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Review

Aging and the Krüppel-like factors

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ABSTRACT

The mammalian Krüppel-like factors (KLFs) are a family of zinc-finger containing transcription factors with diverse patterns of expression and a wide array of cellular functions. While their roles in mammalian physiology are well known, there is a growing appreciation for their roles in modulating the fundamental progression of aging. Here we review the current knowledge of Krüppel-like factors with a focus on their roles in processes regulating aging and age-associated diseases.

KEYWORDS: KLF, Krüppel-like transcription factors, aging, chronic disease, aging-associated disease, lifespan, healthspan.

1. Introduction

1.1. Biologic regulation of aging

Mammalian lifespan is limited. Over time, risk of mortality increases, as well as risk of age-associated debility and disease. Although this process is complex and heterogeneous, it is not entirely passive. Long-lived organisms share common molecular and cellular features, and an extension of lifespan can be coupled to extension of time spent free of age-associated disease (healthspan) [1, 2]. Caloric restriction, the most robust intervention known to extend lifespan, extends both lifespan and healthspan of rhesus monkeys [3, 4]. Further, in recent decades, genetic approaches in short-lived model organisms including the budding yeast *Saccharomyces cerevisiae*, nematode *Caenorhabditis elegans*, and fruit fly *Drosophila melanogaster*, have linked particular cellular signaling pathways to aging. For example, insulin/insulin-like signaling (IIS), the target of rapamycin (TOR) pathway, and AMP-activated protein kinase (AMPK) have all been implicated as modifiers of the aging process, as the activity of these pathways has been shown to alter lifespan and health of organisms across phylogeny [5-12].

Over time, aging organisms display characteristic changes in genomic maintenance, systemic inflammation, proteostasis, stem cell maintenance, mitochondrial health, epigenetic modifications, resistance to oxidative stress, susceptibility to development of senescence, and metabolic pathways [13]. These cellular changes likely contribute to tissue dysfunction over time, leading to features of organismal aging which may be clinically observable. These include impairments in hearing and vision, glucose intolerance, decline in bone density and exercise capacity, deterioration of cognitive function, alterations in blood pressure and sympathetic activity, decreased immune function, and changes in renal and pulmonary function. Coincident with these changes is a dramatic rise in age-associated pathology, notably cardiovascular and neurodegenerative diseases [14-16]. Studies using DNA methylation prediction methods suggest that aging does not occur at the same rate across tissues [17] and is non-cell autonomous, as systemic inflammation and circulating factors can affect aging in every organ in the body [18, 19].

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The Krüppel-like factors (KLFs) are a family of transcriptional regulators with a C-terminal DNA-binding domain containing three C_2H_2-type zinc fingers recognizing a 5'-C(A/T)CCC-3' sequence as well as other GC-rich sequences. While this domain is well-conserved across the family, the N-terminal regions are much less so, allowing diverse protein-protein interactions as well as transactivation or repression. There are at least 18 mammalian KLFs with roles in nearly every major organ system regulating an array of cellular functions [20]. Recently, using the model organism *Caenorhabditis elegans*, two of the three nematode KLFs have been shown to be regulators of lifespan [21, 22], providing the first evidence linking the KLFs to longevity and directing attention towards the question of whether functions controlled by mammalian KLFs might have similar effects on mammalian age-related health and longevity. While formal analyses of the KLFs and their influence on mammalian lifespan have yet to be performed, the KLFs regulate many hallmark molecular and cellular features of aging and have been implicated in mammalian diseases of aging (Figure 1). In this review, we discuss KLF regulation of several of these recently identified features of aging and briefly survey roles in age-related disease.

2. Maintenance of genomic integrity and cancer

2.1. Telomere attrition

Telomere attrition over time contributes to cellular senescence and organismal aging. Telomeres are complexes at the terminal ends of DNA strands, which allow the cell to distinguish them from free

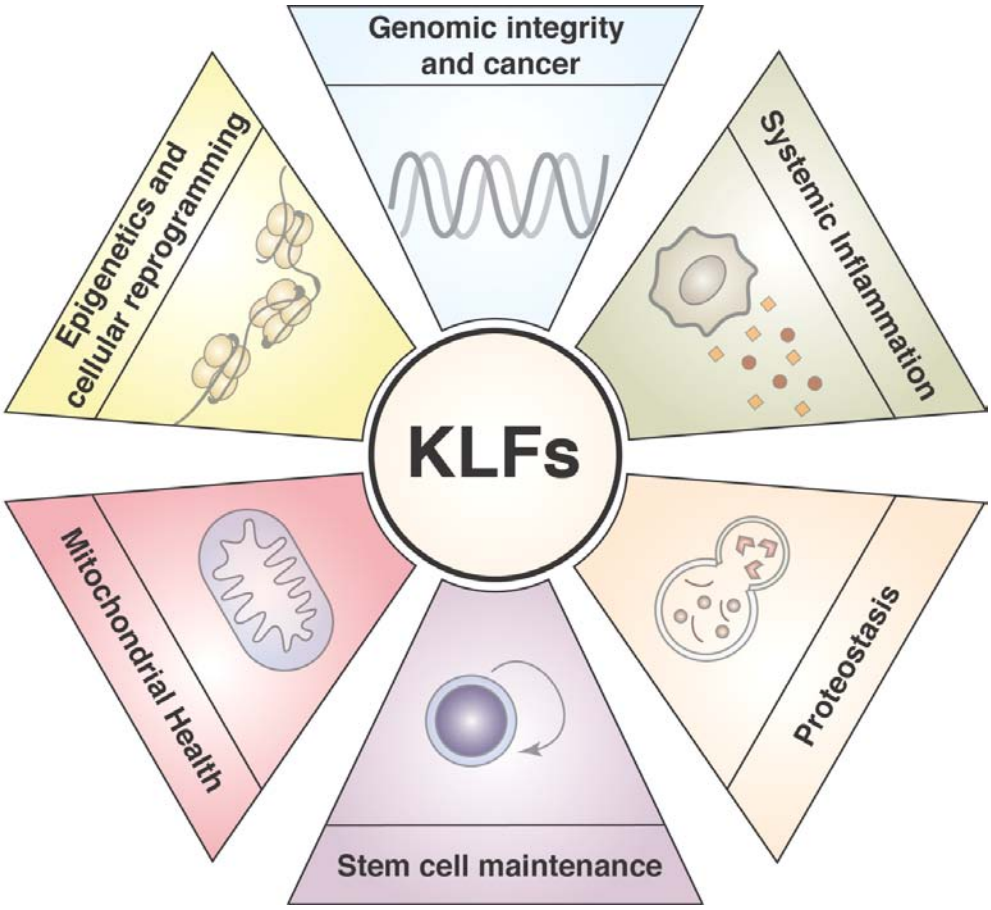

Figure 1. Krüppel-like factor regulation of aging. This review discusses the current state of knowledge regarding aging and the KLFs.

ends generated upon a DNA double strand break and avoid initiating DNA repair mechanisms [23, 24]. They consist of telomere-specific G-rich tandem repeats (in humans TTAGGG) in physical association with multiple DNA-binding proteins. Due to the inability of DNA polymerase to replicate to the very ends of DNA strands, telomeres are replicated *via* activity of telomerase, which utilizes an RNA template to repetitively add the telomeric sequence to the ends of genomic DNA. This enzyme is active in embryonic stem cells and many types of cancer, but not in somatic cells [25, 26]. Therefore, cells with low expression of telomerase over a period of time eventually may experience telomere shortening, which can contribute to organismal aging through complex mechanisms including direct activation of apoptosis or defects in stem cell renewal [27].

KLF4 is a direct transcriptional regulator of the telomerase reverse transcriptase (TERT) [28]. In human cells, the *Tert* promoter contains a KLF4-binding site near its transcriptional start site, and overexpression or knockdown of KLF4 correspondingly altered mRNA transcript levels of *Tert* [28]. In embryonic stem cells and cancer cells, KLF4 interacts with β-catenin and poly(ADP-ribose) polymerase 1 to control *Tert* expression [29, 30]. In T cells, KLF2 has also been shown to repress *Tert* expression; this repression is relieved upon T-cell activation [31].

2.2. Genomic stability

In addition to regulation of TERT, KLFs have roles in maintaining genomic stability. KLF4 is crucial for p53-mediated cell arrest [32], and it differentially regulates the expression of several cell cycle checkpoint proteins, including the cyclin dependent kinase inhibitor 1A (CDKN1A), cyclin B1, and cyclin D1 in response to DNA damage [33-35]. Further, *Klf4* null mouse embryonic fibroblasts exhibit centrosome amplification, numerous chromosomal aberrations, and aneuploidy, which can be rescued upon re-introduction of *Klf4*, and KLF4 is protective against γ-irradiation-induced damage *via* inhibition of cyclin E [36-38]. KLF5 suppresses expression of the CDK inhibitor p27 and therefore promotes cell proliferation and oncogenesis in a triple negative breast cancer cell line [39, 40]. Conversely, KLF6 positively regulates CDKN1A to inhibit cell proliferation in prostate cancer [41]. In a human endometrial epithelial cell line, KLF9 has been shown to upregulate CDKN1A [42]. KLF14 also has roles in protecting the genome, as its deletion in a mouse leads to centrosome amplification and aneuploidy while promoting spontaneous tumorigenesis through its regulation of polo-like kinase 4 [43].

2.3. Cancer

Genomic instability and telomere shortening, which are integral to the aging process, have long been implicated in cancer [44, 45]. Cancer is gaining increasing recognition as an age-associated disease; the incidence of cancer rises dramatically after sexual maturity [46]. Indeed, KLF regulation of these processes is reflected in the abundant literature surrounding roles for the KLFs in tumorigenesis or tumor suppression, although direct mechanistic links are still being established. Numerous members of the family have complex roles in cancers; these roles have been elegantly reviewed in detail [47]. In particular, several KLFs (KLF4, KLF5, KLF6, KLF10, KLF13) exert their influence on cancer by targeting genes involved in regulating the cell cycle and therefore cell proliferation [47]. For example, KLF4 functions mainly as a tumor suppressor. In colorectal cancer, elevated activity of von Hippel-Lindau (pVHL) protein degrades KLF4 and reduces expression of its target gene p21, leading to escape from cell cycle arrest, while expression of a mutant KLF4 lacking pVHL ubiquitylation sites greatly reduces colony formation *in vitro* in a colorectal cancer line [48]. *KLF4* transcript levels are low in multiple types of tumors, and KLF4 is anti-proliferative in cervical carcinomas, pancreatic cancer, bladder cancer, gastric cancer, and lung cancer [49-57]. However, reflective of its complex role in cancer, KLF4 has been reported to be overexpressed in breast cancer and squamous-cell oropharyngeal cancers [58, 59], and in specific contexts such as genetic p21 inactivation or ectopic expression of mutant RAS^{v12}, KLF4 induces expression of p21 and represses p53 [60]. Splice variants of KLF4 can also be oncogenic. KLF4α is upregulated in pancreatic cancer cell lines and increased KLF4α expression enhances *in vivo* tumor formation [61].

3. Systemic inflammation and inflammatory disorders

Chronic, systemic inflammation in the absence of infection, also termed inflammaging, is a hallmark of aging. It is characterized by elevated levels of interleukin 6 (IL-6), IL-1β, and tumor necrosis factor-α (TNF). Inflammaging is also mediated by transcriptional regulators such as nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), as blockade of NF-κB activity removes markers of aging and extends mouse lifespan and healthspan [62-64]. Several factors contribute in a complex fashion to inflammaging, including responses to endogenous damage-associated molecular patterns (advanced glycation end-products, free radical modified proteins, etc.), mitochondrial activation of the Nlrp3 inflammasome, cellular senescence and secretion of proinflammatory cytokines (the senescence-associated secretory phenotype), and alterations in coagulation pathways and immunity [65-73]. Chronic inflammation has been linked to the acceleration of age-related diseases such as type 2 diabetes, atherosclerosis, and degenerative arthritis [74, 75].

3.1. KLF regulation of inflammation

The Krüppel-like factors are expressed in various cells of the innate and adaptive immune system. Several KLFs have pro- or anti-inflammatory functions in myeloid cells, including KLF1, KLF2, KLF3, KLF4, KLF5, KLF6 and KLF10 [76-79]. In particular, KLF2, KLF4, and KLF6 have established roles in macrophage inflammatory gene expression. KLF2 is a negative regulator of monocyte activation and inhibits the activity of NF-κB and activator protein 1 [80]. As a result, mice with myeloid-restricted loss of KLF2 have higher plasma levels of IL-1β and TNFα [81]. *In vivo*, KLF4 modulates macrophage polarization, cooperating with STAT6 to induce expression of arginase-1, the mannose receptor, and resistin-like α, while loss-of-function of KLF4 enhances expression of TNF-α, COX-2, MCP-1, and RANTES [82]. KLF6 loss *in vitro* and *in vivo* strongly reduces the induction of pro-inflammatory genes such as IL-1α, IL-1-β, and TNF-α by lipopolysaccharide (LPS) [83, 84].

Dendritic cells (DCs) function both as antigen-presenting cells to facilitate T cell education and activation and as major producers of cytokines and chemokines. Recent studies have identified KLF2 and KLF4 as mediators of DC functions. KLF4 is expressed in DCs; its depletion impairs development of pre-classical DC progenitors in the bone marrow, and its presence is required for maintenance of $CD11c^{hi}$ DCs in the spleen [85, 86]. KLF4 in DCs influences adaptive immunity, as it has been shown to be required for type 2 helper T cell responses to pathogens such as *Schistosoma mansoni* [86]. Importantly, KLF4 in DCs contributes to systemic inflammation *via* production of IL-6 and may drive differentiation of inflammatory DC subtypes [87, 88].

3.2. Arthritis, atherosclerosis, and metabolic disease

The functional consequences of KLF regulation of inflammation are most well known in the contexts of arthritis, atherosclerosis, and metabolic disease; the incidence of these diseases increases with age. KLF2 regulation of monocyte activation has functional consequences as methylated-bovine serum albumin and IL-1β induced arthritis is exacerbated in KLF2 hemizygous mice [89]. Additionally, KLF2 deletion in the myeloid compartment exacerbates atherosclerosis in an LDLR null mouse model [90]. In an ApoE null model of atherosclerosis, loss of myeloid KLF4 also increases atherosclerotic lesion burden [91]. Macrophage KLF4 also has roles in metabolic syndrome; *KLF4* levels in macrophages isolated from human adipose tissue is correlated with adiponectin and obesity [82]. In these same patients, *KLF4* levels in visceral fat were found to be lower than in subcutaneous fat [82]. In mouse models, KLF4-deficient macrophages have a higher glucose intake, and mice with myeloid-specific deletion of KLF4 gain more weight on high-fat diet and develop insulin resistance [82]. Further, myeloid KLF4 deficiency delays wound healing due to increased iNOS and TNF-α while not affecting cell migration [82]. Finally, DCs with loss of KLF2 express higher levels of CD40 and CD86, and mice with DC-specific deletion of KLF2 (*Itgaxcre-cre* mice) in a model of atherosclerosis develop larger atherosclerotic lesions, but without an increase in macrophage content within the lesions [92].

4. Proteostasis

Homeostasis of the proteome, or proteostasis, is crucial to the health of the organism and requires constant surveillance by the cell. The cell achieves proteostasis *via* mechanisms including control of translational efficiency, autophagy (primarily for the degradation of protein aggregates), proteasome-mediated degradation (degradation of misfolded proteins), and molecular chaperones (assisting in protein folding) [93]. An aging-associated decline in a functioning proteome results in the accumulation of misfolded proteins, which contribute to a wide array of pathology, neurodegeneration (Alzheimer's, Parkinson's, Huntington's diseases) being particularly well-recognized [94].

4.1. Autophagy and molecular chaperones

In *C. elegans*, increased activity of either *klf-1* or *klf-3* not only extends nematode lifespan but also delays the appearance of age-associated phenotypes such as a decline in locomotory speed [22]. This lifespan extension is mediated through KLF regulation of autophagy, and this function is conserved by mammalian KLF4. KLF4 directly regulates genes involved in the autophagy molecular machinery across multiple steps in the pathway, and this broad transcriptional regulation of autophagy by KLF4 occurs in the cardiovasculature [22, 95]. In mouse embryonic fibroblasts and a multiple myeloma cancer model, loss of KLF4 leads to reduced autophagy [96-98]. In endothelial cells, KLF2 and KLF4 have also recently been shown to regulate autophagy, potentially in response to laminar shear stress [22, 99]. In the liver, KLF6 has also been shown to be a positive regulator of autophagy related genes *Atg7* and *Becn1* [100].

Several KLFs regulate the expression of molecular chaperones. In BALB/c 3T3 cells, KLF6 binds to a cis-acting element in the first intron of the collagen-specific *Hsp47* gene to regulate its expression [101]. Recently, KLF4 has also been shown through gain and loss-of-function studies to affect expression of heat shock proteins 84 and 86 (the two versions of heat shock protein 90) and heat shock cognate 70 in C2C12 and RAW264.7 cells and is upregulated by heat shock transcription factor 1 in response to heat stress [102-104]. Finally, during epidermal keratinocyte differentiation, the unfolded protein response is strongly activated simultaneously with increases in *Klf4* mRNA transcript levels and treatment with ER stress-inducing reagents such as tunicamycin upregulates *Klf4* [105].

4.2. Vascular aging and heart failure

Proteostasis is linked to numerous aging-associated diseases [94]. KLF regulation of autophagy has been linked to vascular aging, as a transgenic mouse overexpressing KLF4 in an endothelial cell-specific manner experiences delayed endothelial senescence and improved vascular reactivity with age [22]. Importantly, the maintenance of vascular reactivity with age was abolished with blockade of autophagy by chloroquine [22]. Additionally, KLF4 is a broad regulator of autophagy-related genes in cardiomyocytes, and in a model of heart failure, loss of KLF4 exacerbated cardiac dysfunction [95]. The contribution of the endothelium to organismal aging is an important question which is recently attracting attention. With aging, an increasing number of endothelial cells undergo senescence and secrete soluble, usually pro-inflammatory factors (IL-1, IL-6 and IL-8) which contribute to low-grade systemic inflammaging and the development of age-associated cardiovascular disease [106]. KLF2 and KLF4 have well-known functions in the endothelium as anti-inflammatory, antiadhesive, and antioxidant factors, and overexpression of KLF4 in the endothelium is protective against atherothrombosis. Whether these functions are dependent on KLF regulation of autophagy remains to be seen [107-110].

5. Stem cell maintenance and regeneration

Stem cells experience a decrease in regenerative capacity with age, as well as dysregulation of self-renewal mechanisms, potentially leading to depletion of the stem cell pool or skewed lineage commitment. This has several consequences including a reduced immune response (immunosenescence) as circulating immune cells are not replenished [111], an increased susceptibility to development of cancers, a reduction in osteogenic capability leading to osteoporosis and poor fracture healing [112], and reduced muscle response to injury, among others [113, 114].

5.1. Intestine, skin, breast, and muscle

The KLFs regulate stem cell renewal in a variety of tissues. In the intestinal crypt, proliferating stem cells express KLF5, which controls stem cell maintenance and proliferation [115]. In addition, KLF5 regulates epithelial differentiation and migration expression in part through regulation of genes including Ki-67, cyclin B, cyclin-dependent kinase 1 and cyclin D1 [115]. As a result, intestine-specific loss of KLF5 leads to neonatal lethality due to impaired epithelial barrier function [116-119]. KLF4 is expressed in the differentiated compartment of the intestinal epithelium and serves to arrest growth and maintain those cells in a terminally differentiated state [115]. Additionally, both KLF5 and KLF4 are implicated in the development of intestinal cancers [57, 60, 120-123]. In the skin, KLF4 also contributes to epithelial barrier integrity [124] and is expressed in hair follicle bulge stem cells. Its loss has been shown to inhibit cutaneous wound healing, suggesting a role in maintaining stem cell numbers in this niche [125], and lowered expression of KLF4 has been correlated with incidence of squamous cell carcinoma and basal cell carcinoma [126]. KLF4 expression is also elevated in mammary gland stem cells [127] and hematopoietic stem cells [128]. In muscle, KLF5 is induced after injury in differentiating myoblasts, and satellite cell-specific loss of KLF5 impairs muscle regeneration [129].

5.2. Hematopoiesis

A number of KLFs are major regulators of aspects of hematopoiesis. KLF1 is restricted to erythroid cells and promotes erythropoiesis while inhibiting megakaryopoiesis [130, 131]. KLF2 is also expressed in erythroid cells and KLF1 and KLF2 promote erythropoiesis through regulating embryonic β-like globin gene expression [132, 133]. In thymocytes, KLF4 represses CDKN1b/$p27^{Kip1}$ to decrease thymocyte proliferation [134]. Loss of KLF6 in mouse embryonic stem cells reduces their capacity to differentiate into hematopoietic and vascular cells, although the mechanism remains unclear [135]. KLF7 is expressed in hematopoietic progenitors, and overexpression of KLF7 suppressed myeloid progenitor cell growth while sparing T cells [136]. Finally, in human bone marrow stromal cells, overexpression of KLF2 increases cell proliferation and upregulation of *Oct4*, *Nanog* and *Rex1* [137].

5.3. Embryonic stem cells

In mouse embryonic stem cells, KLF2, KLF4, and KLF5 are recognized to be involved in maintaining a pluripotent state, and they form an internal regulatory circuit by binding to promoter regions of *Oct4*, *Sox2*, and *Nanog*, which then bind to promoters of the KLFs [138, 139]. *Oct4* regulates KLF2, while the leukemia inhibitory factor/Stat3 pathway regulates KLF4 expression. Expression of KLF2 or KLF4 in postimplantation embryo-derived, epiblast-derived stem cells restores naïve pluripotency [140]. KLF4 itself has well known roles in embryonic stem cell differentiation and self-renewal [141, 142]. Recently, acetylation status of KLF5 has been shown to suppress expression of genes related to differentiation and enhance the ability of KLF5 to maintain pluripotency in mouse embryonic stem cells [143]. Interestingly, expression profiling of mouse embryonic stem cells undergoing differentiation identifies expression changes in nearly all the members of the KLF family, an observation which may be explained by competition by each KLF for occupancy of the same promoter regions in genes determining self-renewal [144].

5.4. Nerve regeneration

An intriguing role for the KLFs in regulating axon growth of central nervous system neurons has been described [145]. KLF6 and KLF7 promote neurite growth, while nine KLFs (KLF1, KLF2, KLF4, KLF5, KLF9, KLF13, KLF14, KLF15, and KLF16) suppress it [146, 147]. Schwann cells overexpressing KLF7 grafted into mice improve sciatic nerve regeneration and enhance myelination after nerve injury [148] and overexpression of KLF7 engineered to be transcriptionally active promote regenerative axon growth in cortical slice cultures after axon injury [149]. Further investigation into KLF regulation of stem cell renewal in the context of aging will improve efforts to delay the effects of aging on stem cell maintenance.

6. Mitochondrial health

The role of mitochondria in aging has been proposed for decades, primarily through the

mitochondrial free radical theory of aging [150]. This theory postulates that the necessary result of aerobic metabolism is the release of highly reactive oxygen species (ROS) by mitochondria. Over time ROS can cause oxidative damage to diverse types of molecules in the cell leading to age-related dysfunction. In recent years, this view has fallen out of favor and increasing attention has been drawn to other roles of mitochondria in aging, namely ROS localized in the mitochondria, the role of mtDNA mutations, and the important influence of mitochondria in conserved nutrient sensing pathways known to influence longevity [151]. Because of the central role of mitochondria in cellular homeostasis and metabolism, mitochondrial function and its decline are implicated in the pathogenesis of nearly every age-related disease.

Krüppel-like factors have a critical role in the maintenance of mitochondrial health and function. Within the kidney, mitochondrial health has been linked to several glomerular pathologies, including congenital human nephrotic syndrome, collapsing focal segmental glomerular sclerosis, and adriamycin-induced nephropathy [152-155]. Podocyte-specific loss of KLF6 leads to the appearance of dysmorphic mitochondria and reduced expression of genes involved in mitochondrial replication, such as *Nrf1*, *Polrmt*, and *Tfam* as well as other genes involved in mitochondrial function [156]. Further, these mice were more susceptible to adriamycin-induced injury to the kidney. In humans with focal segmental glomerular sclerosis, KLF6 expression is lower [156]. In the heart, loss of KLF15 leads to formation of megamitochondria, suggestive of a defect in cellular control of mitochondrial fission [157]. Additionally, cardiomyocyte KLF4 regulates mitochondrial biogenesis, dynamics, and energetics in part *via* synergistic interaction with estrogen-related receptor α and PPARγ coactivator 1α [95]. Mice with early (E9.5) cardiac-specific deletion of KLF4 demonstrated 50% mortality two weeks after birth and surviving mice displayed reduced mitochondrial volume density, increased fragmentation, and a 30% decrease in mitochondrial genomic DNA content [95]. These mice also had severely reduced cardiac contractile function, presumably due to the requirement of mitochondrial biogenesis for cardiac adaptation to postnatal developmental conditions [95]. In mice with KLF4 deletion after birth, KLF4 deficiency resulted in an inability to adapt to pressure overload induced by transaortic constriction, and mice aged 9 months exhibited reductions in cardiac contractile function compared to control mice [95].

7. Epigenetics and cellular reprogramming

Epigenetic changes, or regulated alterations in gene expression independent of changes in DNA sequence (e.g. DNA methylation, post-translational modification of histones), function as a layer of regulation in nearly every aspect of biology. The importance of these changes in the aging process has recently been recognized. Particular changes in single epigenetic marks and broader changes to the epigenetic landscape have been identified as being highly associated with aging [158], and manipulation of these marks or the enzymes responsible can modulate lifespan in model organisms like *C. elegans* [159-163]. The reversal of these changes through cellular reprogramming approaches modulates health and lifespan. With the identification of four transcription factors, Yamanaka factors (*Oct3/4*, *Sox2*, *Klf4*, *c-Myc*), capable of returning cells to a pluripotent state, many have recognized that this reprogramming process necessarily removes chromatin marks, including those associated with age [164, 165]. This has led to efforts to delay aging by intervening at the level of epigenetic regulation, while avoiding the tendency of *in vivo* dedifferentiation to cause formation of teratomas or other tumors [166-168]. In this respect, KLF4 is perhaps the most well-known of the KLFs and has been used in many reprogramming approaches. As with *Oct4* and *Sox2* (but not *c-Myc*), *Klf4* acts as a pioneer factor, accessing target sites in areas of DNaseI-resistant, unmodified, “silent” chromatin to activate transcription [169]. Indeed, short-term, cyclic systemic expression of *Oct4*, *Sox2*, *c-Myc* and *Klf4* restores epigenetic markers of aging such as levels of H3K9me3 and H4K20me3 while improving age-associated tissue decline and extending lifespan in a murine progeria model [170].

8. Concluding remarks

Modern efforts to understand aging couple insights from short-lived model organisms with observations from higher order mammals. The Krüppel like factors are relatively new to the field of aging. Their roles in diverse mammalian processes have been fruitful areas of investigation, yet until recently, they have not been evaluated as factors regulating the aging process. We propose that many of the functions of the KLFs, previously viewed through the lens of a particular physiologic or disease process, in fact modify the fundamental progression of aging. The KLFs therefore deserve consideration and further investigation as bona fide aging regulators, contributing to the burgeoning body of knowledge on biologic regulation of aging.

While hallmarks of aging are emerging, it remains to be seen whether these diverse molecular, cellular, and organismal features can or ought to be unified under an overarching theory. Pathways implicated in the biologic regulation of aging likely cooperate, perhaps synergistically and in complex fashion, to modulate aging. This must be reconciled with the partly stochastic nature of aging and the role of environmental factors. Future investigations will benefit from systems approaches to provide a holistic view of aging.

Additionally, the mechanistic links between processes promoting aging and age-related chronic disease are far from clear. Although it is widely accepted that many chronic diseases share age as the predominant risk factor, merging basic knowledge of aging with chronic disease research remains nontrivial. A central challenge remains: how to leverage our understanding of the underlying mechanisms of the aging process to produce an intervention which can prolong health and lifespan in humans. Indeed, studies of aging raise the exciting possibility that this type of intervention can simultaneously modify the progression of multiple age-associated diseases at once by targeting their common primary risk factor, age. Already, several approaches are yielding results in this area, including IIS inhibition (e.g. metformin), dietary regimens (e.g. periodic fasting), rapamycin or other mTOR pathway inhibitors, AMPK activators, sirtuin activators, and even inhibitors of Ras-Erk-ETS signaling (e.g. trametinib) [171, 172]. In this respect, the KLFs may represent attractive targets. Many of the agents identified thus far correspondingly induce or suppress KLF expression depending on their effects, such as metformin [173], fasting [174], rapamycin [175], the AMPK activator AICAR (5-amino-1-β-D-ribofuranosyl-imidazole-4-carboxamide) [176], and the sirtuin activator resveratrol [177], and importantly, the KLFs have been shown to be broadly required for lifespan extension mediated through many of these targeted pathways [22].

Finally, aging researchers are bringing increased scrutiny to the way medical research funding and clinical medicine are structured. Currently, novel treatments are developed for a single disease in isolation, while the treatment of multiple comorbidities in an aging population is increasingly complex. Combating chronic disease therefore may require stepping towards an integrated approach which recognizes the overlapping pathology and interdependent nature of many age-associated chronic diseases. Devising novel scientific funding and clinical strategies to target a fundamental, shared driver of chronic disease, aging, will provide enormous insight into diseases which represent some of the greatest threats to human health of our times.

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CONFLICT OF INTEREST STATEMENT

The authors disclose no conflicts of interest.

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