Wnts constitute a large family of secreted proteins that activates evolutionarily conserved intracellular signaling cascades. Wnt signaling regulates diverse cellular responses during embryonic development, as well as various physiological and pathological processes in the adult, including mammalian aging.1–5 The β-catenin–dependent Wnt pathway is the most understood signaling cascade (Figure 1). In the absence of Wnt ligands, cytosolic β-catenin is phosphorylated by glycogen synthase kinase (GSK)-3β and casein kinase 1 in a so-called β-catenin destruction complex, leading to proteasomal degradation of β-catenin. Binding of Wnt ligands to Frizzled (Frz) receptor and low-density LRP5/6 (lipoprotein receptor-related protein 5/6) coreceptor disrupts the β-catenin destruction complex and increases the amount of cytosolic β-catenin. β-Catenin then translocates to the nucleus, where it functions as a cofactor for T-cell factor (TCF) and activates Wnt target genes.1,4 Organismal aging is a complex process characterized by impairment of multiple organ functions and a decline in regenerative capacity following tissue injury and is regulated by multiple factors including genetic backgrounds and environmental stresses. Aging is also associated with various heart diseases, in part, due to the prolonged exposure of the heart to hypertension, hypercholesterolemia, diabetes, and other cardiovascular risk factors. However, there is also an age-dependent increase in the prevalence of left ventricular hypertrophy, diastolic dysfunction, and atrial fibrillation that is not necessarily associated with classic risk factors for cardiovascular diseases.6 Together with the observation that Wnt signaling is involved in mammalian aging, these results suggest a possible mechanistic link between Wnt signaling and heart diseases or heart disorders that are observed in the elderly.

In this part of the review series on Wnt signaling and the cardiovascular system, we first summarize the general aspects
of cellular senescence and organismal aging. We then review the recent reports linking Wnt signaling to cellular senescence, organismal aging, and aging-related phenotypes. Finally, we discuss the possible involvement of Wnt signaling in heart diseases or heart disorders that are associated with aging.

**Cellular Senescence**

Cellular senescence is characterized by the loss of replicative capacity in primary somatic cell culture, and this decline in cellular division capacity seems to be linked to the shortening of telomeres.7 Telomeres are nonnucleosomal DNA–protein complexes located at the ends of chromosomes. As a result of DNA replication, telomeres get shorter as cells divide and act as an intrinsic molecular clock that signals the eventual growth arrest. In certain cancer cells and tissue stem cells, however, an enzyme called telomerase adds telomeres to the ends of chromosomes and enables sustained proliferation.8 It is interesting to note that TERT, a protein component of telomerase, modulates Wnt/β-catenin signaling as a cofactor of TCF,9 although the link between TERT-dependent Wnt activation and cellular senescence remains elusive. Various stress stimuli also induce a replicative senescence-like phenotype in cultured cells. These stimuli include oxidative stress, DNA damage, and constitutive activation of oncogenic signals such as the RAS-RAF-MEK signaling pathway.10–13 These types of cellular senescence are collectively called stress-induced premature senescence.

The p53-p21 pathway and the p16-retinoblastoma protein (p16-pRB) pathway are the signaling pathways that are known to control cellular senescence induced by various stimuli (Figure 2).8,14 p53 and pRB are well-established tumor suppressor proteins and constitute a potent anticancer mechanism. p53 is required for both replicative senescence and RAS-induced senescence.15–17 p53 promotes cellular senescence, at least in part, through the induction of its direct target gene p21, which encodes a potent cyclin-dependent kinase 2 inhibitor p21 that induces cell cycle arrest. p21 expression is increased in senescent cells, overexpression of p21 induces cellular senescence, and disruption of p21 gene results in loss of replicative senescence.14,16,17 pRB is also an important regulator of cell cycle progression. Hypophosphorylated form of pRB inhibits E2F-dependnet transcription necessary for cell cycle progression, and p16 inhibits cyclin-

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**Figure 1. The β-catenin–dependent canonical Wnt pathway.** In the absence of Wnt ligands, cytosolic β-catenin is phosphorylated by GSK-3β and casein kinase 1 (CK1) in a so-called β-catenin destruction complex composed of Axin, APC (adenomatous polyposis coli), CK1, and GSK-3β, leading to proteasomal degradation of β-catenin (left). Wnt stimulation disrupts the β-catenin destruction complex and induces β-catenin stabilization. β-Catenin then translocates to the nucleus, where it functions as a cofactor for TCF and activates TCF–dependent transcription (right). (Illustration credit: Ben Smith/Cosmocyte)
dependent kinase-mediated pRB phosphorylation. In senescent cells, p16 is upregulated, pRB is hypophosphorylated, and E2F target genes are repressed.\textsuperscript{14,16,17} Although inactivation of p16 does not prevent replicative senescence, p16 is required for senescence induced by RAS and other stresses.\textsuperscript{8,16} Thus, it is conceivable that p53-p21 pathway primarily regulates cellular senescence attributable to telomere dysfunction, whereas p16-pRB pathway preferentially regulates stress-induced premature senescence. p16 is upregulated during organismal aging, and the number of p16-positive cells increases with age. Together with senescence-associated β-galactosidase activity,\textsuperscript{18} p16 is a well-established biomarker of senescent cells.\textsuperscript{8,19} In addition to these classic markers of cellular senescence, Binet et al recently reported that Wnt16B is a marker of senescent cells.\textsuperscript{20} Wnt16B is overexpressed in cells undergoing replicative or stress-induced senescence in vitro and those in K-Ras–induced adenomas in vivo, and forced expression of Wnt16B accelerates, whereas knockdown of Wnt16B attenuates, the onset of cellular senescence. However, because these authors failed to detect the activation of Wnt/β-catenin signaling by Wnt16B, the link between Wnt signaling and cellular senescence is not evident in this context.

One intriguing question is whether cellular senescence plays a causal role in organismal aging. As mentioned above, the number of senescent cells detected by p16 expression and/or senescence-associated β-galactosidase activity is increased with age in multiple tissues in humans and rodents.\textsuperscript{8,16,17} Moreover, these cells are observed at sites of various age-related pathologies.\textsuperscript{21–25} Thus, although these observations are correlative and do not directly demonstrate that organismal aging is attributable to an increase in senescent cells, cellular senescence may, in part, contribute to the pathogenesis of aging-related phenotypes or diseases.

Organismal Aging
Organismal aging is usually defined as the progressive decline in the function of multiple organs to maintain baseline tissue homeostasis and to adequately respond to environmental stresses.\textsuperscript{26} Genetic dissection of aging was greatly advanced by the identification of mutations that extend lifespan of the nematode Caenorhabditis elegans,\textsuperscript{27} and it is now evident that aging process in various organisms is regulated by evolutionarily conserved signaling pathways.\textsuperscript{28} The most extensively investigated signaling pathway that regulates lifespan is the insulin/insulin-like growth factor (IGF) pathway.\textsuperscript{29,30} In C elegans, mutations in daf-2 lead to increased activity of FOXO/Daf-16, insulin/IGF signaling therefore promotes organismal aging through down-regulation of FOXO/Daf-16.
encoding an insulin/IGF receptor or those of chico gene encoding an insulin receptor substrate (IRS)-like protein result in lifespan extension.\textsuperscript{36–38} In mice, heterozygous deletion of igf1r gene encoding type1 IGF receptor, adipose tissue-specific deletion of insulin receptor gene, and brain-specific deletion of irs2 gene all result in extension of lifespan.\textsuperscript{39–41} These results collectively suggest that the conserved insulin/IGF-IRS-PI3K-Akt-FOXO signaling axis regulates lifespan in multiple organisms (Figure 3).

Because insulin/IGF signaling downregulates FOXO/Daf-16 through Akt-mediated phosphorylation (Figure 4), mutations that extend lifespan lead to upregulation of FOXO/Daf-16 activity, suggesting that FOXO/DAF-16 targets are downstream effectors that influence lifespan. Candidate FOXO/DAF-16 targets include genes involved in oxidative stress response (superoxide dismutase, catalase), cell cycle arrest (p27), and DNA repair (Gadd45).\textsuperscript{42–45} Of note, overexpression of superoxide dismutase or catalase induces the extension of lifespan in Drosophila,\textsuperscript{46} and overexpression of catalase targeted to mitochondria results in lifespan extension in mice.\textsuperscript{47} Although the target genes of FOXO/DAF-16 that are thought to affect lifespan are increasing in number,\textsuperscript{48,49} it is conceivable that one mechanism of lifespan extension by mutations in insulin/IGF pathway is the increased stress resistance conferred by FOXO/DAF-16.

\section*{Wnt Signaling and Aging}

In 2007, 3 studies directly linked β-catenin–dependent canonical Wnt signaling to cellular senescence or organismal aging.\textsuperscript{50–52} Ye et al provided evidence showing that cellular senescence of human diploid fibroblast cell line WI-38 was triggered by downregulation of Wnt2 gene.\textsuperscript{50} The authors found that Wnt2 gene expression was downregulated in senescent cells and, as a consequence, GSK-3β kinase activity was upregulated and cytosolic β-catenin levels were downregulated in senescent cells. The authors also showed that inhibition of Wnt signaling induced premature senescence, whereas activation of Wnt signaling promoted cell proliferation and delayed replicative or RAS-induced senescence. Mechanistically, downregulation of Wnt2 leads to increased kinase activity of GSK-3β, which results in increased phosphorylation and enhanced recruitment of HIRA to acute promyelocytic leukemia nuclear bodies. HIRA is a histone chaperone, and translocation of this protein to promyelocytic leukemia nuclear bodies is the initial step for the formation of SAHF (senescent-associated heterochromatin foci), which is a distinct heterochromatin structure specifically observed in senescent cells.\textsuperscript{53,54} Taken together, these observations suggest that canonical Wnt signaling inhibits cellular senescence through inactivation of GSK-3β. It remains to be elucidated how Wnt signaling is downregulated in the initial phase of cellular senescence. Furthermore, given the conserved role of insulin/IGF signaling in organismal aging, it will be interesting to test whether HIRA phosphorylation and SAHF formation are increased by insulin/IGF that inactivates GSK-3β through Akt-dependent phosphorylation.

In contrast to Ye et al,\textsuperscript{50} 2 studies demonstrated that canonical Wnt signaling rather promotes mammalian aging and aging-related phenotypes.\textsuperscript{51,52} Liu et al\textsuperscript{51} used the klotho mouse model of accelerated aging, in which klotho gene expression is severely impaired.\textsuperscript{55} Klotho gene encodes a type I transmembrane protein with a large extracellular domain that is cleaved by ADAM family of proteases.\textsuperscript{56} The resultant secreted form of Klotho protein was shown to directly bind to Wnt3 and inhibit canonical Wnt signaling. The activity of Wnt signaling was increased in klotho mice, which was associated with decreased number of stem cell population and increased number of senescent cells in the skin and the small intestine. Moreover, continuous exposure to Wnt3A induced cellular senescence in mouse embryonic fibroblasts, and overexpression of Wnt1 in the skin resulted in increased expression of senescence markers in hair follicle stem cells. These findings suggest that activation of canonical Wnt signaling plays a causal role in premature aging in klotho mice. It should be noted, however, that Klotho protein was shown to inhibit insulin/IGF signaling and mediate FGF23 signaling as a coreceptor for FGF23, and both of these signaling pathways are implicated in the regulation of lifespan and aging-related pathology.\textsuperscript{56–57} Thus, the extent to which the increased Wnt activity contributes to premature aging phenotypes in klotho mice is unclear.

A decline in tissue regenerative capacity is a hallmark of mammalian aging and is, in part, attributed to the impairment of tissue stem/progenitor cell function. It was previously shown that the age-related decline in tissue-specific progenitor cell activity is modulated by factors that are present in the serum.\textsuperscript{59} In line with this study, Brack et al\textsuperscript{52} provided evidence showing that systemic factors in the serum of aged mice activate canonical Wnt signaling and contribute to
age-related decline in skeletal muscle regeneration. The authors showed that skeletal muscle stem cells (satellite cells) convert from a myogenic to a fibrogenic lineage when exposed to aged serum and that canonical Wnt signaling is enhanced in skeletal muscle of aged mice and in cultured satellite cells exposed to aged serum. Moreover, skeletal muscle regeneration in young animals was attenuated by Wnt3A treatment, whereas impaired muscle regeneration in aged mice was restored by inhibition of canonical Wnt signaling. These observations suggest that activation of Wnt signaling by the “Wnt-like substance” present in the serum of aged organisms contributes to a decline in tissue stem cell function and impaired tissue regeneration associated with aging. The nature of this Wnt-like substance in the serum is unknown at present and remains to be elucidated. Because Wnt proteins are tightly associated with the cell surface and/or extracellular matrix and are thought to act in a short-range manner, it is presumed that the Wnt-like substance in the serum are distinct from conventional Wnt proteins.

How can we reconcile the apparent discrepancies among these three studies? The conclusion of the study by Ye et al50 depends solely on the results of cell culture experiments, whereas the conclusions by Liu et al51 and Brack et al52 deal with premature aging or aging-associated phenotypes in mice. This difference in the experimental system may be one explanation for the discrepancy. In addition, it was previously shown that Wnt signaling has pleiotropic and sometimes antagonistic effects on multiple biological processes, depending on the timing, strength, and duration of the signal. For instance, Wnt signaling promotes self-renewal and expansion of hematopoietic stem cells, whereas constitutive activation of this signaling pathway in hematopoietic stem cells resulted in a rapid exhaustion of long-term stem cell pool and widespread hematologic abnormalities. Likewise, it was shown in one study that β-catenin functions as a cofactor for FOXO/DAF-16 and promotes FOXO/DAF-16-dependent resistance against oxidative stress, whereas another study demonstrated that Wnt signaling activates mitochondrial biogenesis and promotes the production of reactive oxygen species. It was also shown in young animals that Wnt activation is required for myogenic specification of CD45+ resident stem cells during muscle regeneration and that Wnt signaling promotes de novo hair follicle regeneration after skin wounding, suggesting that downstream targets of Wnt signaling may change with age. Taken together, another explanation for the discrepancy among the 3 studies mentioned above may be the cell-, tissue-, and stage-specific pleiotropic effects of Wnt signaling on cellular senescence, organismal aging, and tissue regeneration (Figure 5).

**Wnt Signaling and Age-Related Heart Disorders**

The incidence of left ventricular hypertrophy, diastolic dysfunction, and atrial fibrillation increase with age.6 Aging is also associated with an increase in intimal thickening and vessel stiffness that precede clinical diseases.68 The observation that some systemic factors activate Wnt pathway in aged animals suggests that cells in the aged heart are also targets of Wnt signaling.51 Indeed, aged TOPGAL mice (a transgenic mice in which β-galactosidase transgene is under the control of multimerized TCF binding sites) exhibit increased β-galactosidase expression in the heart, indicative of increased activity of Wnt signaling in the aged heart (our unpublished observation, 2009).

**Wnt Activation in Myocytes**

Cardiomyocyte hypertrophy is one of the characteristic features of aged heart. It was previously shown that stabilization of β-catenin promotes myocyte growth and is required for both physiological and pathological cardiac hypertrophy.69,70 Subsequently, activation of Wnt signaling in cardiac myocytes in vivo was achieved by cardiomyocyte-specific conditional deletion of exon 3 of the β-catenin gene, which renders β-catenin resistant to GSK-3β-mediated phosphorylation/degradation and results in β-catenin stabilization.71 These mice exhibit impaired cardiac growth with normal contractile function at baseline and attenuated hypertrophic growth response and impaired contractility following continuous angiotensin II infusion.71 Thus, contrary to the previous studies, it was suggested that Wnt activation in cardiac myocytes results in attenuated response to hypertrophic stimuli and failure to undergo adaptive remodeling under stressed conditions. The precise reason for such discrepancy is unknown but may reflect the pleiotropic effects of Wnt signaling depending on the experimental conditions. It should also be noted that downregulation of Wnt/β-catenin signaling in cardiac myocytes is implicated in fibrofatty replacement of the myocardium observed in arrhythmogenic right ventricular cardiomyopathy.72,73 Whether Wnt activation in cardiac myocytes contributes to aging-associated disorders in the heart remains to be elucidated.
Wnt Activation in Fibroblasts
Cardiac fibrosis is another feature of the aged heart and is linked to the pathogenesis of atrial fibrillation and diastolic dysfunction, typical clinical features of the aged heart. It was shown that Wnt signaling is activated in type II lung epithelial cells and plays a causal role in the pathogenesis of lung fibrosis. Likewise, Wnt signaling is upregulated in the α-smooth muscle actin–expressing activated fibroblasts in the kidney, and Wnt inhibition prevents fibrosis after renal injury. These findings suggest that chronic activation of Wnt signaling induces transformation of fibroblasts into activated fibroblasts or myofibroblasts and contributes to cardiac fibrosis in the aged heart.

Wnt Activation in Vascular Cells
Vascular calcification is one of the features of age-related alterations in vascular structure, and fate-mapping studies revealed that smooth muscle cells are the origin of osteochondrogenic precursors in arterial calcification. It was shown that an osteogenic transcription factor Msx2 in adventitial myofibroblasts upregulates the expression of Wnt agonists and that these Wnt agonists act in a paracrine fashion on vascular smooth muscle cells and induce osteogenic differentiation of these cells by activating Wnt/β-catenin pathway. It was also shown that lithium, a GSK-3β inhibitor and therefore an activator of Wnt signaling, induces endothelial cell senescence, although this effect appears to be independent of GSK-3β inhibition by lithium.

Wnt Activation in Cardiac Resident Stem Cells
In contrast to a classic view that the heart is a postmitotic organ, accumulating evidence suggests that the myocardium has the potential to regenerate and self-renew. It was previously shown by a genetic fate-mapping technique in mice that stem or progenitor cells contribute to the renewal of adult heart cells after injury but do not play a significant role in cardiomyocyte renewal during normal aging. Subsequently, by measuring the integration of carbon 14 generated by nuclear bomb tests during the Cold War into cardiomyocytes, it was found that human heart has the capability to generate new cardiomyocytes, although with a low turnover rate. Moreover, it was shown that mammalian myocardium contains a small number of resident cardiac stem or progenitor cell population, characterized by the expression of specific markers. Those include c-kit+, Sca-1+, islet1+, and side-population cells. It is therefore conceivable that resident stem/progenitor cells in the adult heart contribute to myocyte renewal during aging and after injury, although the situation may be slightly different in zebrafish heart, in which myocardial regeneration after injury occurs primarily through expansion of preexisting myocytes. Aging-associated decline in organ function is attributed, at least in part, to the aging of stem/progenitor cells in various tissues, including bone marrow, pancreas, and the brain. In the heart, it was shown that the number of c-kit+ cardiac stem cells was increased and that the percentage of stem cells that express a senescence marker p16 was higher in aged animals. Apoptotic cardiac stem cells were p16-positive, and the number of apoptotic stem cells was increased in aged hearts, resulting in a decline in the number of functional cardiac stem cells. Of note, IGF signaling attenuated the aging-associated decline in stem cell function. Although this is inconsistent with the notion that the reduction in insulin/IGF signaling extends lifespan in multiple organisms, a similar observation was made in skeletal muscle expressing IGF-1 transgene.

Whether Wnt activation in resident cardiac stem cells plays a causal role in aging-associated heart disorders is presently unknown. However, it was shown that genetic ablation of β-catenin in the second heart field results in the loss of second heart field-derived tissues and that stabilization of β-catenin in the second heart field results in massive accumulation of isl1+ progenitors and inhibition of further differentiation of these cells into mature cardiomyocytes. Thus, Wnt signaling promotes expansion of isl1+ cardiac progenitor cells and subsequently inhibits further differentiation into mature cell types in the heart. This may partly explain why the number of cardiac stem/progenitor cells is increased, whereas the function of those cells to differentiate into mature cardiomyocytes is declined in the aged heart.

Concluding Remarks
Our present knowledge on the role of Wnt signaling in aging-related malfunction of the heart is extremely limited. However, the observation that systemic factors in the serum of aged animals activate Wnt signaling and promote aging-related phenotypes at least in some tissues suggests that the aged heart is also a target of Wnt signaling. Further studies will be required to delineate whether aging-associated disorders in the heart are caused by enhanced Wnt signaling and whether inhibition of Wnt signaling is a novel therapeutic strategy for heart diseases in the elderly.

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