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Age-related Cardiac Deterioration: insights from *Drosophila*

Robert J. Wessells and Rolf Bodmer

The Burnham Institute of Medical Research, Center for Neuroscience and Aging, 10901 North Torrey Pines Read, La Jolla, CA 92037, USA

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1. ABSTRACT

As the average lifespan in Western countries continues to expand, health care for the aged has become an increasingly important research focus. While clinicians and vertebrate researchers have frequently concentrated on specific age-related diseases, particularly neurodegenerative diseases such as Alzheimer's and Parkinson's Diseases, researchers working with invertebrate genetic model systems have gained important insights into global mechanisms of lifespan determination. Still others have employed biochemical and molecular approaches to elucidate processes contributing to common diseases of the elderly, such as cancer and diabetes. In between the broad focus on organismal aging and the more narrow focus on cellular dysfunction is the study of aging at the level of individual organ function. This review will attempt to highlight recent advances in the area of age-related deterioration of organ function provided by the use of transgenic model organisms, with a view toward incorporating these observations into a framework provided by both broader theories of the aging process and studies of cellular function during aging.

2. CONCEPTS OF ORGANISMAL AGING

2.1. Antagonistic pleiotropy

Each species, despite evident individual variation in lifespan, is observed to have a maximal, as well as a reproducible mean lifespan. The lifespan of a particular species often differs significantly from other species occupying similar evolutionary niches (1,2), suggesting that lifespan cannot solely be a product of environmental hazard, but must be under some sort of genetic control as well. However, one of the principal evolutionary questions inherent in the study of aging is how selection could favor a genetic program that promotes the demise of the individuals expressing the program.

One attempt to explain this apparent paradox is the idea of "antagonistic pleiotropy" (3). This concept, similar in some respects to the "disposable soma" idea (4), is based on the assumption that evolutionary selection has its maximal effect during early life, when reproductive facility is maximized. Thus, selection could favor retention of alleles with pleiotropic effects, which simultaneously increase fitness during early life, while reducing fitness in later life. Despite the intuitive attractiveness of this proposal, to this point there have been surprisingly few examples of genes that can be unambiguously classified as examples of antagonistic pleiotropy (5). On the other hand, selection programs in fruit flies have demonstrated that flies can be successfully bred for late-life reproduction and extended lifespan, but that such selection indeed results in a corresponding decline in early-life reproductive fitness (6,7).

Some of the best examples of individual genetic components that have the potential to behave as antagonistically pleiotropic genes are genes that regulate signals produced in the gonad which affect fertility and lifespan in nematodes (8,9) and fruit flies (10). Such mutations can also fit into the "disposable soma" paradigm, which predicts that lifespan may be dependent on the relative level of resources allocated to germ-line maintenance and fertility as opposed to maintenance of somatic tissue. According to this theory, lifespan is primarily a reflection of tradeoffs between reproductive levels in early life and late-life somatic repair and maintenance (11,12). Other experiments have demonstrated, however, that the connection between fecundity and lifespan is not absolute and that manipulation of lifespan can occur without recourse to changes in reproductive fitness (13). Another frequently cited example of a genetic pathway, which acts in an antagonistically pleiotropic fashion, is the Insulin/IGF signaling pathway, which will be discussed in the following section.

2.2. Genetic program of lifespan

In contrast to the idea that aging occurs as an accidental consequence of selection for early development and fertility is the idea that there exists a conserved genetic program to regulate lifespan across the animal kingdom (14). In this theory, the immense variety of lifespan in varying species can be accounted for in much the same way as the immense variety of morphological forms, by the careful tweaking of timing and levels of expression of highly conserved regulatory modules and by levels of circulating hormonal messengers (15). This idea is supported anecdotally by the fact that different breeds of domesticated animal species exhibit high variation in lifespan, suggesting that lifespan is itself a "trait" which can be bred for. In contrast to developmental regulation, however, genetic regulation of lifespan is still poorly understood, and inherently difficult to separate from environmental factors which have profound, but frequently stochastic, effects on an individual's lifespan.

The best example of such a genetic module for lifespan regulation is the insulin/IGF signaling pathway (16). Lowering the levels of signaling through this pathway by mutation in several different components or upstream regulators has been shown to extend lifespan in worms (17-20), flies (21-23) and mice (24-26). This pathway exerts its effects on lifespan by inactivating a conserved transcriptional regulator FOXO, also known to affect lifespan in multiple organisms (27-30). Adipose tissue appears to be a key tissue for the activity of insulin/IGF signaling to influence lifespan, as tissue specific knockdowns or overexpression of negative components in adipose derivatives increases lifespan in worms (31), flies (32,33) and mice (34).

Another source of examples of genetic regulation of lifespan comes from studies of stress response. Acute exposure to stress, termed "hormesis", can induce a transcriptional response of genes, which protect cellular macromolecules and DNA against stress-induced damage (35). Overexpression of such genes can also extend lifespan under some circumstances (36-40). These observations suggest a possible modular genetic program whereby lifespan can be set by modifying the level of expression of factors which protect cells and organs from accumulating damage. Ultimately, each of these broad attempts to explain the general process of aging begs the question of how these mechanisms actually work. Such questions have led to more specific theories of how aging occurs at the cellular level, which we discuss in the following section.

3. CONCEPTS OF CELLULAR AGING

3.1. Cellular senescence

A critical component in maintenance of somatic tissues is the preservation of DNA integrity of the mitotic cell. Somatic cells are capable of a variable, but finite, number of cell divisions after which they reach a state of terminal growth arrest, frequently termed "cellular senescence". This state can be brought on either by progressive telomere shortening, or by various forms of accumulated damage to their genetic material. Many sources of potential damage to nuclear as well as mitochondrial DNA exist. External environmental sources, such as radiation, toxins and diet, as well as toxic byproducts of cellular reactions, such as Reactive Oxygen Species (ROS), can produce damage to DNA (41,42). Such damage can produce point mutations and genomic rearrangements, which may contribute to organismal senescence. Indeed, such mutations have been demonstrated to increase with age (43). Additionally, cells of several tissue types undergo "replicative senescence", in which cells exit the cell cycle and undergo characteristic changes in morphology (44). These changes are frequently accompanied and predicted by a progressive shortening of telomeres (45) and mutations affecting telomere maintenance can accelerate aging (46,47).

Maintenance of the complicated cellular machinery dedicated to DNA repair is essential to delaying cellular senescence and one of the best characterized "progeroid" disorders in humans has been attributed to a mutation in a key upstream regulator of DNA repair, the ataxia-telangiectasia mutated kinase (ATM) (48). A complex relationship also exists between induction of the repair response and induction of the stress-induced apoptotic response (49), and it has been proposed that cellular senescence itself involves tradeoffs between protection against cancer and long-term maintenance of mitotic cell populations (50).

3.2. Oxidative stress

Another postulated determinant of the rate of aging of various species (51) is the rate at which cells undergo oxidative stress as a consequence of mitochondrial ROS generation (52). ROS are generated as a byproduct of essential biochemical reactions during cellular respiration, but can accumulate to damaging levels if produced more rapidly than antioxidant proteins such as Cu, Zn Superoxide Dismutase (SOD) and catalase can remove them. Thus, an organism with an increased activity level may be expected to accumulate higher ROS.

In support of this model, houseflies exhibit increased levels of ROS generation and protein modification, as well as a decreased lifespan (53). Interestingly, the amount of oxygen consumption by houseflies during an average lifespan is the same under different metabolic conditions, while the rate of oxygen consumption varies inversely with the length of life (54). Additionally, replicative senescence of primary cell cultures can be delayed by culturing under conditions of lowered oxygen (55) or by increasing levels of ROS-scavenging enzymes (56). The correlation with lifespan is bolstered by recent microarray experiments establishing that changes in gene expression induced by oxidative stress are broadly similar to changes in gene expression during aging (35).

Further evidence is provided by RNAi-based screens in worms for mutations which increased lifespan, as the largest class of such genes identified were genes affecting mitochondrial electron transport (57). Additionally, mutations in components of respiratory-chain complexes have been shown to extend lifespan (58). In both worms and flies, upregulation of the stress-activated JNK kinase increases both lifespan and stress resistance (40,59,60).

On the other hand, evidence also exists to suggest that the relationship between metabolic rate and lifespan is not a simple inverse relationship as the free radical-dependent "rate of living" hypothesis (61) would suggest. For example, the most consistent and well-characterized intervention that causes lifespan extension in multiple model organisms is that of "Caloric Restriction" (62). However, a regime of reduced caloric intake does not reduce, and, if anything, actually increases metabolic rate in yeast (63). Furthermore, mutations that increase lifespan in flies do not necessarily reduce metabolic rate (22,64).

In summary, each of the ideas touched on in this section has a wide experimental backing, but none can satisfactorily explain the phenomenon of aging by itself. Each of these mechanisms plays a significant role, and indeed, there are many points at which each mechanism can interact with the others. For example, the Sir2 family of histone deacetylases is known to affect both lifespan and stress resistance (62-64). This family, known as sirtuins, can also be linked to apoptosis through its interactions with p53 (65-67) and to regulation of insulin signaling through its interactions with the Foxo family of forkhead transcription factors (68-70).

Foxo, in turn, is a direct target of insulin signaling (27,29,71) and its transcriptional targets include genes necessary to respond to oxidative stress (72-75).

The fact that such genes as p53 and Foxo can be used as examples supporting the roles of antagonistic pleiotropy, conserved genetic plan, oxidative stress and cellular senescence is strong evidence that these mechanisms of aging operate in a cooperative, overlapping fashion and are far from being mutually exclusive. Perhaps the best paradigm for understanding the aging process is to view it as a complex network of ongoing changes and responses to a lifetime's accumulated internal and external threats to cellular and tissue integrity. Such a network can be theorized to consist of many linkages connected through a few critical "nodes", of which p53, Foxo and Sir2 would be examples (76,77).

Although understanding the global aging process is of immeasurable value to human health, the most immediate promise for therapeutic intervention is likely to be at the level of modulating age-related changes in specific organ function. How then does this network of mechanisms operate at the level of organ systems to cause the operative dysfunction that ultimately results in the limiting of an individual's lifespan? The next section of this review will discuss evidence for genetic and metabolic mechanisms for age-related changes in specific organs. Since cardiovascular dysfunction is the leading cause of mortality in the elderly population (78), we will focus on the heart as an operative example of such mechanisms at play in an aging organ system.

4. CARDIOVASCULAR AGING IN MAMMALS

4.1. Age-related changes in the cardiovascular system

A number of physiological changes in the cardiovascular system are associated with advanced age in humans (78-81). Increase in cardiomyocyte cell size and heart weight without changes in cardiomyocyte numbers correlate with increased incidence of left ventricular hypertrophy (82). Although stroke volume and ejection fraction are unchanged, defects in diastolic filling increase with age (83), resulting from prolonged action potential delaying relaxation (84). These changes have clear physiological import, as incidences of atrial fibrillation and congestive heart failure also increase with age, even absent evident pathology (78). Response to stimulation by exercise or catecholamines is also reduced with age (85,86), perhaps due to a degeneration of sympathetic nerve supply (87). Aging hearts are also less tolerant to transient ischemic stress (88,89).

Age-related changes in the heart also occur at the cellular level as cardiomyocytes from aging hearts display reduced contractility, with prolonged contraction duration (90). Gene expression also alters in the heart with age as a gradual switch from the alpha form to the beta form of myosin heavy chain takes place (91,92), while expression of key regulators of contractile activity, such as Troponin T and SERCA2 are downregulated (93).

Additional changes occur in both the cardiac conduction system and the vasculature of vertebrates with age, and these changes are further compounded by increased susceptibility to environmental hazard-induced pathologies, such as atherosclerosis and hypertension (79). Since these changes are not modeled well in invertebrate systems with open circulatory systems, the remainder of this review will focus on adverse age-related change in the myocardium itself. How do these changes in a single organ relate to the broad mechanistic regulation of lifespan discussed in the first section? In the following sections, we

discuss the role of two major aging mechanisms in mammalian cardiac tissue, Insulin/IGF signaling and oxidative stress.

4.2. Mechanisms of mammalian cardiac aging

The relationship between insulin/IGF signaling and cardiac performance is complex (94). The interactive axis of deterioration in metabolic control, known as the "metabolic syndrome", relates obesity, insulin resistance and cardiovascular dysfunction in a self-perpetuating web of regulatory abnormality which is a major target of ongoing research (95,96). With respect to cardiac aging, insulin/IGF signaling may have both indirect systemic effects as a result of bodily alteration in hormone concentrations and direct effects in the myocardium and vasculature. Further complicating analysis, these effects need not always act in the same direction with regard to cardiac health.

It is clear that insulin signaling is necessary for the proper growth and development of the mammalian heart, as cardiac-specific knockout of the insulin receptor gene in mice generates hearts which are reduced both in overall size and in the size of individual cardiomyocytes (97), whereas overexpression of IGF1 Receptor (98) or the downstream kinase Akt (99) increases the size of murine hearts dramatically.

The effect of such signaling on cardiac function, on the other hand, remains somewhat controversial and appears to be highly context-dependent (100). For example, dwarf mice which exhibit low circulating levels of IGF-1, GH and prolactin have dramatically extended lifespan, but exhibit reduced cardiac contractility with age (101). Conversely, IGF-1 administration has been shown to protect hearts from apoptosis following myocardial infarction (102). This protective role may be confined to damage-induced apoptosis rather than normal aging, as studies tracking markers of apoptosis in aging hearts suggest that apoptosis is not dramatically elevated in the myocardium of aging hearts, but is greatly increased in diseased hearts (103).

In addition to its role as a survival factor protecting against damage-induced apoptosis, chronic overexpression of IGF-1 in cardiomyocytes under normal physiological conditions can improve calcium uptake by the sarcoplasmic reticulum and improve diastolic function (104). However, chronic IGF-1 administration in murine hearts counterbalances such short-term benefits by eventually inducing pathological cardiac hypertrophy (105).

Cardiac-specific inhibition of the kinase PDK1, which is essential for Insulin signaling through PI3K in diverse organisms (106), generates hearts with thinner ventricular walls and enlarged ventricles which are susceptible to spontaneous heart failure (107). It should be noted, however, that such hearts have a reduction in cardiomyocyte cell size but not cell number, exactly the opposite profile seen in normal aging (81), suggesting that Insulin/IGF activity may have binary effects in the aging heart, protecting against accumulated damage, while causing changes in cell size and number which, in turn, contribute to age-induced cardiomyopathy. In support of this idea, cardiac-specific knockout of the PI3K antagonist PTEN leads to hypertrophy and also causes a decrease in contractility (108). Meanwhile, cardiac-specific knockout of the insulin receptor (97) or its downstream kinase Akt (109) reduces cardiomyocyte size postnatally, while increasing glucose uptake and cytosolic glycolysis at the expense of fatty acid oxidation, thus retaining a fetal pattern of cardiac energy utilization (97).

An additional role in age-related heart failure may come from the effect of insulin signaling on endothelial cell senescence (110). Upregulation of AKT in vascular cells decreases replicative lifespan and increases apoptosis in a Foxo-dependent manner, thus promoting atherosclerosis and, indirectly, heart failure (111). Returning to the idea of oxidative stress as a critical force in driving age-related dysfunction, decreasing AKT activity in these cells causes upregulation of ROS-scavenging enzymes, such as MnSOD, and this activity is important for promoting endothelial cell survival (111).

Indeed, the accumulation of ROS in cardiac tissue may be a major contributing factor to the aging of the myocardium as well, as canine hearts undergoing pacing accumulate ROS and activate a stress response dependent on the adaptor protein $p66^{Shc}$ which increases apoptosis of cardiomyocytes (112). Mice engineered to undergo increased levels of spontaneous mutation in mtDNA develop a syndrome of age-related symptoms including cardiomyopathy early in life (113), although, interestingly, the ROS levels are not dramatically increased.

There is likely to be significant linkage between levels of ROS accumulation and levels of insulin signaling, as caloric restriction (CR) in mice lowers oxidative stress (114) and circulating IGF-1 levels (115,116), and CR-dependent ROS reduction in rat liver tissue can be reversed by insulin treatment (117).

Thus, the role of insulin/IGF signaling in the aging heart is complex (Figure 1), with protective effects against acute damage and an essential role for postnatal growth, but also exhibiting effects on later regulation of cell size and cell number, as well as intermediary metabolism, which may contribute to the chronic age-related deterioration in function known as "senescent cardiomyopathy". Insulin/IGF signaling in the heart may thus be an example of antagonistic pleiotropy in action. Clearly, the transition from fetal to adult cardiac form and function requires insulin/IGF activity, insuring selection pressure for retaining such activity in cardiomyocytes. However, later in life, the same signaling axis seems to promote deleterious age-related changes. Additional complexity is added by the variation in activity between insulin and IGF-1 as well as the highly variable effects of various isoforms of IGF-1 and other downstream factors (118). In the last section, we will turn our attention to the role of invertebrate model systems, where fewer pathway components and a lower degree of complexity may

contribute to clarifying the role of insulin signaling in aging cardiac tissue.

4.3. Cardiac aging in invertebrate model systems

Invertebrate model systems have traditionally been employed primarily to address broad experimental questions regarding lifespan, where the tractability of such genetic models as yeast, worms and flies has been invaluable. Recently, invertebrate models have begun to show their facility for addressing questions of age-related changes in organ systems as well. This section will discuss recent work on aging hearts in fruit flies and attempt to integrate results from this simpler genetic model with what is known about aging vertebrate hearts.

In both worms and flies, valuable descriptions of age-related muscle deterioration in wild-type stocks have set the background for future work to unravel genetic combinations that may slow or reverse such muscle wasting. For example, in aging fly muscle, accumulation of ROS contributes to age-related upregulation of apoptosis (119). Meanwhile, worms have been nimbly employed to demonstrate that even genetically identical animals undergo stochastic variation in rates of muscle deterioration with age, and that nervous tissue tends to be much better preserved during normal aging than muscle (120). Well-characterized locomotor defects that accumulate with age in worms have been solidly attributed to a decline in muscle integrity, rather than an inability to respond to sensory cues (121). Interestingly, these declines are delayed in animals carrying mutations in homologs of the insulin receptor (120) or in PI3K (121). Additionally, age-related declines in the structural integrity of the epidermis have been described in worms, and resulting increases in bacterial infection have been proposed as the primary cause of death for nematodes in the laboratory (122).

Since worms do not have a fluid-pumping organ with developmental homology to the vertebrate heart, the fruit fly is the most suitable genetic model for modeling human heart disease and age-related pathology (123). Age-related changes in fruit fly cardiac function have recently been described, with changes in resting heart rate (124,125), maximal heart rate (124), rhythmicity (K. Ocorr and R.B., unpublished.) and ability to respond to external electrical pacing (23,125), showing clear age-dependent declines.

Since insulin/IGF signaling plays a complicated role in aging human heart performance (see above), the simpler fly system which only has one insulin family receptor (21), which plays diverse roles relating regulation of size, nutrient intake and lifespan (15,16), was employed to examine the direct role of insulin signaling in cardiac tissue. Heart-specific overexpression of the Insulin Receptor hastens the increase in pacing-induced failure rate in young flies, while reducing insulin signaling in the heart by cardiac-specific overexpression of the PI3K antagonist PTEN or of Foxo prevents any perceptible age-related decline in heart function (23). Reduction of levels of circulating insulin-like peptide cause developmental delays and high early mortality, but produce flies which are both long-lived and maintain youthful levels of cardiac performance at advanced ages (23). Significantly, cardiac-specific interventions affecting insulin signal transduction in flies do not affect overall lifespan (23), suggesting insulin signaling controls lifespan through systemic circulatory effects, but that there exist separate Insulin Receptor-dependent mechanisms, acting autonomously within single organs in parallel to systemic effects, which regulate the "aging" rate of those organs.

In both flies and vertebrates, insulin action on cardiac tissue appears to involve production of secondary hormones, since hyperglycemia-induced cardiac dysfunction in vertebrate cell culture can be reversed by treatment with 17beta-estradiol (126) or other isoflavones (127). In flies, the abrogation of cardiac age-related decline caused by mutation in the Insulin Receptor substrate homolog *chico* can be reversed by treatment with the sesquiterpenoid Juvenile Hormone analog methoprene (R.J.W., M. Tatar and R.B., unpublished).

The relationship between cardiomyocyte growth and cardiac aging may also be conserved between flies and vertebrates. In vertebrates, cardiomyocyte size increases with age while numbers decrease (82). Meanwhile, in flies, while no evidence exists for changes in cell number with age, inhibition of growth-promoting genes such as S6K and TOR, improves cardiac function with age, while overexpression of the translation initiation factor eif4E hastens age-related cardiac deterioration (R.J.W. and R.B., unpublished), suggesting that activation of the growth program in cardiomyocytes may be a causative agent in the age-related decline in cardiac function.

When comparing data from flies and vertebrates, it is possible to hypothesize that in vertebrates, which undergo significant postnatal growth, insulin signaling is necessary for proper size control of the cardiac tissue. In addition, IGF-1 seems to perform a critical task in promoting regeneration and replacement of damaged tissue through its roles as a mitogen and cell survival factor. In addition to these positive roles, insulin signaling may also be involved in allocation of resources to somatic maintenance as opposed to growth (Figure 1). In this context, insulin signaling is likely to contribute to age-related changes that lead to "senescent cardiomyopathy", characterized by gradual changes in energy utilization, increases in ROS-induced stress responses and apoptosis.

In flies, where no post-natal growth exists and the number of cardiac cells is determined during metamorphosis, the positive roles in adult cardiac maintenance are not observed, leaving the fly heart as a clear, simplified model for regulation of cardiac metabolism during aging by insulin signaling (Figure 1). Indeed, genetic screens in flies have recently been employed to identify mutations, which improve late-life cardiac performance (R.J.W. and R.B., unpublished).

Using the fly heart to understand the relationship between changes in Insulin signaling and metabolic changes in cardiac tissue will be one of many ways in

which invertebrate models will continue to contribute to the understanding of aging mechanisms in the heart and elsewhere.

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Send correspondence to: Dr. Rolf Bodmer, The Burnham Institute of Medical Research, Center for Neuroscience and Aging, 10901 North Torrey Pines Read, La Jolla, CA 92037, USA, Tel: 858-713-6295, Fax: 858-713-6293, E-mail: rolf@burnham.org

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