Introduction

Growth hormone (GH) and insulin like-growth factor-I (IGF-I) play a central role in normal growth and development in humans, as indicated by the fact that GH deficiency and states of GH resistance, severe growth failure is observed in humans. In contrast, excess GH, and the concomitant increase in circulating IGF-I levels, results in acromegaly, which is associated with tissue overgrowth, comorbidities, including hypertension, diabetes, heart failure, increased susceptibility to certain types of cancer, and increased mortality. It is well known that decreased insulin/IGF-I signaling is associated with an extended lifespan. Although it has been shown that, downstream of insulin/IGF-I receptor signaling, the transcription factor FOXO plays important roles in the regulation of lifespan, the underlying mechanisms have not fully been elucidated. Recently, it has been reported that IGF-I induces reactive oxygen species (ROS) generation and enhances cellular senescence via ROS and the tumor suppressor protein p53. Given that cellular senescence is closely related to the development of age-related diseases such as atherosclerosis, diabetes, and heart failure, and that cellular senescence plays an important role in the aging process itself, it is suggested that IGF-I-induced cellular senescence may explain, at least in part, increased comorbidity and mortality of acromegaly, and the mechanisms underlying longevity.

KEY WORDS: IGF-I, longevity, ROS, cellular senescence, acromegaly

Insulin/IGF-I and longevity

Components of the GH and IGF-I pathways have been shown to affect processes involved in aging and longevity. In Caenorhabditis elegans, mutations that decrease the activity of daf-2, which encodes a hormone receptor similar to the insulin and IGF-I receptors, more than double the lifespan, and mutations affecting the downstream phosphatidylinositol 3-kinase (PI3K)/AKT/PDK kinase cascade extend life span as well. Inhibition of insulin/IGF-I signaling extends lifespan through DAF-16, a FOXO transcription factor; the heat-shock transcription factor; and SKN-1, an Nrf-like xenobiotic-response factor. These transcription factors regulates diverse genes that act cumulatively to produce large effects on lifespan. Downstream genes shown to be functionally significant, including stress-response genes such as catalase, glutathione S-transferases, and metallothioneins, as well as genes encoding antimicrobial peptides, chaperones, apolipoproteins, lipases, and channels.

The effect of the insulin/IGF-I pathway on lifespan has been evolutionarily conserved. In Drosophila, inhibiting insulin/IGF-I signaling or increasing activity of FOXO extends lifespan. In mammalian systems, growth and glucose metabolism are regulated by two independent pathways, with IGF-I and insulin having separate receptors, suggesting that these pathways are more complicated. Nevertheless, a series of genetic manipulations in the mouse have provided evidence that these pathways affect longevity and aging in mammals. Mutations that
result in GH deficiency or impair the GH receptor caused reduced body size, lowered IGF-I and insulin levels, increased stress resistance, and an extended lifespan. Heterozygous deletion of the IGF-I receptor gene in mice causes a modest reduction in size, improved stress resistance, and extended lifespan in females only. Disruption of insulin receptors in adipose tissue in mice results in an extended lifespan in both sexes. Knockout of the downstream signaling adaptor protein insulin receptor substrate 1 (IRS1), which is a major effector of both insulin and IGF-I, also resulted in increased longevity in female only, irrespective of mild insulin resistance. Intriguingly, irs-2-/- mice were short–lived, whereas Irs1+/- and Irs2+/- mice of both sexes showed normal lifespans. In humans, a 22-year-cohort study in Ecuadorian individuals with a mutation in GHR revealed that GHR deficiency is associated with a major reduction in pro-aging signaling, cancer, and diabetes, suggesting that pathways involved in the control of aging and disease burden are evolutionarily conserved in humans.

In vitro studies have demonstrated that insulin/IGF-I may affect lifespan through several mechanisms. Calorie restriction (CR) is recognized as one of the most successful means of delaying aging. Interestingly, many phenotypic characteristics of animals subjected to CR are shared by many, but not all, animals, with repression of activity of the GH/IGF-I axis. CR results in reduced body weight, reduced GH and IGF-I levels, decreased plasma levels of insulin and glucose, reduced fertility, and delayed puberty. Several studies have attempted to distinguish whether the mechanisms by which CR and decreased activity of GH/IGF-I axes lead to increased longevity are distinct or overlapping by using CR on dwarf mouse models. Intriguingly, when long-lived Ames dwarf mice, which are deficient in GH, PRL, and TSH because of PROP1 deficiency, were subjected to CR, both mean and maximal lifespan were increased as compared to non-dwarf controls subjected to CR or Ames dwarf mice fed ad libitum. This suggests that CR and the effects of IGF-I are controlled by distinct mechanisms. Moreover, specific genes associated with insulin signaling were reported to be altered differently when Ames dwarf mice were subjected to CR. However, when GHR-deficient mice were subjected to CR, mean lifespan was unchanged in both males and females, and only a slight impact was observed in maximal lifespan of females. Although this may suggest that GH resistance and CR operate via similar mechanisms, the results of gene expression profiling suggest that some differences exist. A comprehensive study that simultaneously compared hepatic gene expression in Ames, Snell, GHR−/−, and lit/lit mice, subjected to CR showed different gene expression profiles between these groups and it has been suggested that GHR−/− mice are not merely mimetics of CR animals. However, co-existence of reduced insulin levels and enhanced insulin sensitivity are among the most prominent endocrine features shared by these mice, which were subjected to CR and were deficient in GHR signaling. In addition, studies of genetic models resulting in form of GH resistance such as Laron dwarfism and GH deficiency due to GHRH receptor mutations demonstrated increased serum levels of adiponectin. These results suggest that enhanced insulin sensitivity and elevated adiponectin levels are attributable to extended lifespan. At the molecular level, FOXO3A, a family of FOXO transcription factor, may play an important role downstream of insulin/IGF-I signaling in mammals. FOXO3A can act as a brake on cell cycle progression and as a stimulus to activate DNA repair mechanisms, and thus potentially limit tissue aging due to cell loss and perhaps decrease the risk of malignancy. However, the underlying mechanisms in which decreased insulin/IGF-I signaling extends lifespan in mammals have not yet been completely elucidated.

\[ \text{Fig. 1. Possible involvement of ROS-induced cellular senescence in the regulation of lifespan.} \]

In addition to the FOXO protein, several mechanisms, including cellular senescence, may be attributable to the extension of lifespan via decreased GH/IGF-I signaling.
Human genetics

Recently, genetic studies have revealed the link between insulin/IGF-I signaling and longevity in humans. A study of Ashkenazi Jewish centenarians found that rare non-synonymous mutations in the IGF-IR gene were significantly more common in female centenarians 33,34. Female centenarians carrying those mutations had lower IGF-IR levels and decreased IGF-I signaling than the female centenarians without those mutations. In a study of long-lived Italians, individuals carrying a variant allele at the IGF-IR locus had lower plasma IGF-I levels than those without the variant allele; this allele was over-represented in long-lived Caucasians 35. Variants of AKT and FOXO3A have been linked to longevity in three 22 and seven cohorts 4, respectively. FOXO1 gene variants have also been linked to longevity 24,25. These data strongly suggest that insulin/IGF-I signaling is also linked to longevity in humans.

Acromegaly as a human model of IGF-I excess

Patients with acromegaly have excess GH secretion and increased circulating levels of IGF-I, accompanied by disproportionate skeletal, tissue, and organ growth. In most cases, chronic GH hypersecretion is caused by a benign pituitary adenoma. This disease is associated with increased morbidity and premature mortality; the reported standardized mortality ratio ranges between 1.3 and 3.0 11,26. The constellation of hypertension, glucose intolerance, heart failure, premature atherosclerosis, and malignancies such as colon and thyroid cancer, which may be intractable, are associated with elevated GH and IGF-I levels. Importantly, normalization of GH/IGF-I levels by surgery or medical treatment reduces the risk of these morbidities and premature mortality, indicating that elevated levels of GH and IGF-I are closely associated with the increased morbidity and mortality, although the precise underlying mechanisms have not been clarified.

In patients with acromegaly, biventricular cardiac hypertrophy manifests in 20% of young patients and in up to 90% of patients with long-standing disease independent of the presence of hypertension 27. Approximately 50% of patients are at intermediate–to–high risk of coronary atherosclerosis. Insulin resistance caused by excess GH results in glucose intolerance and diabetes 27. The prevalence of diabetes ranges from 19–56%. Ostearticular manifestations are also a feature of the disease; symptoms or signs referable to articular joint disorders such as osteoarthritis (OA) are present in a great majority of patients with acromegaly at their diagnosis. Acromegalic arthropathy affects both axial and peripheral sites. The appendicular skeleton is involved in up to 74% of patients, and the knee is the most commonly involved peripheral joint, followed by the shoulder, hip, ankle, elbow, and joints of the hand 28. Acromegalic arthropathy is generally noninflammatory, although features of OA frequently develop in later stages of the disease 29. These morbidities are closely associated with elevated levels of GH and IGF-I; however, the underlying mechanisms have not fully been elucidated.

Cellular senescence

In contrast to germ cells and certain stem cells, most somatic cells permanently stop dividing after a finite number of cell divisions in culture and enter a state termed cellular or replicative senescence. Cellular senescence was originally described by Hayflick 29 as a process that limits cell division of normal human cells in culture. The definition of cellular senescence has expanded to include the similar phenomenon of growth arrest, which is caused by various cellular stresses, including DNA damage and oxidative stress 30. Critically shortened telomeres resemble damaged DNA and thus trigger cellular senescence via a p53-dependent pathway 31. Irreversible growth arrest is also induced by the expression of activated oncogenes, such as Ras 32, and by activation of tumor suppressor genes 33,34. Cellular senescence is characterized by enlarged, flattened cell morphology 35, the expression of senescence-associated β-galactosidase (SA-β-gal) 36, and activation of tumor suppressor networks 37. Induction of cellular senescence results in accumulation of tumor suppressors p19Arf and p53. Cellular senescence caused by p53 is associated with the regulation of p53-dependent genes (e.g., p21), which participate in cell cycle arrest. Accordingly, recent studies demonstrated that cellular senescence functions as an important tumor-suppressive mechanism to restrict tumor development 38,39. Intriguingly, activation of cellular senescence and the aging process are closely related. The number of senescent cells increases with age, senescent cells are present at sites of age-related pathology, and increased numbers of senescent cells are causally related to tissue aging—decrements in neurogenesis, hematopoiesis, and pancreatic function 40. A trade-off between tumor suppression and aging is seen in mice that express constitutively hyperactive forms of p53, whereas these animals are remarkably tumor-free, they show multiple signs of accelerated aging 39.

Cellular senescence and age-related disease

Epidemiological studies have shown that age is the major risk factor for lifestyle-related diseases such as cardiovascular disease and diabetes. Recently, genetic analysis using animal models has identified molecules that are related to aging. These include components of the pathways of DNA repair, tumor suppression, telomere maintenance, and insulin/Akt signaling, in which most of the molecules regulate cellular senescence, suggesting a causative link between cellular senescence and aging 41. In addition, the close relationship between cellular senescence and age-related disease has recently emerged. Fenton et al detected SA-β-gal-positive vascular cells in damaged rabbit carotid arteries 42. Minamino et al. reported that SA-β-gal-positive vascular cells were detected in atherosclerotic plaques obtained from the coronary arteries of patients with ischemic heart disease 43. SA-β-gal-positive cells in human atheroma exhibit increased expression of p53 and p16, which is another evidence in favor of senescence in vivo.

With respect to mechanisms, several factors may regulate cellular senescence in vivo. Angiotensin II (Ang II) has been reported to induce premature senescence of human vascular smooth muscle cells (VSMCs) via the p53/p21-dependent pathway 44. Intriguingly, disruption of the Ang II type I receptor (Agtr1a) extended the lifespan of mice 45. Agtr1a/- mice developed less cardiac and vascular injury, and multiple organs from these mice displayed less oxidative damage than wild-type mice. The underlying mechanisms suggest that oxidative
stress and DNA damage have been shown to induce premature senescence in vascular cells and contribute to atherosclerosis \cite{44,46}. Exposure to chronic oxidative stress such as oxidized low-density lipoprotein enhances telomere shortening and accelerates the onset of senescence in human vascular endothelial cells (ECs) \cite{47}. These results suggest that development of atherosclerosis, a representative age-related disease, is closely associated with cellular senescence. Similar to atherosclerosis, the prevalence of heart failure increases with age, through various mechanisms. Accumulating evidence suggests that aging and pathological stimuli promote both senescence and apoptosis of cardiac progenitor cells (CPCs), thereby decreasing the number of functional components of CPCs \cite{48}. The number of p53- or p16-positive CPCs with short telomere is greater in the aged animal heart \cite{49}, as well as in the human ischemic heart \cite{50}. Aging is also known to increase the prevalence of metabolic disorders such as diabetes. Recently, it has been shown that p53 in adipose tissue is crucially involved in insulin resistance, which underlies age-related cardiovascular and metabolic disorders \cite{51}. Upregulation of p53 in adipose tissue induced the expression of pro-inflammatory cytokines and accumulation of macrophages in adipose tissue, resulting in insulin resistance. Excessive calorie intake led to the accumulation of oxidative stress in the adipose tissue of type 2 diabetic mice and promoted senescence-like changes, thereby increasing the production of pro-inflammatory cytokines. Furthermore, p53-induced adipose tissue inflammation is critically involved in the development of insulin resistance in heart failure \cite{52}. OA is the most common cause of chronic disability in older adults and is strongly linked to aging. Accumulating evidence suggests that cellular senescence in chondrocytes plays a pivotal role in the development of OA \cite{53}. OA is characterized by major extracellular matrix changes, including reduced thickness of cartilage, proteolysis, advanced glycation, and calcification. The associated cellular changes include reduced cell density, cellular senescence with abnormal secretory profiles, and impaired cellular defense mechanisms and anabolic responses. These data indicate that age-related diseases, including atherosclerosis, heart failure, insulin resistance, and OA, are closely related to cellular senescence of the component cells of the tissues; however, it is uncertain whether a common factor regulates the senescence of the various type of cells.

**IGF-I and cellular senescence**

It has been reported that growth factors, including IGF-I, induce ROS and modulate intracellular signaling via redox regulation \cite{54}. Regarding the role of ROS in IGF-I signaling, it has been reported that IGF-I stimulates ROS production and regulates proliferation and migration in VSMCs via ROS, suggesting that ROS induced by IGF-I play a role in the development of atherosclerosis \cite{55,56}. Fukuoka \textit{et al.} reported that ROS induced by IGF-I inhibit insulin-dependent glucose uptake in adipocytes \cite{57}. ROS also play an important role in the IGF-I-induced hypertrophy in C2C12 myocytes \cite{58}. These results suggest that ROS play an important role in IGF-I action in a tissue-dependent manner. Furthermore, it has been reported that IGF-I enhances cellular senescence via the ROS-p53 pathway \cite{59}. IGF-I induced markers for DNA damage and cellular senescence in the confluent state in rat VSMCs and human and mouse fibroblasts. In the presence of ROS scavengers or in p53-null cells, IGF-I did not enhance cellular senescence, suggesting that it acts via the ROS-p53 pathway. Given that excess ROS deteriorate tissue function and are causally associated with age-related diseases such as atherosclerosis and diabetes, and that cellular senescence is closely associated with the process of aging \cite{60}, it is speculated that IGF-I contributes to the regulation of aging through ROS production and induction of cellular senescence, in addition to downregulating FOXO proteins, as the underlying mechanism (Fig. 1). Intriguingly, oxidative stress is enhanced in acromegaly in animal models and humans \cite{61}, suggesting that excess levels of IGF-I increase oxidative stress \textit{in vivo} and play a role in the increased morbidity and mortality of patients with acromegaly (Fig. 2). In addition, these results imply that drugs that have an anti-oxidant effect may be effective in treatment of acromegaly.

Interestingly, it has also been reported that IGF-I induces the tumor suppressor protein p53 and its target genes \cite{59}. It has been shown that IGF-I regulates p53 activity in several different ways, depending on conditions. IGF-I acutely induces MDM2-dependent degradation of p53 in response to DNA damage via p38 MAPK \cite{62}. On the other hand, IGF-I chronically induces the p53-p21 pathway via PI3K \cite{63}, although the physiological relevance has not been elucidated. The role of this IGF-I-p53-p21 pathway may be inducing cellular senescence by IGF-I through the p53 protein. IGF-I is a growth factor involved in cell

**Fig. 2** Hypothetical pathophysiology of acromegaly.
IGF-I-induced ROS generation and related cellular senescence may play a role in the various comorbidities and increased mortality associated with acromegaly.
p53 and p53 may interact in the progression of cancer, and cross talk between IGF-I signaling and the p53 pathway has previously been reported. For example, wild-type but not mutant p53 suppresses IGF-IR gene transcription. Indeed, loss-of-function mutations in tumor suppressors, which is a common feature in human cancer, can lead to aberrant regulation of IGF-IR gene expression. Furthermore, IGF binding protein-3 (IGFBP-3), which modulates IGF-I bioactivity, is also regulated by p53. Taken together, it is possible that IGF-I induces cellular senescence via p53 to restrain cancer development as a feedback mechanism, while simultaneously stimulating proliferation. Thus, when p53 function is impaired by mutations, IGF-I may promote more aggressive tumor growth. Indeed, mutations in the p53 gene play a pivotal role in the development of colon and thyroid cancer, which are reportedly associated with acromegaly.

Conclusion

Although IGF-I plays an essential role in cell proliferation, growth, cancer development, and aging, these processes are strictly regulated by tumor suppressor cascades. It is suggested that cellular senescence regulated by the novel IGF-I-ROS-p53 pathway plays an important role in these biological actions of IGF-I.

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Conflict of interest statement

The author declares no financial or other conflicts of interest.

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