Cardioneural Aging in the Context of Cardiomyocyte Aging

Introduction

Research in the past few decades has alluded to several triggers for aging, and in recent years, has revealed many different cellular pathways that are catalyzed during this aging process, thereafter, generalizing these findings to all the organ systems situated in the body. However, it is vital to understand the aging process in the context of all individual cell types. Additionally, while studying the aging process of all cell types may prove to be insightful on a broad spectrum, paying close attention to the diverse effects of various pathways on internal organs such as the heart and brain. Currently, several studies focusing on the underlying caveats of aging in neural cells are in progress. While the outcomes from these studies are both beneficial and necessary to shed light on the aging process, it is important to consider the aging of the brain in context of the rest of the body. One key organ that has been heavily associated with the neurological aging process recently is the heart. Its vital functionality helps maintain brain function in many capacities, but its slight dysfunction can prove to be incredibly devastating for the brain.

Review

Mechanisms of Aging

Oxidative Stress

One of the main underlying mechanisms of aging is attributed to oxidative stress. When oxygen participates in certain chemical reactions, it produces free radicals often called Reactive Oxygen Species (ROS). Accumulation of ROS has consistently shown to be associated with cardiomyocyte(CM) aging pathways including cellular senescence, a major attribute of cellular

aging(Xie et al., 2023). Nevertheless, ROS are unavoidable because they are produced by various metabolic pathways and become harmful especially when there is a lack of antioxidants present to counteract their adverse effects((de Almeida et al., 2022).

ROS accumulation may occur for several reasons. Researchers recently revealed that a transcription factor called KLF9 increases ROS levels of neonatal cardiomyocytes in rats by downregulating Txnrd2, an enzyme critical for antioxidative pathways. It was also uncovered that KLF9 is found in higher levels in ischemic cardiomyocytes indicating that ischemia may be an enhancing trigger for this pathway(Yan et al., 2019).

Another factor in ROS accumulation that is commonly overlooked is Angiotensin II, which is critical to the Renin-Angiotensin-Aldosterone(RAS) system that is tasked with regulating blood pressure and ion imbalances. Through a ROS assay and comparing CypA expression in one group of rat cardiomyocytes treated with Angiotensin II and then another treated with Angiotensin II and the antagonist for the AT_2R receptor, it was determined that there was a significant association between ROS accumulation and the activation of the AT_2R receptor by Angiotensin II(Tian et al., 2018). Another group of ROS accumulation factors that is also sidelined and unthoroughly explored is the β-adrenergic receptor(β-AR) and β-arrestin pathway. After treating neonatal mice cardiomyocytes(NMCM) with isoproterenol, a β-AR agonist, ROS levels were observed to be significantly increased after DHE staining. Further assays revealed that β-arrestin was associated with ROS production in the mitochondria due to β-AR activation(Zhang et al., 2017).

Interleukins also remain eminent in the aging process, but they make their presence known in various ways. A recent study focused on ROS production in HL-1 cardiomyocytes and revealed that IL-39 is associated with high ROS levels in these cells. Furthermore, the

researchers associated with the study also revealed that ROS production can also be concentration dependent with the highest observed ROS levels at 60 ng/mL and 80 ng/mL(Xiong et al., 2021). Other factors that retain high relevancy in accentuating cellular oxidative stress include Nicotinamide Adenine Dinucleotide Phosphate Oxidase(NOX), Xanthine Oxidase/Oxidoreductase(XO), and Nitric Oxide Synthase(NOS). NOX enzymes are generally involved in the transfer of electrons to create superoxide, in turn, significantly increasing oxidative stress. Similarly, XO helps catalyze the oxidation of hypoxanthine to xanthine and produces superoxide and hydrogen peroxide as a by product. What both groups of enzymes share is that they are heavily involved in hyperglycemia-induced ROS production(Kaludercic & Di Lisa, 2020). Studying the effect of these enzymes further under diabetic conditions may reveal more about the pathway that is induced in a diabetic demographic. NOS is also involved in superoxide formation, like the other two enzymes, but this happens when NOS is uncoupled(Kaludercic & Di Lisa, 2020).

While inducing oxidative stress is an effective way of accelerating the aging process, there are several promising remedies being studied. Resveratrol, a naturally occurring polyphenol seems to decrease the load of oxidative stress that is experienced by the cell because of its antioxidant properties(Aguilar-Alonso et al., 2018).

Shortening of Telomeres

Telomeres serve as a form of protection for DNA, located at the ends of every chromosome, and an indicator for a cell's lifespan. Each time a cell divides, the telomeres shorten because of the incapability of DNA Polymerase to replicate the entire DNA strand. However, assessing telomere shortening in cardiomyocytes is different than most other cells because they do not divide as frequently. Furthermore, while telomere shortening leads to a

relatively consistent senescence-associated secretory phenotype(SASP) in aging cells, the SASP triggered in cardiomyocytes proves to be associated with a few key components that are not otherwise abundant at the mRNA level in other aging cells: the corresponding proteins were Edn3, Tgfb2, and Gdf15(Anderson et al., 2019).

While telomere shortening may not seem like an aging mechanism worth noting in a cell type that does not divide frequently, genetic predispositions coupled with a cell's epigenetic markers can increase the need to observe telomeric shortening. For example, loss of certain epigenetic tags like H3K9me3 can lead to increased proliferation in cardiomyocytes which would then put the cells at a significantly higher risk of telomeric shortening(Chen et al., 2022).

Individual and individual factors can also produce telomeric shortening. For instance, Xray irradiation in murine cardiomyocyte caused both telomeric DNA damage and non-telomeric DNA damage, but only the telomere-associated DNA damage was significantly persistent while non-telomeric DNA damage was resolved fairly efficiently by means of the DNA Damage Response(DDR). The functional conclusion from this information is that telomeric DNA damage cannot be repaired with the damage lasting for a prolonged period of time(Anderson et al., 2019). When considering this knowledge in the context of epigenetics, it is safe to conclude that telomere shortening is a key mechanism to be explored in regard to CM aging.

Varied regulation of certain genes and proteins will also lead to telomere shortening. Rap1, a telomere-associated repressor protein was found to be present in trace quantities within the hearts of aged mice. Reduced Rap1 levels were also associated with elevated levels of p53 expression, a tumor suppressor gene, as well as reduced peroxisome proliferator-activated receptor (PPARα) expression(Cai et al., 2021). Additionally, RAP1 was shown to negatively

regulate telomere length in human mesenchymal stem cells(hMSC), while human neural stem cells(hNSC) sustained an insignificant effect on their telomeres(Zhang et al., 2019) While neither of these observations involve CMs, they do reveal that telomere aging mechanisms have as many similarities as they do differences between different cell types; both the differences and similarities are equally important to take note of.

Additionally, different disorders may accelerate telomere attrition or trigger telomere shortening in unique ways. For example, it was revealed that Duchenne Muscular Dystrophy(DMD) increased the muscle stiffness of cardiomyocytes which was found to be associated with telomere shortening. Interestingly, telomere shortening progressed the most in human induced pluripotent stem cell(hi-PSC) derived CMs in their post-mitotic phase, contributing to about a fifty percent telomere reduction over about a thirty-day time period(Chang et al., 2021). Diabetes mellitus(DM), as lethal as it is on its own in this day in age, continues to amplify numerous health issues including accelerating aging through telomere attrition. In recent years, it has been concluded that there appears to be a significant association between presence of diabetes and shortened telomere length, specifically in individuals with alcohol use disorder(Inomata et al., 2024). Hypertension, commonly found with DM, was found to have a measurable association with attrition as well. A study on hypertension-induced mice revealed this fact along with the underlying relationships between NOX2-induced ROS accumulation, as previously discussed, and other major mechanisms including augmented HDAC6 activity and depletion of the antioxidant PRDX1(Brandt et al., 2022).

Other major diseases, viruses, and malformations may also contribute to telomere attrition including HIV and cancer. Recent publications have revealed significant associations between decreased telomere length and presence of HIV or cancer in different cohorts of

patients(Liang et al., 2024). Without fail, smoking makes its way onto this list, among many others, as an associate of telomere attrition. Mean telomere lengths in a cohort of Pakistani individuals yielded a very significant moderate correlation with smoking habits(Tariq et al., 2024). Unfortunately, it is possible, and seemingly very likely, that many more diseases will be associated with telomere attrition in the future.

Mitochondrial Dysfunction

Mitochondria are associated with several aging pathways at the cellular level. Since mtDNA is more prone to mutations than nuclear DNA, the associated pathways are heavily affected as well. One such example is ROS accumulation by elevated levels of mutations in mitochondrial DNA(mtDNA) which can be damaging to numerous aspects of a cell including essential macromolecules(Guo et al., 2023). As aforementioned, in the face of significant ROS imbalances cells are likely to experience a notable acceleration in aging. Damaged mtDNA also has similarly harmful implications on the aging process especially in the absence of 8oxodeoxyguandine glycosylase, which is tasked with the function of repairing mtDNA(Hoppel et al., 2017).

A lack of respiratory capacity of the mitochondria may also lead to mitochondrial dysfunction which leads to senescence-associated aging. Declining respiratory capacity in the mitochondria can lead to lower overall ATP turnover which sends the cell into a senescent state. Furthermore, this can be attributed to the inhibition of the mitochondrial complexes within a cell(Guo et al., 2023). While the underlying reasons for this is unknown, this mechanism of mitochondrial dysfunction seems to be based on a disruption in the phosphorylative system of the mitochondria(Rosa et al., 2023).

Pathways Associated with Aging

Cellular Senescence

Cellular senescence is a common indicator of cellular aging. Senescence is referred to as the part of a cell's life where it ceases to grow and divide, and the byproducts of these cells can taint the viability of the neighboring healthy cells. In the context of CMs, the associated SASP calls for the release of different factors such as tumor necrosis factor-alpha(TNF-α), interleukins, tumor growth factor-beta(TGF-β), and multiple others that play a vital role in regulating nearby non-CMs by decreasing cardiac function(Tang et al., 2020).

Senescence can be induced and maintained by a number of factors, many of which were previously mentioned including telomere shortening, mitochondrial dysfunction, and oxidative stress. Usually, these factors do not directly contribute to senescence, but rather trigger a senescence-inducing pathway. There are many genes that contribute greatly to accelerating the aging process when silenced or enhanced in expression. Evidence suggests that certain tumor suppressor genes including p16 and p21 are upregulated during cellular senescence, with the former maintaining senescence and the latter being triggered at the start of senescence(Huang et al., 2022). REDD1 is yet another gene that, when enhanced, promoted cardiac senescence in mice. This gene promotes NF-κB which promotes a senescence phenotype that is familiar to CMs(P. Huang et al., 2021). The NF-κB pathway in particular seems to be important in senescence and aging because it is regulated by multiple other genes. Studies suggest that the CAMKII gene, when upregulated, is responsible for accentuating the NF-κB pathway in aged cardiomyocytes(Li et al., 2023). Further studies have also suggested that the FOXP1 gene is another associate of cellular senescence. FOXP1 acts as a transcriptional repressor and serves to regulate proliferation in CMs. Intuitively, downregulation of FOXP1 should lead to increased

CM proliferation, but it was found that downregulation contributes to aging(Zhang et al., 2023). ACE2, tasked with regulating the renin angiotensin system(RAS), is another gene that when upregulated is associated with cellular aging. Additionally, it might prove to be beneficial to observe the genes that are responsible for upregulating ACE2 which are IL1β, IFN-α, and IL7. It is also worth noting that IL1β and IFN-α are both senescence associated genes while IL7 is an aging-associated inflammatory cytokine(Ma et al., 2021).

Previously, the notion of telomere shortening leading to cellular senescence was raised, but there was no mention of how this occurs. One way this occurs is when the FOXC1 gene is upregulated in hi-PSC CMs which appeared to trigger senescence in the hi-PSC CMs(Li et al., 2024). Telomere shortening is also the culprit of calcium ion overaccumulation in the mitochondria which helps activate the p53/PGC1α pathway of CMs(Liu et al., 2024). Considering that the p53 pathway is a tumor-suppressor gene that limits proliferation, it is not a task to conceive an idea of how this mechanism may result in cellular senescence within CMs.

Discussion

While CM aging provides valuable insight into heart health, it can be a great tool to assess the acceleration of aging in the rest of the body, specifically in the brain. While damage to the heart may affect blood supply to the rest of the body, even the slightest amount of anoxic injury can be devastating in the brain among other disruptions. One study even revealed a significant association between ischemic heart disease(IHD) and decreased volume of brain structures(Rauseo et al., 2023). Furthermore, decreasing ejection fractions in the heart may even aid in this process of anoxic brain injury. Left ventricular ejection fraction in patients with cardiovascular disease(CVD) and heart failure was shown to be strongly associated with smaller total brain volumes(Moore & Jefferson, 2021). Impairment of cardiovascular output is also

indicative of neurodegenerative disease with studies showing that it can be linked to Alzheimer's Disease(AD) and irregular brain aging. Specifically, CVD patients with attenuated cardiac output performed significantly worse on neuropsychological tests than their control group counterparts. Even in dementia patients, cardiovascular impairment is an established risk factor despite being insignificantly explored(Jefferson, 2010).

While all these associations have been drawn, research is still needed to uncover the real pathways of cardiovascular aging-induced brain damage. Some potential biomarkers to be explored include those associated with mitochondrial dysfunction, considering that reduced blood flow to the brain will likely lead to reduced mitochondrial function. This list can include markers such as Neurofilament light chain(NfL), and creatine kinase(CK), as well as even more common markers such as lactate and pyruvate(Shayota, 2024). NfL and CK are especially important because of the brain selectivity of NfL and the high concentrations in which CK is present within the heart and the brain that allow fluctuations to be more easily detectable. The Amyloid-β hypothesis also proves to be a linkage between the heart and the brain that needs to be further explored. The plaque build-up associated with this phenomenon shows evidence of being linked to an increase in oxidative stress and arterial stiffness, ultimately being linked to neurodegenerative diseases such as AD(Stakos et al., 2020). It is possible that this pathway could be linked to the RAS system as well which is already heavily involved in regulating oxidative stress in CMs and many other integral physiological processes.

While there are many proteins and pathways related to aging in the heart and the brain, there are also many that went undiscussed that could also have potential effects on the cardioneural aging process. A large-scale proteomics study recently discussed the following proteins as having a causal relationship with the general aging process: KLOTHO, UMOD,

MYL7, CPLX1, CPLX2 and NRXN3(Oh et al., 2023). In other work, further work needs to be done in the field of cardioneural aging and the relation of these proteins to the process. While these relationships have not yet been established, future work will make headway into the specific roles that these proteins play and their relations to other heavily discussed pathways and gene expression.

While these pathways are numerous, a few that should be highlighted is the FOXC1 pathway because of its role in triggering telomere shortening. Telomere attrition seems to recur often as a trigger for cellular senescence and aging which is what makes FOXC1 so important. What makes FOXC1 particularly interesting is the fact that it is known to induce cell proliferation which questions its relevance to cellular senescence. However, a relationship between FOXC1 and NF-κB was revealed where FOXC1 activates NF-κB signaling which helps mediate FOXC1-induced cell proliferation(Han et al., 2017). However, what remains to be uncovered is all the ways in which FOXC1 is regulated apart from its negative regulatory pathway with NF-κB that boldens the impact of FOXC1 on cellular senescence.

Conclusion

This review provides a comprehensive reference of various aging factors and pathways in cardiomyocytes along with potential pathways that have been explored in other organs that may also have similar implications on cardiomyocytes. Moreover, pathways that were especially promising were highlighted in the context of aging in the brain. Since much of these suggested pathways are speculative, this calls for further research to aid in shedding light on the aging process in heavily concomitant organs such as the heart and the brain as well as the general acceleration of aging.

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372

373

374

375