expression resulting differential apoptotic signaling. Studies on rodents models of ageing showed increase in activity of caspases including caspase-2,3,6,7,9 in different organs in older rodents as compared to young ones [58-59] and also elevation in the level of cytochrome c [60-61]. The study by Wang et al., (2014) concluded that apoptosis is associated with ageing leading to sarcopenia, apoptosis of satellite cells and capillary functions impairment [62]. Apoptosis is a mechanism at molecular level which involves free radicals that can lead to aura of longevity through signaling molecules which acts beneficially. These signaling molecules can also be manipulated to mechanism which leads to slowing of ageing. This manipulation leading to stimulating signal mechanism that has an aura of longevity effects which is beneficial for neurodegenerative diseases [63]. These apoptotic signals in the brain prevent killing of cells instead provide resistance on damaging effect and this is because it is difficult to replace dead neurons due to complexity of their connections.

Cellular Senescence: Cellular senescence is a natural phenomenon that precludes the multiplication of the normal human cells and tumor cells, in-vitro and it has been established almost 5 decades back by Hayflick and Moorhead [64-65]. That is why it has been linked with the tumor suppression processes and aging. This cellular senescence is all due to its encounter with stress caused by the oncogene. Cellular senescence causes the induction of the myriad of stimuli's. We all know about the telomeric activity (loss of Telomeric DNA with each S-phase) which results in the generation of DNA damage response (DDR). This generation of DDR will in turn results in maintenance and initiation of senescence arrest [66-67].

Cellular senescence is irreversible physiological process with due exceptions that some cells do recommence growth as they do not show ectopic expression of CDKi (cyclin-dependent kinase inhibitors) i.e. p21IWAF1 [68-69]. The cells which undergone senescent transformation shows increase in size up to two-fold [64]. It is one of the hallmarks of senescent cells who show SA-Bgal (senescence related β-galactosidase) [70]. Senescent cells are not quiescent [71]. These senescent cells having tenacious DDR signals to conceal factors such as proteases, cytokines and growth factors that have potent paracrine and autocrine functions [72-73].

Cancer is may be due to age related or due to anomalous alterations in the cells genomic structure [74-75]. While ageing does not show any aberrant alterations in the cells or it is mostly due to failure in cellular functioning. As we all know p53 is serious tumor suppressor. So study done by Tyner et al., 2002 and Maier et al., 2004 on mouse models

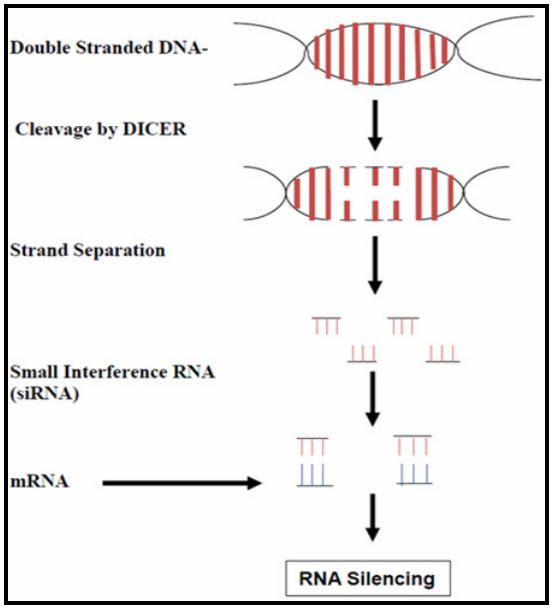
by artificially and naturally altering p53 protein showed hyperactive p53 [76-77]. This hyperactivity in the mouse model was related with their cancer-free life. It has been seen that this hyperactivity in the mouse models also show shortened lifespan [76-77] and normal longevity. The mechanisms of senescence affect the tissues of the stem or progenitor cells which further leads to delayed healing, decrement in tissue repair and regeneration [78]. Senescence has been linked with cancer pathologies and age related diseases which functions as a brake to the growth and maturation of the cells which alters the surrounding environment. The advancement in cellular senescence will help in increasing the knowledge about the mechanism involved in it and strategies it for interventional and therapeutic purpose.

Some Beneficial Parameters

Gene Silencing: Gene silencing is in general, silencing or change in gene expressions by certain base pairs or we can say "turning off" a gene to express and this should not be mixed up with gene knockout as gene knockout is simply complete removal of a particular gene from the organisms as in knockout mice while silencing will only reduce the expression in contrast with knockout [79-80]. Such as RNAi (RNA interference) and miRNA (micro RNA) reduces or suppresses the expression of gene but does not completely eliminate it [81]. It can be seen in Figure-1 that small interference in genomic structure alters the mechanism and causes gene silencing. Gene silencing in cells may be transcriptional gene silencing, post-transcriptional gene silencing and meiotic gene silencing by unpaired DNA (MSUD) [82].

Gene silencing is often used for research purposes as it may be used for therapeutics purpose in treating various cancer and neurodegenerative disorders. There has been two major techniques of gene silencing which are Antisense gene therapy and RNAi gene therapy (Post transcriptional gene therapy) for treating various neurodegenerative diseases for example Huntington's Disease and atherosclerosis a cardiovascular disease. KYNMARO [83] has been formulated by antisense technology will be helpful in patients with homozygous familial hypercholesterolemia (HoFH). There are other drugs also which has been produce by antisense technology for neurodegenerative disorders such as Huntington's disease but drug is under trial for humans. RNAi can be used in modulation of HIV-1 replication (Hannon, 2002). Selective silencing of viral gene expression in HPV- Positive human cervical carcinoma cells treated with siRNA, a primer of RNA interference (le Bon et al, 2002). RNAi will be helpful in establishment of retroviral siRNA vectors (higher efficiency, infection of suspension cell lines).

Figure-1: Schematic flow Chart showing Gene Silencing



Hormesis: The word hormesis has been acknowledged in the field of toxicology [84] and radiation biology, is considered to be a dose response (Bi-Phasic dose [85]) therapy but not all show response to this therapy. The concept of hormesis is basically an adaptive response of cell and organism to slow down ageing or delaying in age related diseases by stress induced pathways [86]. The effects of hormesis is defined as exposure to low dose of chemical agents or biological agents that may include antibiotics, vitamins, minerals, chemotherapeutic agents or ionizing radiation [87-88].

For example if vitamin-E taken on normal diet or daily doses in food are beneficial in protecting cell membranes from damage or enzymes destruction or preventing cancer but if taken in high doses as supplements it will

more likely to cause cancer study done by national cancer institute on prostate cancer in 2001 showing a U-shaped or inverted U-shaped response [87, 89]. Hormesis may enhance plasticity of characteristics which are observable phenotypicaly [90]. Hormesis in general is condition of the organisms which respond to the mild level of doses on exposure to environmental agents. This mild level of doses produces a advantageous response producing a conditioning hormesis. This makes the organism's to develop ability to uphold more stronger level of doses in comparison to those who are directly exposed to higher level of doses [91]. Thus hormesis leads to irretrievable alterations in there phenotype which promote organisms resistance to heavy doses of stress.

Spermidine (Age Promoting Supplement): Spermidine is an anti-ageing supplement, naturally occurring polyamine involved in important cellular and molecular process such as inflammation, Autophagy, DNA stability, cell growth, transcription and translation etc. The molecular mechanism of spermidine's action of pathway is mainly as an Autophagy enhancer and reduction in inflammation by inhibiting the action of interleukin-1β and tumor necrosis factor-α. Addition of spermidine in food increases the life span of worms, yeast cells and fruit flies and immune cell survival in-vitro [92]. Its intracellular level decreases as the age increases [93]. Studies on old mice on supplementation with spermidine showed increase in aortic pulse wave velocity and increase formation of advanced glycation end-products (AGEs) [94].

It reverses large elastic artery stiffening and restores NO-mediated endothelial function and also reduces oxidative stress [94]. Spermidine plays a role in life-extension mechanism by inducing apoptotic like reaction for the protection of the mammalian cells [95]. With spermidine there are two extra polyamines are there which plays role in regulating cellular mechanism i.e. spermine and putrescine. These polyamines such as spermine and spermidine are derived by SAM (S-adenosyl- methionine) [95]. Spermidine has been found to have potent stimulation effect in the growth of human hair by promoting elongation of hair shaft and regulating the appearance K15 and K19 (keratins associated with the epithelial stem cell) [96]. Spermidine administration reduces the loss of one of the hallmarks of Parkinson's disease i.e α-synuclein induced loss of dopaminergic neurons in nematodes [97]. It has been seen that spermidine has a neuro-protective effects In-vitro. It has a protective action by inhibiting the action of ROS by acting as a inherent free radical forager. The study by Noro et al., (2015) found that everyday ingestion of spermidine prevent the damage of retinal ganglionic cells (RGC) following optic nerve injury [98]. They concluded that spermidine can be used for the treatment as a neuro-regenerative agent for glaucoma [98].

Research in this field is at most important as spermidine is found to be increasing lifespan in rodents so that it can be kept into the leagues of resveratrol and rapamycin to enhance longevity. As rapamycin do have side effects [99] whiles spermidine has not been shown to have any side effects yet. On the other hand these polyamines (spermidine) can be taken as supplements in our diet which includes mushrooms and soya beans etc and these supplements tends to increase in the level of polyamines in blood. So a observant study is required to strategies the implementation of spermidine as supplementation in the diets of humans.

Caloric Restriction: Caloric restriction (CR) is categorized in brief as "Under-nutrition without being Malnourished". In this dietary plan is involving low calories intake without getting under nourished. It is understood as only procedure which prolongs longevity and delay onset of various cardiovascular and neurodegenerative disorders [100]. It was first came into notice of world in 1930s, that restriction in diet is valuable and increases lifespan in rodents [101]. CR is the strongest non-molecular nutritional intercession involving experiments for the extension of life or longevity in species such as nematodes, fruit flies, yeast, rats, dogs, fish and mice [102-103].

In rodents reduction in the diet of 30-60% early in life enhances life span by 30-60% [102-103]. In rodents it has been seen that cancer is 80% cause of death and CR had been seen to reduced the underlying cause of tumor

induced by radiation and chemicals [104]. It is also beneficial in reducing pathological diseases in cardiac and neural system of rodents [105]. CR also results in decrease in neurodegeneration, β-amyloid deposition in the brain and enhancement of neural regeneration in animal models of Parkinsonism, Huntington and stroke [106-107]. It has been seen in rhesus monkeys also when they were restricted with the 30% of their diet since there young adulthood shows decrease in mortality [108]. CR significantly improves age-related and all-cause survival in monkeys on a long-term ~30% restricted diet since young adulthood. In overweight humans also CR has shown to decrease cardiac diseases, improving mitochondrial function and sensitivity to insulin [109-110].

As per Madeo et al., (2014) the mechanism involved in CR is deacetylation of cellular proteins involving three classes of compounds [111]. It has been shown by many authors that CR results in decrease in ROS creation thus led to decrease in the oxidative stress causing damage [112-113]. There are many neuroendocrine adaptations which are mediated by CR as an anti-ageing effect i.e. (a) decrease in the level of hormones that are involved in the regulation of heat production and cell metabolism (e.g., Nor-epinephrine, leptin, thyroid hormone), (b) decrease in the production of anabolic hormones (e.g., estradiol, testosterone) (c) Hormones that suppresses inflammation are increased in volume (e.g., ghrelin, glucocorticoids) [114]. So there are many mechanisms which are involve or work in conjunction with CR such as down regulation of Insulin growth factor-1 (IGF-1) [115].

As per future aspect a key purpose to study the effects of caloric restriction is to see whether it is beneficial for all humans but not only in obese or overweight peoples. Another purpose is to find out whether it is beneficial to have intermittent fasting in prolongation of life span, delaying age-associated disorders without doing CR [116-117].

Discussion and Conclusion

This is a brief enumeration of various parameters of ageing which categorize them into two; one as who affects ageing, accelerate ageing, leads to cancerous conditions, various diseases are deleterious markers and other one are those which leads to decrease in cancerous conditions and overall longevity are age enhancers. One of the main features of the deleterious markers are that they all act negatively for the body which involves oxidative stress causing cellular damage which can be pathological. ROS which act as signaling molecules is having both negative and positive aspects.

These negative effects are most commonly seen in pathologic conditions where equilibrium between antioxidants and ROS is disturbed. As antioxidants prevent from the oxidative damage produce by the accumulation of ROS. Proteostasis and Epigenetic manipulation such as loss of function of SIRT1 and SIRT6 holds for extending lifespan. Overview of cellular senescence shows tumor suppressor activity by extending longevity. The mechanism involved is also eliminating senescent cells. On the other hand age enhancers delays age related diseases by decrease in ROS, DNA stability and proper functioning of cellular pathways such as apoptosis. Gene silencing is kind of deleting gene or manipulation in genomic structure to enhance lifespan. A study published in Cell Metabolism, state that gene LOS1 if removed showed increase in life span in yeast by 60%. So the above overview is appropriate in the pathological conditions.

Hormesis review shows the agents which can be helpful in enhancing ageing and spermidine can be considered an hormesis agent. While caloric restriction review showed that it needs human testing on large scale to prove it as effective to be called as Hallmark in enhancing age. This review will build futuristic aspect for various molecular studies on ageing and will help in promoting human health by improving their life span. However there are still many aspects which are proposing a challenge in understanding this multifaceted biological process.

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