Heart Failure as an Aging-Related Phenotype
Wnt/β-Catenin Signaling and p53 Pathway
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Summary
The molecular pathophysiology of heart failure, which is one of the leading causes of mortality, is not yet fully understood. Heart failure can be regarded as a systemic syndrome of aging-related phenotypes. Wnt/β-catenin signaling and the p53 pathway, both of which are key regulators of aging, have been demonstrated to play a critical role in the pathogenesis of heart failure. Circulating C1q was identified as a novel activator of Wnt/β-catenin signaling, promoting systemic aging-related phenotypes including sarcopenia and heart failure. On the other hand, p53 induces the apoptosis of cardiomyocytes in the failing heart. In these molecular mechanisms, the cross-talk between cardiomyocytes and non-cardiomyocytes (e.g., endothelial cells, fibroblasts, smooth muscle cells, macrophages) deserves mentioning. In this review, we summarize recent advances in the understanding of the molecular pathophysiology underlying heart failure, focusing on Wnt/β-catenin signaling and the p53 pathway.

Key words: Senescence, Sarcopenia, Apoptosis, DNA damage

Heart failure, which is a complex pathophysiological syndrome, is one of the leading causes of mortality in the world. The prevalence of heart failure is predicted to increase markedly (46% in the United States from 2012 to 2030) due to aging of the population. A better understanding of the underlying molecular pathophysiology, in particular, common molecular pathway towards heart failure, is needed.

In the cardiovascular area, there is an age-dependent increase in the prevalence of left ventricular hypertrophy, diastolic dysfunction, and atrial fibrillation, which are not necessarily associated with classical risk factors for cardiovascular diseases. There is also an aging-related increase in vascular intimal thickening and vessel stiffness. In addition, maladaptation and/or abnormal response to stress (e.g., pathological hypertrophy, apoptosis, replacement fibrosis, progression to heart failure) can be aging-related. Taken together, cardiovascular diseases can be partly regarded as aging-related phenotypes. From this viewpoint, in this review, we will discuss the fundamental molecular mechanisms underlying the onset and/or development of heart failure focusing on the molecular pathways related to the aging process, although a plethora of other molecular mechanisms for heart failure are being identified. In particular, we will review the contribution of Wnt/β-catenin signaling and p53 pathway, both of which play an important role in aging, to the progression of cardiac remodeling and dysfunction in the failing heart.

Wnt/β-Catenin Signaling and Aging
In an analysis of the klotho mouse model of accelerated aging, the activation of Wnt/β-catenin signaling was shown to cause premature aging. Basically, Wnt signaling is initiated by the Wnt receptor Frizzled and the coreceptors LRPS/6 and consists of β-catenin dependent canonical and β-catenin independent non-canonical pathways. The canonical pathway requires Frizzled and LRP 5/6, whereas the non-canonical pathway only requires Frizzled. In the canonical pathway, upon Wnt stimulation, cytosolic β-catenin is stabilized and translocates to the nucleus, where it binds to T cell factor/Lymphoid enhancer factor (TCF/LEF) and induces TCF/LEF-dependent transcription of Wnt responsive genes (Figure 1). Wnt/β-catenin signaling plays critical roles in stem cell self-renewal, development as well as adult homeostasis, and augmented Wnt/β-catenin signaling is also implicated in aging, aging-related phenotypes, and various diseases.

Aging-Promoting Substances
An experiment using parabiotic pairings between young and aged mice (heterochronic parabioses) suggested that there should be systemic factors changing with age which can modulate the age-related decline of progenitor cell activity. These aging-promoting substances in the serum from aged mice were shown to bind to the Frizzled family of proteins, activating Wnt/β-catenin signaling. Because the classical Wnt ligands (e.g., Wnt3A) are hydro-
Cleavage of the extracellular domain of Wnt co-receptor LRP6. This cleavage is consequently induces C1s-dependent cleavage of the extracellular domain of Wnt co-receptor LRP6, results in the recruitment of β-catenin destruction complex to the receptors, followed by the stabilization of β-catenin. Then, cytosolic β-catenin accumulates and translocates into the nucleus, where it functions as a cofactor for T-cell factor (TCF) and activates TCF-dependent transcription of Wnt responsive genes (quoted from Clevers H, et al. Cell 2012; 149: 1192-1205.8).

Figure 1. Wnt/β-catenin signaling. A: In the absence of Wnt ligands, cytosolic β-catenin is phosphorylated by glycogen synthase kinase 3 (Gsk3) and casein kinase 1 (Ck1) in a β-catenin destruction complex composed of Axin, adenomatous polyposis coli gene product (Apc), Gsk3β, and Ck1, targeting β-catenin for β-Trcp (an E3 ubiquitin ligase subunit)-mediated ubiquitination and its subsequent degradation by the proteasome. B: In the presence of Wnt ligands, the formation of the Wnt-Frizzled receptor (Fz)-low-density lipoprotein receptor-related protein 5/6 (Lrp5/6) co-receptor complex, together with the recruitment of the scaffolding protein Dishevelled (Dvl), results in the recruitment of β-catenin destruction complex to the receptors, followed by the stabilization of β-catenin. Then, cytosolic β-catenin accumulates and translocates into the nucleus, where it functions as a cofactor for T-cell factor (TCF) and activates TCF-dependent transcription of Wnt responsive genes.

Figure 2. Wnt/β-catenin signaling induced by C1q. Upon binding to Frizzled receptor (Fz), C1q activates C1r/C1s, which results in Lrp5/6 cleavage and activation of Wnt/β-catenin signaling (modified from Clevers H, et al. Cell 2012; 149: 1192-1205.8).

phobic glycolipoproteins, and tightly bind to the cell surface and/or extracellular matrix, the aging-promoting substances in the serum that could systemically activate Wnt/β-catenin signaling were assumed to be distinct from such “classical” Wnt ligands.

As a protein binding to the cysteine-rich domain of Frizzled 8 in the serum from mice with heart failure, complement C1q was found to be a novel activator of canonical Wnt/β-catenin signaling, and responsible for an age-dependent increase in β-catenin activity and aging-related phenotypes including impaired tissue regeneration. With aging, serum C1q concentration is increased, and accordingly, Wnt/β-catenin signaling activities are augmented in tissues of various organs, which are not observed in C1qa-deficient mice. C1q binds to Frizzled 8 receptor and subsequently induces C1s-dependent cleavage of the extracellular domain of Wnt co-receptor LRP6. This cleavage is essential for aging-associated activation of Wnt/β-catenin signaling triggered by C1q (Figure 2). Skeletal muscle regeneration in young (2 months old) mice is inhibited by C1q treatment, whereas impairment of skeletal muscle regeneration via attenuated satellite cell proliferation and stimulated fibroblast proliferation in aged (2 years old) mice is restored by C1s inhibition (with neutralizing antibody M241) or C1qa gene disruption, independently of the classical complement pathway activity.

C1q Secreted from Macrophages Infiltrating into Injured Tissue

Our next question is where Wnt ligand C1q comes from. In injured skeletal muscles, C1q secreted by macrophages activates Wnt/β-catenin signaling, suppressing muscle regeneration and repair. The angiotensin II type1 receptor blockade with irbesartan treatment was shown to reduce C1q expression in cryoinjured muscles and in cultured macrophages. Consequently, the irbesartan treatment could improve skeletal muscle regeneration after injury through a decrease in macrophage-derived C1q followed by inactivated Wnt/β-catenin signaling.

In the aortic tissues from 1-week angiotensin II-infused hypertensive mice, activation of Wnt/β-catenin
signaling in vascular smooth muscle cells (VSMCs) was shown to cause proliferation of VSMCs and pathological arterial remodeling, which are attenuated by macrophage depletion or C1qa gene disruption. Macrophage-derived C1q and VSMC-derived C1r/s might compose the C1 complex. Under hypertensive conditions, M2-type macrophages recruited into the aortic adventitia secrete complement C1q, which induces Wnt/β-catenin signaling-dependent proliferation of VSMCs in the aortic media, resulting in the progression of hypertension-induced pathological arterial remodeling (Figure 3).

Taken together, C1q secreted from macrophages infiltrating into the affected tissues could locally exert its pathological properties through the activation of Wnt/β-catenin signaling. Further studies are warranted to clarify how C1q systemically or locally contributes to the promotion of aging-related phenotypes. In addition, the molecular mechanisms of the Wnt/β-catenin-related pathological responses to various stimuli in various affected tissues should be investigated.

**Wnt/β-Catenin Signaling in the Pathogenesis of Sarcopenia**

Sarcopenia is considered to be a syndrome characterized by progressive loss of skeletal muscle mass and strength with advancing age. Sarcopenia leads to disabilities, reduced activities of daily living, reduced quality of life, a higher risk of falls and fractures, and eventually an increased risk of mortality. Along with the imbalances between muscle protein synthesis and degradation, changes in nutritional intake and hormone levels, reduction in physical activity, increased oxidative stress and inflammation, age-related impairment of muscle regenerative potential of skeletal muscle could account for the pathogenesis of sarcopenia. Activation of the Wnt/β-catenin signaling pathway in aged myogenic progenitors was found to play a pivotal role in the age-related decline of myogenic activity followed by an increase in replacement fibrosis. As shown above, in aged mice, increased C1q activates Wnt/β-catenin signaling in skeletal muscle, leading to the impairment of skeletal muscle regeneration. In humans, serum C1q can be a novel biomarker of sarcopenia. A correlation was observed between serum C1q levels and aging-related reduction of muscle mass and strength even after adjustment for serum concentrations of TNF-α and IL-6. More interestingly, 12-week resistance training in 11 healthy older adults (60-81 years old) decreased serum C1q levels, and the training effects on serum C1q levels were significantly correlated with the changes in the cross-sectional area of the thigh.

Recently, C1q-induced activation of Wnt/β-catenin signaling in skeletal muscle during chronic heart failure was shown to contribute to skeletal myopathy via the functional interaction between β-catenin and transcription factor Forkhead box O1 (FoxO1). In cultured C2C12 mouse myoblasts, serum of DCM model mice (knock-in mice with deletion mutation K210 in cardiac troponin T gene) activated both Wnt and FoxO signaling and induced a fiber type shift of myosin heavy chain towards fatigable fiber IIb (encoded by Myh4) (Figure 4) as were observed in the skeletal muscle of DCM model mice. The Wnt inhibitor DKK1 and a C1 inhibitor attenuated FoxO signaling and fiber type shift both in C2C12 cells and skeletal muscle of DCM model mice, suggesting that systemically-increased Wnt ligands (e.g., C1q) contribute to sarcopenia in patients with chronic heart failure. Collectively, heart failure can be regarded as a systemic syndrome of aging-related phenotypes induced by activation of Wnt/β-catenin signaling.
**Wnt/β-Catenin Signaling in the Heart**

What is the relevance of Wnt/β-catenin signaling to cardiac dysfunction? In the failing heart, the expression levels of Axin2, c-Myc, Nkd-1, Nkd-2, Wisp1, and Wisp2 are increased, that is, Wnt/β-catenin signaling is activated. In a previous report, cardiac-specific overexpression of Dvl-1 (Dishevelled-1), which is a positive regulator of Wnt/β-catenin signaling, was found to cause extensive hypertrophy, heart failure, and premature death in mice.\(^\text{19}\) On the other hand, it was reported that stabilization of β-catenin attenuates adaptive cardiac hypertrophy and leads to impaired cardiac function under angiotensin II treatment.\(^\text{20}\) The Wnt1/β-catenin injury response activated cardiac fibroblasts to promote cardiac repair after acute ischemic cardiac injury, preserving cardiac function.\(^\text{21}\) In other reports, blocking of Wnt/β-catenin signaling was shown to avert adverse remodeling or improve cardiac function in animal models of myocardial infarction.\(^\text{22-25}\) In spite of such a context-dependency, Wnt/β-catenin signaling is thought to play a pivotal role in the progression of cardiac dysfunction/heart failure.\(^\text{26}\)

![Figure 4. Functional interaction between β-catenin and Forkhead box O1 (FoxO1) in skeletal muscle cells during chronic heart failure. Wnt ligands (eg. C1q) activate Wnt/β-catenin signaling, leading to β-catenin translocation into the nucleus with FoxO1. Activated FoxO1 promotes fiber type shift of myosin heavy chain towards type IIB fatigable fiber. TCF; T cell factor (modified from Okada K, et al. Circ Heart Fail 2015; 8: 799-808.\(^\text{18}\)).](image-url)

In the canonical Wnt/β-catenin pathway, DKK1 (Dickkopf1) basically inhibits the formation of a ternary complex consisting of LRP5/6, Frizzled, and the Wnt ligand followed by inhibition of the canonical Wnt/β-catenin pathway. DKK1 itself is a target gene of Wnt/β-catenin signaling, thereby establishing a negative feedback loop.\(^\text{7}\) Recently, it was shown that LRP5/6 can directly bind to Frizzled and inhibit the Frizzled-regulated non-canonical pathway.\(^\text{27}\) In ischemic injury, LRP5/6 and β-catenin play a crucial role in attenuating and enhancing H₂O₂-induced DNA damage in cultured cardiomyocytes, respectively. Accordingly, DKK1 enhanced but IGFBP-4 prevented H₂O₂-induced DNA damage in vitro, findings that are consistent with the opposing effects of DKK1 and IGFBP-4 on ischemic injury in vivo.\(^\text{28}\)

 Basically, IGFBP-4 is expressed at later stages of embryogenesis, when the heart is already formed at the ventral portion and starts to grow and remodel to maintain embryonic circulation.\(^\text{29}\) Wnt/β-catenin signaling is known to exhibit developmental stage-specific biphasic effects on cardiomyogenesis in murine ES cells: Wnt signaling promotes cardiomyocyte differentiation in the early phase, whereas it inhibits cardiomyocyte differentiation in the late phase.\(^\text{30}\) In agreement with these findings, IGFBP-4 promotes cardiomyocyte differentiation via the suppression of Wnt signaling at later stages of embryogenesis. IGFBP-4 could potentially exert cardiomyogenic properties in adult hearts in the same manner as that observed at later stages of embryogenesis.

Of note, serum from 2 different mouse models of
heart failure (pressure overload and DCM) more potently increased Wnt/β-catenin signaling activities than serum from aged mice, indicating that activators of canonical Wnt/β-catenin signaling (e.g., C1q) are secreted increasingly under heart failure. The downregulation of basal Wnt/β-catenin signaling (expression levels of Axin2 mRNA) in C1q-deficient mouse hearts suggests that C1q as well as the classical Wnt ligands activate Wnt/β-catenin signaling even in unaffected hearts. In the failing heart, increased C1q could activate Wnt/β-catenin signaling. Although these findings clearly show us that activators of Wnt/β-catenin signaling, including C1q, induce heart failure as a systemic syndrome of aging-related phenotypes, the extent to which C1q plays a pathophysiological role as one of the potent Wnt ligands in the failing heart remains to be further elucidated.

**Wnt/β-Catenin Signaling in Endothelial Cells and Angiogenesis**

Endothelial function is impaired in the failing heart and its dysfunction might induce heart failure, whose molecular mechanisms remain to be investigated. Activation of Wnt/β-catenin signaling in endothelial cells is known to promote proliferation and migration of vascular endothelial cells during neovascularization in the infarcted myocardium. However, the same Wnt factor or receptor may induce distinct and sometimes opposite responses in endothelial cells depending on the context. Recently, Wnt/β-catenin signaling in endothelial cells was found to play a critical role also in the pathophysiology of heart failure. Sustained activation of Wnt/β-catenin signaling in arterial endothelial cells and endocardium in Bmx/CA mice (endothelial cell-specific, tamoxifen-inducible β-catenin stabilized mice) could cause severe heart failure without involvement of cardiomyocyte death, cardiac ischemia, inflammatory changes, or fibrosis. Electron microscopic analysis revealed characteristic changes such as dilatation of T-tubules and degeneration of mitochondria in cardiomyocytes, which are similar to the changes in hearts of cardiomyocyte-specific ErbB2 receptor or ErbB4 receptor conditional knockout mice, suggesting that ErbB signaling might be involved in the pathophysiology of cardiac abnormalities of Bmx/CA mice. Activation of Wnt/β-catenin signaling in endothelial cells suppresses the expression/secretion of neuregulin-1 in endothelial cells. Low neuregulin-1 levels suppress ErB signaling in cardiomyocytes and induce heart failure, which can be ameliorated by administration of recombinant neuregulin-1 protein. Like this, endothelial cells might play a primary role in the pathophysiology of heart failure. Moreover, in heart failure, the decrease in secretion of VEGF (vascular endothelial growth factor) and angiopoietin-1 from cardiomyocytes induces the dysfunction of endothelial cells (Figure 5). Taken together, a cross-talk between cardiomyocytes and arterial endothelial cells profoundly contributes to the development of heart failure.

**p53 Signaling and Aging**

The p53 pathway also plays an important role in the pathophysiology of heart failure through the induction of aging-related phenotypes. Replicative senescence induced by telomere dysfunction and stress-induced premature senescence are mediated by p53- and pRB (retinoblastoma protein)-dependent pathways. p21 encoded by CDKN1A is a direct target of p53 and mediates p53-induced cell cycle arrest. p16 inhibits pRB phosphorylation, which results in the inhibition of E2F-dependent gene transcription essential for cell cycle progression. A critical tumor suppressor protein, p53, activates a cellular response to stress signals (e.g., DNA damage) that leads to a halt in proliferation via apoptosis or senescence. Such a depletion of cells could compromise the structure and function of tissues, which are the processes towards aging-related phenotypes and tumor suppression. In particular, because cardiomyocytes do not proliferate after birth, p53 exerts a pathogenic effect on cardiomyocytes through the induction of apoptosis.

**p53 Induces Cardiomyocyte Apoptosis and Heart Failure**

p53 induces apoptotic cell death which accelerates the progression of heart failure. The number of cardiomyocytes positive for TUNEL (TdT-mediated dUTP nick end labelling) is significantly increased in the late phase of pressure overload, and this increase is attenuated by p53 deficiency. As in the hypertrophied heart, p53 is accumulated under hypoxic stress in the heart after myocardial infarction. Downregulation of CHIP (carboxyl terminus of heat shock protein 70 interacting protein) under hypoxia is responsible for this p53 accumulation followed by cardiomyocyte apoptosis. In rat hearts after myocardial infarction, a significant downregulation of mitochondrial ALDH2 (aldehyde dehydrogenase 2) was shown to induce an elevation of the reactive aldehyde 4-hydroxy-2-nonenal, which enhances cardiomyocyte apoptosis through downregulation of heat shock protein 70 and activation of JNK and p53 and thereby promotes the development of
DNA Damage Causes Heart Failure

DNA damage and the subsequent activation of the DNA damage response (DDR) are observed in failing hearts. Although the relevance of oxidative DNA damage to heart failure has been clearly demonstrated in doxorubicin-induced cardiomyopathy, it is unknown whether DNA damage is the cause of heart failure. In the experiments using fibroblasts, p53 induced by a DNA damage response was shown to associate with the promoters of PPARγ coactivator1-α (PGC1α) and PGC1β to repress expression of these genes, leading to the inhibition of mitochondrial biogenesis and function, which consequently increases reactive oxygen species (ROS) production, forming a positive feedback loop. It remains to be elucidated whether such a positive feedback loop between oxidative stress, DNA damage, and p53 accumulation exists also in cardiomyocytes, playing a critical role in the pathophysiology of heart failure.

Under pressure overload, the accumulation of unrepaired DNA single-strand break (SSB)-induced DNA damage was shown to activate the DDR, thereby causing heart failure. DNA double-strand breaks were not increased. Pressure overload induced more severe heart failure in mice lacking XRCC1, an essential SSB repair enzyme, as compared with the control mice, suggesting the causative role of SSB accumulation and DDR activation in the pathogenesis of pressure overload-induced heart failure. In neonatal rat cardiomyocytes with knockdown of Xrcc1, which is an in vitro model of accumulation of SSB, induced phosphorylation of ATM as well as H2AX and p53. In this in vitro model, the NF-kB pathway was activated and the expression of inflammatory cytokines was increased, which were abolished by simultaneous knockdown of Atm, suggesting that ATM is essential for SSB accumulation-induced acquisition of inflammatory phenotype. After pressure overload, the extent of the increase in ROS production in the hearts of mice lacking XRCC1 was similar to that in the hearts of control mice, whereas cardiac inflammation was significantly exacerbated by Xrcc1 deficiency. Also, ATM gene deletion was shown to rescue the increased cardiac inflammation and severe heart failure after pressure overload in the mice lacking XRCC1, suggesting that ATM plays a critical role in DDR activation followed by accumulation of SSB.

The Interplay between Wnt/β-Catenin Signaling and p53 Pathway

In this review, we have summarized recent advances in understanding the molecular pathophysiology of heart failure, focusing on the contribution of Wnt/β-catenin signaling and the p53 pathway, both of which are key regulators of aging. It is not surprising that there might be a tight interplay between these molecular pathways. In mouse embryonic stem cells, p53 was shown to induce the transcription of many Wnt ligand genes upon DNA damage to delay the differentiation of neighboring cells. In cancer cells, p53 inhibits the epithelial-to-mesenchymal transition-like process which allows the initiation of metastases during tumor progression. Concordantly, in metastatic tumors compared with non-metastatic tumors, the p53 pathway is down-regulated, whereas the Wnt and Notch pathways are significantly activated. In cancer cells, p53-mediated pro-apoptotic degradation of β-catenin has been reported, which might explain the concomitant reciprocal changes in the p53 and Wnt/β-catenin pathways. However, in the cardiovascular area, there is a dearth of experimental findings on the interplay between the p53 and Wnt/β-catenin pathways.

Conclusions

Heart failure can be regarded as a systemic syndrome of aging-related phenotypes. Wnt/β-catenin signaling plays a critical role in the pathogenesis of heart failure. Circulating C1q was identified as a potent activator of Wnt/β-catenin signaling, promoting systemic aging-related phenotypes including sarcopenia and heart failure. Also, p53 mediates stress-induced premature senescence in the heart, for example, inducing the apoptosis of cardiomyocytes. In the understanding of the molecular pathophysiology of heart failure, the cross-talk between cardiomyocytes and non-cardiomyocytes deserves mention. Further investigations with multidisciplinary approaches will be required to fully clarify the molecular mechanisms underlying heart failure.

Disclosures

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