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Important roles of Akt/PKB signaling in the aging process

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1. ABSTRACT

The increased costs associated with an ever-growing aged population are expected to pose a significant burden on health care resources. From a biological standpoint, aging is an accelerated deteriorative process in tissue structure and function that is associated with higher morbidity and mortality. The Akt / protein kinase B (PKB) is a family of serine / threonine protein kinases, which play prominent roles in a diverse number of processes including cell survival, cell growth, gene expression, apoptosis, protein synthesis, energy metabolism and oncogenesis. It is likely that age-related changes in tissue structure and function are related to alterations in Akt expression and Akt-dependent signaling. Here we review the role that Akt may play in the aging process and attempt, where possible, to highlight how these data may lead to new directions of inquiry and clinical relevance to the aged.

2. INTRODUCTION

Population aging is becoming a worldwide issue. The number of people aged 60 and above accounted for 10% of global population in 2000, and it is projected that this will increase to be 21.8% in 2050 and 32.2% in 2100 (1). Aging exerts a significant economic burden on human society. In the United States, the health care spending per capita for the elderly population is more than 3 and 5 times higher than that for working-age person and child, respectively (2). According to the United States Department of Health and Human Services, the national health expenditure, which accounted for 16.2% of the Gross Domestic Product in 2007, is projected to increase 6.2% per year over the next decade.

Aging is a complex biological process that is associated with decreased physiological function leading to

Table 1. Phonotype alteration in Akt gene knockout studies

Isoform	Phenotype	Reference
Akt1	Fetal and postnatal growth, stress response, apoptosis, lipid metabolism	26, 33, 34, 40
Akt2	Growth retardance, insulin sensitivity, glucose homostatisis	32, 35, 36, 40, 41
Akt3	Brain development	31
Akt1/Akt2	Growth deficiency (neonatal death), insulin sensitivity	34, 37
Akt1/Akt3	Embryonic lethality (defects in the cardiovascular and nervous systems)	38
Akt2/Akt3	Growth retardance, smaller brain and testis size, glucose and insulin intolerance	39

increases in morbidity and mortality. For example, the age-related loss of skeletal muscle mass leads to decreases in muscle strength that if severe can result in disability (3, 4). Similar to skeletal muscle, the cardiovascular system also experiences decreased function with age, manifest as diminished cardiomyocyte contractility, prolongation of contraction and relaxation, and eventually cardiovascular failure, a major cause of death in the elderly (5-7). The aged brain also undergoes atrophy and degeneration, which if allowed to proceed unchecked can lead to neurodegenerative diseases, such as Alzheimer's (7-9). Similarly, the aged respiratory system experiences an increase in dead space and collapsed airways, both conditions that are associated with a higher prevalence of asthma and respiratory failure (10). The aged immune system is characterized by diminished development of functional T and B cells and antibody production that can lead to increased susceptibility to inflammation and a higher incidence of infections (11, 12).

The underpinning mechanism(s) for increased age-associated morbidity and mortality are not entirely clear but are likely related to molecular changes within the cells. For example, cellular reactive oxygen species (ROS) are increased with age, which can induce genomic and mitochondrial DNA damage and oxidatively modified proteins and lipids. These latter events can result in cellular apoptosis, impaired protein synthesis and cell survival, and have been linked to various pathological developments, including sarcopenia, neurodegeneration and cardiovascular disease (CVD) (13-15). Aging also appears to affect cellular metabolism. For example, glucose uptake into the cell is diminished with aging, which may contribute to the progressive development of age-associated hyperglycemia, insulin resistance, and cellular ROS (16-18).

The protein(s) and pathway(s) that are responsible for cellular aging are not well understood. The Akt / protein kinase B (PKB) is thought to play a central role in regulating cell survival, apoptosis, protein synthesis and glucose metabolism as it functions to integrate anabolic and catabolic responses by transducing extracellular (e.g. growth factors, nutrients and cytokines) and mechanical stimuli (e.g. load and contraction) to its downstream signaling cascades via the phosphorylation of numerous Akt substrates (14, 19-23). Herein we review the role that Akt may play in the aging progress. As a caveat to the reader, please bear in mind that although we have tried to include much of the relevant literature, it is likely that we have overlooked many as this field of inquiry is growing rapidly.

3. REGULATION OF AKT AND ITS SIGNALING IN THE AGING PROCESS

3.1. Akt / protein kinase B

The Akt / protein kinase B molecules are a family of serine / threonine-specific protein kinases (EC 2.7.11.1) consisting of three different members: Akt1 (also called PKB-alpha), Akt2 (PKB-beta) and Akt3 (PKB-gamma). These isoforms are coded by three different but highly homologous genes and share significant homology in amino acid sequences. The molecular structure of Akt contains three important domains that are crucial for its kinase activity: an amino-terminal pleckstrin homology (PH) domain, which can interact with phospholipid messenger phosphoinositides-(3,4,5)-P3 (PIP3) and other molecules, a catalytic domain, and a carboxyl-terminal hydrophobic domain (14, 19, 24, 25). Within these latter two domains are important serine (Ser473 in Akt1, Ser474 in Akt2 and Ser472 in Akt3) and threonine (Thr308 in Akt1, Thr309 in Akt2 and Thr305 in Akt3) residues that can undergo reversible phosphorylation that acts, in at least in part, to regulate the kinase activity of the Akt molecule.

The Akts are widely expressed in different cell types and tissues. Akt1 is ubiquitously expressed (26), while Akt2 is largely expressed in insulin-responsive tissues, such as skeletal muscle, adipose tissue and liver (27, 28). Akt3, on the other hand, is predominately expressed in brain, lung and kidney (29-31). Genetic manipulation studies have shown that Akt plays a critical role in a number of diverse processes including the regulation of cell growth, survival, energy metabolism, protein synthesis, and apoptosis (Table 1). The function of individual Akt molecules has been examined using gene knockout studies. Although the knockout of Akt1 or Akt2 is not lethal, this alteration leads to growth deficiency in the transgenic animals (26, 32, 33). Akt1-deficient mice also exhibit a shortened life span upon exposure to genotoxic stress, a finding that may be related to increased susceptibility to apoptotic stimuli (26). Gene silencing using small interfering RNA (siRNA) has shown that Akt1 also plays a key role in lipid metabolism, as depletion of Akt1 is associated with increased basal fatty acid uptake and beta-oxidation in myotubes (34). Besides diminished growth, the liver and skeletal muscles of Akt2-null mice are insulin resistant leading to the development of mild diabetes over time, suggesting that Akt2 may play an essential role in mediating insulin signaling and glucose homeostasis (35, 36). Loss of Akt3 gene reduces brain size in transgenic mice, mainly due to reduction of both brain cell number and size (31). Double-knockouts of both the Akt1 and Akt2 genes cause severe growth deficiency in mice, resulting in death shortly after birth (37), while loss of Akt1 and Akt3 caused fetal death mainly due to defects

in the cardiovascular and nervous systems (38). Animals deficient in both the Akt2 and Akt3 genes can survive but have less body weight (~25% reduction), substantial reductions in brain and testis size, and exhibit glucose and insulin intolerance (39).

Many stimuli can modulate Akt activity. Growth factors such as insulin and insulin-like growth factor-1 (IGF-1), can rapidly activate Akt via binding to tyrosine kinase-type growth factor receptors (TKR) (28, 42). Besides TKR, Akt may also be activated by G protein-coupled receptors (GPCR). The GPCRs, a large family of seven-transmembrane receptors composed of perhaps more than 800 different subtypes, are responsive to many different hormones, neurotransmitters, calcium and chemokines, suggesting that Akt may be responsive to a large number of different biological stimuli (43, 44). Akt is highly mechanosensitive, as such, it is readily activated by muscle loading and contraction, which leads to increased muscle glucose uptake and protein synthesis (6, 23, 45, 46). Some heavy metal ions, such as Zn^{2+} and Cu^{2+}, have also been shown to activate phosphoinositide 3-kinases (PI3K) / Akt signaling possibly via inhibition of protein tyrosine phosphatases (PTPases) (47). In addition, some Akt-binding proteins, e.g. Tcl1 and carboxyl-terminal modulator protein (CTMP), can interact with Akt which may be a mechanism to further modulate Akt activity (48-50).

The sequence of events leading to the activation of Akt is highly conserved. Upon binding to ligand, the conformational change of the TKR or GPCR leads to the activation of PI3K, which in turn catalyzes the production of phospholipid messenger PIP3 (43). Generation of PIP3 results in recruitment of PIP3-bindng proteins, including Akt and its upstream phosphoinositide-dependent kinase-1 (PDK1), to the membrane. The phosphorylation of Ser473 within the carboxyl-terminal hydrophobic motif by the mammalian target of rapamycin (mTOR) complex-2 (51) and DNA-dependent protein kinase (DNA-PK) (52), facilitates the phosphorylation of Thr308 by PDK1 and the full activation of Akt kinase activity (53, 54). Alternatively, the dephosphorylation of PIP3 by phosphatase and tensin homolog (PTEN), a PIP3-phosphatase, can lead to quench Akt activation (55).

The physiological function of Akt is mediated via phosphorylation of its downstream molecules. For example, Akt increases glucose uptake via phosphorylation of Akt substrate of 160 kDa (AS160, Thr642) which functions to increase glucose transporter translocation to the cell membrane (56). Phosphorylation of mTOR (Ser2448) (57) and TSC2 (Ser939 / Ser1086 / Ser1088 / Thr1462) (58) by Akt leads to increases in protein synthesis. Phosphorylation of Bad (Ser136) (59), Bax (Ser184) (60), caspase-9 (Ser196) (61) and forkhead box O-3α (FOXO3α, Thr32/Ser253/Ser315) (62) by Akt regulates apoptotic process and cell survival. Phosphorylation / inactivation of glycogen synthase kinase (GSK)-3α (Ser21) and GSK3β (Ser9) by Akt functions to regulate glycogen synthesis and apoptosis (63-65). Finally, phosphorylation / activation of endothelial nitric oxide synthase (eNOS, Ser1177) by Akt regulates blood pressure, vascular remodeling and angiogenesis via generation of nitric oxide (NO) (66). Taken together, these data suggest that Akt signaling regulates several diverse cellular processes, including cell proliferation, growth, migration, survival, and cellular metabolism as reviewed in depth elsewhere (13, 14).

3.2. Potential role of Akt in the aging of skeletal muscle

3.2.1. Aging impairs skeletal muscle structure and function

Skeletal muscle is one of the largest tissues in the body and is largely composed of contractile cells. The primary function of the contractile cells is to produce force that in turn powers the movements of our body. In addition, skeletal muscle is also involved in the maintenance of body posture and temperature (67, 68). As we age, there is a progressive loss of skeletal muscle mass and strength known as sarcopenia. It is thought that the degree of sarcopenia is accelerated with increasing age (3, 4, 15, 69, 70) as clinical studies have demonstrated that the prevalence of sarcopenia increases dramatically (greater than 4-fold) from ages 70-75 to 85 and older (3). Similar age-related changes have also been demonstrated in the laboratory rat. For example, in the aging Fischer 344 / NNIaHSD × Brown Norway / BiNia (F344BN) rat model (71), age-associated decreases in muscle mass, muscle cross-sectional area, and diminished muscle function are accelerated after 30 months of age (15, 69, 72). Sarcopenia is an important health problem as it is directly influences the capacity of an individual to maintain quality of life. Indeed, the development of sarcopenia is associated with a loss of balance, increased muscle weakness and fatigue, increased incidence of falling and fracture, and a higher prevalence of disability (3).

Skeletal muscle exhibits a great deal of plasticity in response to changes in contractile activity (e.g. loading), circulating growth factors, cytokines, and nutrients. However, muscle from aged animals exhibits a diminished ability to adapt compared to that observed in their younger counterparts (73-77). For example, Hwee and colleagues using the F344BN rat model demonstrated that the ability of the plantaris to undergo muscle growth (hypertrophy) in response to functional overload was decreased ~40% at the beginning of middle age (18-weeks old), and by about 60% at 30-months of age (77). Similar findings of decreased plasticity in aging muscle have been found using other models of muscle loading such as the recovery of lost muscle mass following the cessation of muscle unloading (76, 77).

3.2.2. Role of Akt singling in the regulation of muscle protein synthesis

Skeletal muscle mass is determined by the balance between protein synthesis and degradation. Aged muscle has a lower cellular protein-to-RNA ratio and a decreased ability to increase protein synthesis following contractile-, growth factor- or nutrient-stimulation (73-75). Among the signaling regulators governing protein synthesis in skeletal muscle, the Akt / mTOR pathway appears to play a critical role (21, 78). The Akt / mTOR is activated in response to anabolic stimuli and is thought to regulate

Table 2. Expression of Akt in aged skeletal muscle

Model	Gender	Age	Expression	Reference
Human	Male	70 vs. 20 yr.	↑	96
F344BN rats	Male	26 vs. 10 mo.	Akt1: ND	97
F344BN rats	Male	30 vs. 6 mo.	↑	74
F344BN rats	Male	33 vs. 6 mo.	ND	15
Fischer344 rats	Male	24 vs. 3 mo.	ND	16
C57Bl/6 mice	Female	24 vs. 5 mo.	Akt1: ND	95
Sprague-Dawley rats	Female	30 vs. 4 mo.	↑	98

↑: increased; ND: no difference; Akt isoform indicated where appropriate.

protein synthesis via the phosphorylation of various downstream regulators, including activation of S6 ribosomal protein and inhibition of eukaryotic translation initiation factor-4E (eIF4E) binding protein-1 (4EBP1) (79, 80). The ability of muscle to activate these signaling pathways appears to be attenuated with aging, and this may be related to the diminished capacity of aged muscle to undergo muscle growth in response to an anabolic stimulus. For example, Funai and colleagues showed that aging decreased the capacity of skeletal muscle to phosphorylate 4EBP1, an inhibitory regulator of translation initiation when non-phosphorylated (80), in response to high-frequency electrical stimulation (73). Other work from our laboratory has demonstrated similar findings as aging appears to be associated with diminished phosphorylation of the S6 ribosomal protein (Ser235/236) and increased inhibition of eIF4E by binding to 4EBP1 (Wu, unpublished observation), both of which are, in turn, associated with diminished myocyte size and decreased expression of the contractile proteins myosin and actin (15).

In addition to the effect of Akt / mTOR signaling on protein synthesis, it is likely that Akt also participates in regulating protein degradation via FOXO signaling (81). Indeed, the mRNA expression of the muscle-specific E3 ubiquitin ligases muscle RING-finger 1 (MuRF1) and muscle atrophy F-box (MAFbx / atrogin-1) is inhibited by the phosphorylation of FOXO transcription factors by Akt (82). It has been reported that MuRF1 and MAFbx mRNA and proteins are increased with age in several animal models (70, 83), and that the increased expression of these molecules appears to related to diminished Akt signaling (83). Further experiments designed to directly test the role of Akt activity in the regulation of muscle atrophy will no doubt increase our understanding of how this process may be better managed either by pharmacological means or by exercise.

3.2.3. Role of Akt signaling in regulating glucose uptake into muscle

Although not fully understood it is thought that Akt may participate in the regulation of glucose uptake into the muscle cell (84-87). For example, insulin stimulates Akt activation / phosphorylation and glucose transporter-4 (GLUT4) translocation to the plasma membrane within minutes (88, 89). Research using both humans and animal models have demonstrated that aged skeletal muscle exhibits a high incidence of insulin resistance while there are high prevalence of hyperinsulinemia (16, 17). Although aged muscle retains the ability of insulin to stimulate Akt phosphorylation (15, 90), the magnitude of Akt activation and subsequent phosphorylation of its downstream regulators, such as AS160, is significantly decreased with age (16). In addition, skeletal muscle from aged animals also exhibit a decreased ability to activate Akt in response to elevations in extracellular glucose (91).

Why aging may diminish insulin sensitivity in skeletal muscle is not well known. Oh and colleagues found that expression of Akt and caveolin-1, an insulin sensitivity modulator, is down regulated in the muscles of aged C57BL/6 × DBA/2 mice (JYD) mice, and that these changes may be related to why these animals suffer from a higher incidence of type 2 diabetes (92). Consistent with this notion, transient induction of caveolin-1 has been demonstrated able to improve insulin tolerance and muscle glucose uptake in these animals (92). Duan and co-workers demonstrated that SH2-B, a Src homology 2 (SH2) and PH domains-containing adaptor protein, can bind to insulin receptor in response to insulin. Further, they also noted that disruption of SH2-B can result in age-dependent hyperglycemia, hyperinsulinemia, and glucose intolerance (93). Moreno and colleagues showed that chronic 17beta-estradiol treatment is able to decrease the deleterious effect of aging on Akt phosphorylation (Ser473) and GLUT4 membrane translocation, indicating age-associated loss of gonadal function may also play a role in the development of skeletal muscle insulin resistance (94).

3.2.4. Is aging associated with the post-translational modification of Akt?

A review of the literature suggests that aging typically does not result in a decrease in the expression of Akt expression, more often than not, it appears that Akt expression is actually increased with age (Table 2). These data suggest that Akt levels may not be limiting per se in aged muscle. Nonetheless, the literature does suggest that aging may be associated with alterations in Akt signaling. Indeed, several studies have demonstrated an age-related mismatch between the degree of Akt phosphorylation level and Akt-dependent downstream signaling. For example, Hwee and colleagues found that even though the amount of basal and overload-induced levels of Akt phosphorylation are increased with age, the ability of Akt to phosphorylate eIF2B epsilon, which is one of Akt substrates, is actually diminished (77). Li and colleagues reported that although IGF-1 increases Akt1 phosphorylation in muscle to a similar degree in young and aged mice, the IGF-1-induced p70S6k phosphorylation is absent in aged muscle (95). Given these apparent mismatches between Akt phosphorylation and downstream signaling, some have wondered whether these changes in Akt signaling could be ascribed to changes in the Akt molecule itself. Recent work from our laboratory supports this possibility. As an example, Wu and colleagues showed that aged skeletal

muscle exhibits an uncoupling between Akt phosphorylation and the phosphorylation of its downstream substrates GSK3α and GSK3β (15). In addition, these researchers also found that there is a mismatch between the enzymatic activity of Akt and the degree of Akt phosphorylation at Ser473 and Thr308, as higher Akt phosphorylation did not lead to higher Akt activity as determined by *in vitro* activity assays (15). Further, their data also demonstrated that Akt dysfunction, at least in the skeletal muscle of the aged F344BN rat model, is related to increases in the degree of Akt S-nitrosylation, as reduction of S-nitrosylated Akt by chronic acetaminophen administration was found to increase *ex vivo* insulin responsiveness, myocyte size, and expression of the contractile proteins myosin and actin (15).

3.3. Potential role of Akt in the aging of the skeletal system

Continuous bone remodeling throughout life is an important phenomenon that maintains bone mass and integrity (99). Bone mass is determined by the balance of bone resorption by the osteoclasts and the degree of bone formation by the osteoblasts. An alteration in this interplay, results in loss of skeletal development as is observed in several developmental conditions including aging (100). Osteogenesis is an important aspect in bone and skeletal development and is regulated positively by factors such as bone morphogenic proteins (BMPs) and IGF-1. The activation of PI3K / Akt signaling by IGF-1 plays a key role in skeletal development by regulating BMP-mediated bone development and growth (101). The molecular mechanisms responsible for regulating BMP expression by PI3K / Akt signaling are not entirely clear, however recent data has suggested that the activation of runt-related transcription factor 2 (Runx2), which is thought to be a master regulator of osteoblast commitment, may be involved (102). Moreover, it is also thought that IGF-1 and PI3K / Akt may also participate in the regulation of processes related to the development of the skeletal system such as chondrocyte proliferation and maturation and the formation of cartilage, possibly using mechanisms involving the Akt / FOXO, Akt / GSK3 and Akt / mTOR pathways (103). It is well established that bone becomes resistant to the anabolic activity of IGF-1 with aging. Osteoporosis, defined by compromising bone strength with an increasing risk of fracture, is perhaps the most prevalent bone disease in elderly population (104). The factors regulating osteoporosis have not been fully elucidated however impaired IGF-1 receptor signaling and diminished activation of PI3K / Akt signaling have been postulated to be involved in causing an age-related loss in osteogenic capacity (105). Alterations in Akt signaling may also be involved in the development of osteoarthritis, with this process occurring through the inhibition of Akt by Tribbles homolog (TRIB3) protein, an inhibitor of insulin signaling, (106).

3.4. Potential role of Akt in the aging of the cardiovascular system

Cardiovascular disease is the number one cause of death in people over age 65 yrs and 84% of deaths caused by heart disease occur in the elderly population (107, 108). Recent large cross-sectional studies have demonstrated that aging, by itself, confers a greater risk for cardiovascular diseases than do the other major risk factors such as plasma lipid levels, smoking, diabetes, or sedentary life style (109). Although not well understood it is thought that the profound impact of age on the risk of the occurrence, severity and prognosis of cardiovascular disease is due, in part, to age-associated changes in cardiovascular structure and/or function (110).

Aging in healthy adults (20-85 yrs) is characterized by increased left ventricular (LV) wall thickness, alterations in the diastolic filling pattern, impaired LV ejection, diminished reserve capacity, altered heart rhythm, large artery thickening, and endothelial dysfunction (111-113). Although these age-associated changes do not result in clinical heart diseases per se, they do compromise the cardiac reserve capacity and affect the threshold for symptoms and signs, as well as the severity and prognosis of heart failure secondary to any given disease-related challenge.

3.4.1. Role of Akt in cardiac aging

Both the Akt1 and Akt2 isoforms are highly expressed in the mammalian heart (114). Similar to that observed in skeletal muscle, tyrosine kinase receptor binding of various cytokines and growth factors such as insulin and IGF-1 activate PI3K / Akt signaling in the heart. Akt activation participates in several cellular processes including glucose uptake, glycogen synthesis, cellular growth and survival. Akt signaling has been found to play a role in cardiac hypertrophy (115-119), apoptosis (120-123) and angiogenesis (124-127). A time dependent effect of Akt activation in the heart has also been shown (123). Short term activation of Akt1 in cardiac muscle by insulin / IGF-1 or other factors results in cardiac hypertrophy, possibly through the mTOR pathway. Akt activation may also play a protective role, as the Akt-associated activation of vascular endothelial growth factor (VEGF) appears to function in preventing ischemia / reperfusion (I/R) injury. In contrast, long-term activation of Akt is associated with dysregulation of intracellular signaling which can promote cardiac hypertrophy and a concomitant inhibition of VEGF and increased myocyte injury following I/R (128). Akt is also involved in the regulation of heart development. The transcription factors FOXO1 and FOXO3 are downstream targets of PI3K / Akt signaling and regulate the expression of cyclin kinase inhibitors (e.g. Cip/Kip). An increase in FOXO1 protein is thought to inhibit myocyte proliferation which could in turn, affect heart development (129). A similar process is also likely to operate in the vasculature where the inhibition of FOXO3a is essential for the promotion of the Akt-mediated growth arrest (130). Finally, the up-regulation of FOXO proteins by Akt activation may also serve to protect cardiac myocytes against damage due to ROS (131).

Akt expression levels are decreased in the senescent rat and in aged human hearts (123, 132). These age-related changes in Akt expression may be related to increases in myocardial fibrosis which is a known contributor of the cardiovascular disease process (132). A decrease in IGF-1 and downstream Akt-related signaling

with aging may also desensitize the cardiomyocyte to mechanical stimuli (133). Age-associated decreases in Akt expression and activation / phosphorylation has been found to be associated with increased cardiomyocyte apoptosis, altered composition and localization of the cardiac dystrophin glycoprotein complex (DGC), and impaired cardiac membrane integrity (123).

3.4.2. Role of Akt in vascular aging

Akt has a key role in the regulation of vascular permeability, angiogenesis and endothelial function (22, 134-136). The activation of PI3K / Akt in the vasculature promotes macrophage survival and atherosclerosis (137, 138). Knockout of Akt1 enhances the high cholesterol diet induced atherosclerosis in ApoE-/- mice (139, 140). Likewise, insulin and IGF-1 are also implicated in the atherosclerotic lesion development (130).

It is postulated that cell aging (senescence) is one of the triggering mechanism of atherosclerotic lesion development (141). Akt phosphorylation is increased in the balloon injured vascular wall of young rats, but not in old rats. This loss of Akt activation in the vascular wall with aging is associated with a decrease in repair of endothelium after injury (142). *In vitro* studies have shown that late-passage endothelial and vascular smooth muscle cells (VSMCs) exhibit less migration and Akt phosphorylation in response to shear stress than early-passage cells (143). Other studies have begun to establish the role of Akt in cellular senescence and atherogenesis. It is thought that Akt can reduce the lifespan of cultured endothelial cells via a p53/p21 dependent pathway (141). Similarly, oxidized low density lipoprotein (LDL) suppresses telomerase activity through inhibition of the PI3K / Akt pathway. This process if allowed to proceed unchecked leads to pro-atherogenic and aging-mediated effects on endothelial cells (ECs) (144). The loss of telomerase also contributes to hypertension by increasing endothelin-1 expression (ET-1) (145). The maintenance of the EC layer is an essential defense mechanism against vascular disorders (146). This maintenance is performed by neighboring cells in normal conditions or by the participation of endothelial progenitor cells (EPCs) (146). A decrease in EPC telomerase activity (147) possibly occurring through impaired PI3K / Akt signaling can contribute to the senescence of EPCs and the impairment of EC maintenance (144, 148). Interestingly, high density lipoprotein (HDL) can increase NO release by activating eNOS which has been shown to increase PI3K / Akt signaling thereby promoting telomerase activity to slow EPCs senescence (149). Likewise, growth hormone (GH) stimulation can upregulate IGF-1 through PI3K / Akt pathway signaling which in turn is associated with the partial prevention of EPC senescence (150).

Increases in oxidative stress have been posited to promote aging and are a risk factor for the development of CVD (151, 152). The role of PI3K / Akt signaling in modulating oxidative stress has not been fully elucidated. Akt activation may increase oxidative stress through inhibition of FOXO transcription factors (153). Aging also increases the phosphorylation of FOXO1 and the activation of NF-kB signaling via the PI3K / Akt pathway (154). Interestingly, this process can be suppressed by caloric restriction (154). Moreover, there are decreased levels and activation of manganese-superoxide dismutase (MnSOD) in the VSMCs of aging rats. While not fully understood, it has been postulated that an increase Akt activity and the subsequent phosphorylation of FOXO3a may be responsible for MnSOD inhibition (155).

Akt is also implicated in the regulation of thrombotic events. Plasminogen activator inhibitor-1 (PAI-1) promotes the thrombotic process by attenuating the fibrinolytic system (156, 157). Alterations in PAI-1 expression are also an independent risk factor of cardiovascular disease (157, 158). PAI-1 is up-regulated in senescent endothelial cells (159-161) in a process that is inhibited by the activation of the PI3K / Akt pathway (162). Similarly, the apoptotic effects of thromboxane A2 (TxA2) is also facilitated by the inhibition of Akt phosphorylation (163). Changes in Akt activity may also be related to the maintenance of vaso-tone as impaired vaso-relaxation is associated with impaired activation of the Akt / eNOS pathway (164).

Aging is also associated with an increased incidence of hypertension (165). The characteristic pathological change of the vascular wall in aging and hypertension is a progressive increase in the thickness and rigidity of smooth muscle layer in capacitance arteries (166, 167). Whether Akt may play a direct role in this process is not entirely clear however Akt1 expression is up-regulated in the VSMC of capacitance arteries in hypertensive animals (168). This finding may be of importance given that increased Akt1 levels in VSMC have been shown to increase cyclin B degradation and produce polypoid / hypertrophic changes (168). It is thought that these processes might involve the anti-apoptotic effect of Akt1 and its regulation of the Bcl-2 family protein, Bad (169, 170).

It is likely that aging also contributes to the loss of control of vasomotor function (171). The senescent endothelial cells has decreased production of NO (172, 173). This decrease may be related to changes in eNOS expression as the levels of this protein appear to be diminished in the aorta of aged rats, likely by a mechanism involving decreased levels of Akt (172). Similarly, eNOS phosphorylation is directly modulated by Akt (66). The factors that may regulate this process are not entirely understood however it is interesting that TRIB3 has been shown to impair NO production through its ability to inhibit Akt phosphorylation. As expected, the expression of TRIB3 is dramatically induced during insulin resistance and early aging (174). Whether TRIB3 plays a direct role in regulating age-related changes in vasculature function by its affects of Akt is currently unclear.

3.5. Potential role of Akt in the aging of the neuronal system

3.5.1. Age-associated changes of Akt signaling in neuronal system

As the neuronal system ages, it undergoes a variety of morphological, functional, and biochemical

changes that include neuronal loss, synaptic destruction, memory and cognitive impairment, a loss of glutamate receptors, a decrease in the abundance of acetylcholine, dopamine, and norepinephrine, and an increase in oxidative stress and inflammation (175). The formation of senile plaques and neurofibrillary tangles if allowed to accumulate can lead to Alzheimer's disease, the most common neurodegenerative disease in people over 65 years of age (9). Studies have shown that several signaling pathways contribute to age-related changes in neuronal systems including those associated with Akt, a finding that is none too surprising given the role of this molecule in regulating cell survival, cellular apoptotic, and glucose uptake in neuronal system.

The expression and activity of the Akt signaling molecule in aged brain have been investigated by several research groups. Myung and colleagues examined the phosphorylation and expression level of Akt in the frontal cortex, striatum, hypothalamus, hippocampus, cerebellum, cerebral cortex, thalamic area, brainstem, and amygdala-septum-preoptic areas of the brain in infant (1 mo.), young (3 mo.), adult (6 mo.), and aged (20 and 24 mo.) rats. Although they did not observe significant changes in the expression level of Akt during aging, they did note that the degree of Akt phosphorylation was significantly increased in the aged rat hippocampus, cerebellum, striatum, and thalamic area (176). In a similar study, Han and co-workers using a senescence-accelerated (SA) mouse model that simulates the early onset of neurodegenerative dementia investigated the expression and activity of Akt in hippocampal tissues from differently aged (2, 4, 6, 8 and 10 mo.) animals (177). Their analysis demonstrated a learning and memory impairment in the SA mice and that the level of Akt mRNA and protein did not significantly change during aging. However, the phosphorylation of Akt Ser473 was gradually decreased in the SA model compared to that observed in age-matched controls. This finding led them to postulate that age-related reductions in Akt activity may be related to neuronal death, which, in turn, could lead to learning and memory impairment (177). The CTMP has also been reported to play a role in the progress of ischemia-induced neurodegeneration and neuronal death via negative regulation of Akt phosphorylation / activation (50, 178). However, to date, it is unclear whether CTMP play a role in age-associated alterations in neuronal and other physiological systems.

It is thought that the CA1 section within the hippocampus is the most vulnerable to and affected by age-associated stressors such as ischemia and the accumulation of Tau protein or amyloid β (Aβ). Araki and co-workers explored the age-associated changes in Akt1 immunoreactivity in the CA1 hippocampus section from mice of various ages (2, 8, 18, 40-42, and 50-59 weeks old). They observed significant increases in the number of cells demonstrating Akt1 immunoreactivity in neurons and glial cells in the CA1 hippocampus section from mice at the age of 40-42 and 50-59 weeks (179). Similar age-related changes have been found in the hippocampus of F344BN rats. For example, Jackson and colleagues compared the level of phosphorylated Akt (Thr308 and Ser473) in the nuclei of CA1 and CA3 hippocampal sections obtained from F344BN rats of different ages (4, 17, 28 and 37 mo.) and found no significant differences in total Akt expression level between different age groups or between different hippocampal regions (180). However, the amount of phosphorylated Akt Thr308 was reduced with age in the hippocampal CA1 region, while its phosphorylation was increased with age in the CA3 region. The phosphorylation level of Akt Ser473 did not change significantly with age in either the CA1 or CA3 region. They also determined the level of cytoplasmic phosphorylated Akt Thr308 and Ser473, as well as the total expression level of cytoplasmic Akt in the CA1 and CA3 regions in rats of different ages. They observed no aged-associated changes in the total expression level of cytoplasmic Akt, or in the phosphorylation level of cytoplasmic phosphorylated Akt at Thr308 or Ser473 in either the CA1 or CA3 region (180). In addition, the amounts of cytoplasmic Akt and phosphorylated Akt at Thr308 were comparable in the CA1 and CA3 regions from all four age groups. However, it was noted that the phosphorylation of Akt Ser473 was higher in the CA3 as compared to the CA1 region in all four age groups (180). The physiological significance of these findings is currently unclear.

3.5.2. PI3K / Akt signaling pathway and Alzheimer's disease

Alzheimer's disease is often called "type III diabetes" because it is closely linked to insulin resistance in the brain. Recently, it has been found that signaling through the PI3K / Akt pathway is impaired in Alzheimer's disease, and importantly, that this diminished signaling may be linked to the formation of senile plaques, neurofibrillary tangles, and neuronal loss (181, 182). The molecular mechanism(s) responsible for these types of changes are currently unclear. Nonetheless, it is interesting to note that Akt substrates, such as GSK3α and GSK3β, can phosphorylate the tau protein (182). This may be important given the fact that hyperphosphorylation of tau is associated with the increase of neurofibrillary tangles in Alzheimer's disease. The Aβ, a 40- or 42-amino acid product derived from the serial cleavage of the amyloid precursor protein (APP) by β-secretase and γ-secretase, is a major component of senile plaques, whose production is promoted by activated GSK3α. Attesting to the potential role of Akt in these processes is the finding that PI3K / Akt signaling appears to be inhibited in Alzheimer's disease. This reduced activation of Akt is thought to lead to decreased inhibition (i.e., decreased phosphorylation) of GSK3β, which may be associated with the accumulation of hyperphosphorylated tau protein and neuronal death. Similarly, reduced activation of Akt also leads to decreased inhibition of GSK3α, which can lead to the accumulation of Aβ and further inhibition of PI3K / Akt signaling.

Using primary cultures of cortical cells obtained from rats, Querfurth and co-workers investigated the effects of intracellular accumulation of Aβ42 on Akt signaling. With increased Aβ42 levels they observed non change in the amount of total Akt expression, however there was an approximately 30% decrease in Akt phosphorylation when compared to the control group (183). Consistent with the

decreased activity of Akt, an approximately 35% decrease in GSK3β phosphorylation was observed in the Aβ42-induced cultures when compared to that observed in the control group (183). These findings were also followed up with *in vivo* studies, which demonstrated that the phosphorylation level of Akt in the frontal lobe of Alzheimer's patients was decreased when compared to that found in a control group. In addition, they also noted that the activity of Akt1 was also decreased in Alzheimer's brains as compared to controls (183). Although the mechanism(s) underlying these changes are not fully understood, these data led to the postulate that increased levels of intracellular Aβ might act to inhibit the interaction between PDK1 and Akt, thus inhibiting the PDK-dependent activation of Akt (181).

Other data investigating the putative role of Akt in Alzheimer's disease have demonstrated somewhat different results, which may due to differing experimental conditions and/or differences in the way the tissue was collected. For example, Pei and colleagues found that the level of Akt phosphorylation was increased in the frontal cortex of Alzheimer's patients as compared to control groups (184). Similarly, Griffin and co-workers reported an increase in Akt phosphorylation in the particulate fraction of isolates obtained from the brains of individuals suffering from Alzheimer. Interestingly, they observed no significant changes in the total Akt expression level in particulate fractions, whereas there was a significant decrease in total Akt level in the cytosolic fraction in the Alzheimer brains when compared to controls (185). Additional research to explore how aging and Alzheimer's disease may affect Akt signaling will no doubt be useful in furthering our understanding of how differences in Akt activity may be related to this neurological disease.

3.6. Potential role of Akt in the aging of the respiratory system

Aging is associated with decreases in peak airflow, a reduced capability of lung alveolar gas exchange (oxygenation of blood and removal of carbon dioxide), a loss of lung elasticity and defense mechanisms, and a loss of strength in the muscles of the chest, airways, and diaphragm (186).

Thus far there have been only a few studies focused on examining the regulation of Akt signaling in the aging respiratory system. Boriek and co-workers using diaphragm preparations investigated the effect of age on the stretch-induced regulation of transcription factors FOXO in young (2 mo.), adult (12 mo.), and aged (24 mo.) mice (187). Their work demonstrated that Akt and IKK activation is required for the stretch-induced binding of FOXO to DNA. In addition, they also showed that basal Akt and IKK activities were increased in adult and aged diaphragms as compared to the levels observed in the diaphragms of young mice. Further, mechanical stretch-induced activation of Akt and IKK was significantly increased in young diaphragms, whereas this stretch-induced increase was not observed in the diaphragms from adult and aged mice. Taken together, these data suggest that aging in the diaphragm is associated with a loss of muscle mechanosensitivity (187). Whether this loss in the ability of the diaphragm to "sense" mechanical load is related to age-associated decreases in diaphragm extensibility, compliance, and viscoelasticity remains to be determined.

Work from Lorenzini and colleagues detailed the study of how aging may affect the activity of Akt using the lung derived WI-38 diploid fibroblast cell line. In this study cells of different ages: early passage (less than 50% of replicative lifespan) and senescent (at the end of replicative lifespan) were deprived of serum for 3 days, and then stimulated with 10% fetal bovine serum for 15 or 30 minutes (188). The researchers found a comparable level of Akt Ser473 phosphorylation in total cell extracts of the senescent cells and their younger counterparts, but the capability of Akt to phosphorylate nuclear targets was reduced in the senescent cells (188). Therefore, aging may be associated with defects in Akt-related nuclear signaling. Conversely, Bartling and colleagues using pre-senescent and senescent WI-38 fibroblasts found no differences Akt Ser473 phosphorylation with aging (189). How aging may affect Akt-related signaling in the respiratory system is currently unclear. Future studies perhaps using other cell lines or tissues obtained from aged animals will certainly be useful for increasing our understanding of how Akt may function in the aging respiratory system.

3.7. Potential role of Akt in the aging of the digestive system

Aging accompanies an increased prevalence of digestive problems that are associated with changes in the regulation of gastrointestinal motility. Typical age-associated syndromes include dysphasia, dyspepsia, anorexia with the decreased peristaltic contraction (190) and changes in the rate of gastric emptying (191, 192) and lower intestinal transit (193). It is thought that the loss of enteric neurons is responsible for the esophageal (194) and gastrointestinal abnormalities (192, 195). Age-related increases in chronic constipation may be related to increased colonic transit time (193) which are likely related to a decline of submucosal and mesenteric plexuses (196) and diminished NO production (197). Decreases in the expression of glial cell line-derived neurotrophic factor (GDNF) are thought to be regulated, at least in part, by PI3K / Akt (198) and have been shown to contribute to a loss of enteric neurons, a finding that might be associated with the development of age-related digestive dysfunction (199-201). Akt may also play a role in the increased proliferation of mucosa in the aging gastrointestinal tract (202-204) possibly through its effect on EGF receptor activation (205) or by the inactivation of pro-apoptotic regulator Bad (206). Whether or how age-related changes in Akt activity might directly affect digestive function is not well understood and will require additional studies.

3.8. Potential role of Akt in the aging of the immune system

It is thought that immune system function gradually declines with age which leads to an increased susceptibility to inflammation (207-209). The aged immune system produces fewer antibodies, which also results in a reduced response to vaccination (207). The antibodies

produced by the aged immune system have a lower affinity for their antigens, and a decreased capability to distinguish foreign invaders from "self" substances. These changes, together, may result in an increased risk of autoimmune disorders and other diseases, such as cancer (209). In addition, aging is also associated with a reduction in the ability of the disease-fighting T lymphocytes to function properly (210, 211).

Very few studies have been done to explore whether / how alterations in the PI3K / Akt signaling pathway are involved in age-associated changes in immune system. Larbi and colleagues investigated the effects of aging on the formation and regulation of lipid rafts, which are thought to be critical for assembling the T-cell receptor (TCR) signaling machinery that controls T-cell activation. Using cells obtained from elderly humans, they determined that age-associated impairments in lipid raft polarization are accompanied by the alterations in CD4+ (cluster of differentiation 4, a co-receptor that assists TCR-mediated T-cell activation) T cell activation (212). Further, lipid rafts from both resting and stimulated CD4+ T cells displayed a significant decrease in the amount of phosphorylated Akt in elderly human subjects when compared to their younger counterparts. This significant difference in the phosphorylation of Akt was not observed in CD8+ T cells from young and elderly subjects (212). Similarly, Agrawal and co-workers observed comparable levels of Akt but differences in the degree of Akt activation in lipopolysaccharide (LPS)-stimulated monocyte-derived dendritic cells from young and aged human subjects (12). Additional research to explore age-associated modification in Akt signaling in the immune system is needed to better understand the mechanisms behind these changes.

3.9. Potential role of Akt in the aging of the urinary system

Aging results in dramatic changes in renal morphology and physiology. Studies in humans and rodents have shown that aging is accompanied by a loss of renal function, glomerulosclerosis, interstitial fibrosis, the infiltration of inflammatory cells and tubular casts (213, 214). Currently very few studies have explored the role of Akt signaling in the urinary system during aging. In the rodent aging model, age-associated renal cortex dysfunction is accompanied by a significant decrease in Akt activity, which results in the attenuation of cell growth and replacement (215). Similarly, increased oxidative stress and its deleterious effects possibly on Akt activity may also be involved in the age-related renal disorders (216).

3.10. Potential role of Akt in the aging of the adipose tissue

Aging induces declines in body metabolism and energy expenditure which are oftentimes accompanied by an increase in the percentage of body fat relative to overall body weight (18, 217, 218). People aged over 75 years may have double the percentage of body fat than they had in middle age. This increase in body fat, in turn, increases the risk of metabolic disease such as obesity and diabetes, and accelerates the aging process. Approximately eighty percent of our body's fat/lipids are stored in the adipose tissue beneath the skin (subcutaneous fat) and around internal organs (visceral fat). Fat distribution also changes with age, as aging typically increases the proportion of visceral fat. Although the percentage of body fat gained and the distribution of fat are somewhat different in aged men and women, both sexes demonstrate an increase in body fat mass and a preferential accumulation of fat in the abdominal region (219). It is believed that overall glucose utilization decreases with age, and this effect is stronger in white adipose tissue than in the brown. White adipose tissue cells contain a single large fat droplet as an energy store. Conversely, brown adipose tissue cells contain many smaller fat droplets, more mitochondria, and more capillaries than white adipose tissue, which together allow it to generate more body heat. Therefore, age-associated alteration in adipose glucose utilization can affect energy metabolism and body temperature (18, 219-221).

Similar to other tissues, Akt performs several important functions in adipose tissue given its role in the regulation of glucose metabolism. Villar and colleagues examined the effects of insulin on Akt activation and GLUT4 translocation in adipocytes prepared from epididymal adipose tissue obtained from young (3 mo.) and aged (24 mo.) Wistar rats (222). Compared to preparations derived from young animals, they observed a significant increase in the amount of basal Akt phosphorylation in aged rat adipocyte (~24% increase in the plasma membrane and ~57% increase in the cytosol), and a diminished insulin-stimulated phosphorylation of Akt. Associated with this impairment of Akt activity, the insulin-stimulated translocation of GLUT4 from intracellular compartments to the plasma membrane was also significantly decreased in aged rat adipocytes (222). Although not fully understood, it is thought that the reduced activation of Akt and diminished GLUT4 membrane translocation with aging may be related to age-associated differences in the subcellular distribution and phosphorylation of IRS-1 and IRS-3 proteins (222). In a similar fashion, Serrano and colleagues demonstrated that the basal phosphorylation of Akt was increased in epididymal white adipose tissue from aged (24 mo.) male Wistar rats compared to that found in adipose tissue samples of young (3 mo.) rats. In addition, insulin-stimulated Akt phosphorylation was reduced in subcellular fractions of white adipose tissue, including the plasma membrane, internal membrane and cytosol in aged rats when compared to their younger counterparts (90). Carvalho and colleagues observed an insulin-induced increase in serine phosphorylation of Akt in adipocytes from lean young Zucker rats, however, this response appeared to be absent in adipocytes from aged or obese rats (223). Likewise, Akt translocation to the plasma membrane in response to insulin appears to be diminished in the adipose tissues of aged animals compared to that found in young animals (223).

Energy metabolism is thought to be coupled to life span, and calorie restriction has been investigated as an approach to extend life span. Studies have shown that calorie restriction can reduce adipose tissue mass and that this change is accompanied by decreases in oxygen metabolism and the production of mitochondrial hydrogen

peroxide. Park and colleagues studied the effect of calorie restriction (reduction by 30% for 4 months) on insulin signaling in the epididymal adipose tissue of young (7 mo.) and aged (22 mo.) male F344 rats (224). Their findings suggested that the basal level of Akt phosphorylation in the young calorie-restricted group was increased compared to age-matched controls, and that this alteration was associated with differences in the activation of the insulin signaling pathway. Conversely, the basal level of phosphorylated Akt was decreased in the aged calorie-restricted group when compared to their younger counterparts, a finding they attributed to age-associated desensitization of insulin signaling (224).

Of the three Akt isoforms, Akt2 appears to be the most abundant in adipose tissue (32). Using Akt2-null mice model, Coleman and colleagues demonstrated that both male and female mice missing the Akt2 gene exhibited aged-dependent lipoatrophy (i.e., loss of adipose tissue), accompanied by the reduction of adipose depots with age (32), suggesting that Akt2 may function in the regulation of adipose tissue size. Berniakovich and co-workers using p66Shc-null mice (p66Shc facilitates the generation of mitochondrial ROS) observed a reduction in the mass of both white and brown adipose tissue, as well as reduced insulin-stimulated phosphorylation of Akt in the p66Shc-null mice as compared to the control group (225). What other role(s) Akt may play in the aging of adipose tissue is currently unclear.

4. CONCLUSIONS AND PROSPECTIVES

Age-associated alterations in tissue structure and function are postulated to play a role in the physiological impairment seen with aging. The mechanism(s) underlying these changes are not well understood given the complexity of cellular function and the difficulty of studying physiological processes in the aged tissues. Akt / protein kinase B belongs to a family of serine/threonine protein kinases, which play prominent roles in a diverse number of processes including cell survival, cell growth, gene expression, apoptosis, protein synthesis, and energy metabolism (14, 19-21). Given the importance of Akt in regulating such a wide variety of cellular functions, it is likely that age-related changes in tissue structure and function may be related to alterations in Akt expression and Akt-dependent signaling. Recent data from a variety of different animal models and other work using *in vitro* approaches appear to support this notion particularly in some of the larger and better-understood organ systems such as skeletal muscle, adipose tissue and the cardiovascular system. For example, the restoration of Akt function in aged animals has been shown to increase muscle mass, prevent denervation-induced myofiber atrophy and reduce body adipose tissue mass (226, 227). Although these findings are promising, more work remains. For example, a review of the literature indicates that most of the investigations to date have focused on the role of Akt in the aging male. It is well known that gender may have an effect on the aging process while other studies have shown that gonadal hormones can affect Akt activation and other physiological processes such as glucose metabolism (72, 94, 228). Whether gender-related differences in the regulation of Akt occur during aging has to our knowledge not been explored. It is anticipated that additional studies will further our understanding of the role that Akt plays in the aging progress and that this work will yield valuable clinical insight for the increasing aged population.

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Abbreviations: 4EBP1, eIF4E binding protein-1; Akt/PKB, protein kinase B; Akt1, PKB-alpha; Akt2, PKB-beta; Akt3,PKB-gamma; APP, amyloid precursor protein; AS160, Akt substrate of 160 kDa; Aβ, amyloid β; BMPs, bone morphogenic proteins; CTMP, carboxyl-terminal modulator protein; CVD, cardiovascular disease; DGC, dystrophin glycoprotein complex; DNA-PK, DNA-dependent protein kinase; ECs, endothelial cells; eIF4E, eukaryotic translation initiation factor-4E; eNOS, endothelial nitric oxide synthase; EPCs, endothelial progenitor cells; ET-1,endothelin-1; F344BN, Fischer 344 / NNIaHSD × Brown Norway / BiNia; FOXO, forkhead box O; GDNF, glial cell line-derived neurotrophic factor; GH, growth hormone; GLUT4, glucose transporter-4; GPCRs, G protein-coupled receptors; GSK, glycogen synthase kinase; HDL, density lipoprotein; HHS, the United States Department of Health and Human Services; I/R, ischemia/reperfusion; IGF-1, insulin-like growth factor-1; JYD mice, C57BL/6 × DBA/2; LDL, low density lipoprotein; LPS, lipopolysaccharide; LV, left ventricular; MAFbx/atrogin-1, muscle atrophy F-box; MDDCs, monocyte-derived dendritic cells; MnSOD, manganese-superoxide dismutase; mTOR, mammalian target of rapamycin; MuRF1, muscle-specific E3 ubiquitin ligases muscle RING-finger 1; NO, nitric oxide; PAI-1, Plasminogen activator inhibitor-1; PDK1, phosphoinositide-dependent kinase-1; PH, pleckstrin homology; PI3K, phosphoinositide 3-kinases; PIP3, phosphoinositides- (3,4,5)-P3; PTEN, phosphatase and tensin homolog; PTPase s, protein tyrosine phosphatases; ROS, reactive oxygen species; Runx2, runt-related transcription factor 2; SA, senescence-accelerated; SH2, Src homology 2; siRNA, small interfering RNA; TCR, T-cell receptor; TKR, tyrosine kinase-type growth factor receptors; TRIB3,Tribbles homolog; TxA2, thromboxane A2; VEGF, vascular endothelial growth factor; VSMCs, vascular smooth muscle cells.

Key Words: Aging, Akt/protein kinase B, PKB, Skeletal Muscle, Bone, Cardiovascular System, Neuronal System, Alzheimer's Disease, Respiratory System, Digestive System, Immune System, Urinary System, Adipose Tissue, Review

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