Cardiokines
Recent Progress in Elucidating the Cardiac Secretome
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The sets of proteins secreted from cells, ie, secretomes, play crucial roles in intercellular and intertissue communication during tissue development and growth and in response to various pathological stresses. These secretomes produced by the heart encompass a group of proteins that have been referred to as cardiokines. In today's era of molecular sophistication, the number of identified cardiokine candidates is steadily increasing as a result of the desire to identify new biomarkers and targets for cardiovascular disease treatment. It is widely recognized that the cells of the heart, including myocytes, fibroblasts, vascular cells, and progenitor cells, secrete various subsets of regulatory proteins in response to changes in the cardiac environment.1–5 These secreted proteins are required for the maintenance of normal cardiac function, and they control pathological remodeling of the myocardium in response to injury through their ability to modulate myocyte death, fibroblast activation, inflammation, and vascular growth and regression. In addition, some of these factors function systematically, influencing kidney function or cachectic processes.

Examples of well-known cardiokines include atrial natriuretic peptide and brain natriuretic peptide, which are synthesized mainly in the myocardium and upregulated in response to myocardial stretching.6,7 Both atrial natriuretic peptide and brain natriuretic peptide exert beneficial actions on cardiac remodeling by directly affecting cardiac cells in an autocrine and/or paracrine manner. In addition, atrial natriuretic peptide and brain natriuretic peptide influence electrolyte and water excretion in the kidney and regulate vascular tone and vascular cell growth via endocrine mechanisms.7 Cardiac cells produce tumor necrosis factor-α and transforming growth factor-β1 in pathological states, and these factors can promote pathological myocardial remodeling by promoting the recruitment of inflammatory cells or by facilitating hypertrophic growth and fibrosis.8,9 Under conditions of stress, the heart also produces angiotensin II, contributing to cardiac hypertrophy and fibrosis. Recent studies have shown that angiotensin II has a catabolic effect on skeletal muscle, suggesting that it may contribute to muscle wasting in congestive heart failure.10,11

Recently, a number of studies have used gene expression, array screening, cloning, and other techniques to identify new cardiokines and cardiokine networks that are regulated during cardiac stress. With mouse genetic approaches, many of these newly identified factors have been shown to have functional roles in pathological cardiac remodeling. The discovery and characterization of novel cardiokines are of interest because they will lead to a better mechanistic understanding of how alterations in cell-cell communication contribute to heart disease and could identify new diagnostic and therapeutic targets. This brief review focuses on the regulation and function of a few of the more recently identified cardiokines.

Growth Differentiation Factor-15 as a Cardioprotective Cardiokine

Growth differentiation factor (GDF)-15, also referred to as macrophage-inhibitory cytokine 1, is a member of the transforming growth factor-β superfamily.12 GDF-15 was identified as a nitric oxide–inducible gene in cardiac myocytes by microarray analysis.13 GDF-15 is upregulated in cultured cardiomyocytes and murine heart in response to ischemia or ischemia/reperfusion, and GDF-15 expression is enhanced in infarcted human heart.13 GDF-15–deficient mice show increased infarct size and cardiomyocyte apoptosis after myocardial ischemia/reperfusion, and GDF-15 protects cardiomyocytes from apoptosis via activation of PI3K-Akt signaling. GDF-15 expression is also induced in murine heart in response to pressure overload, and cardiac-specific overexpression of GDF-15 leads to attenuation of the cardiac hypertrophic response via activation of SMAD2/3 signaling.14 More recently, it was shown that ablation of GDF-15 in mice contributes to an increased frequency of cardiac rupture after myocardial infarction, which is accompanied by an increased infiltration of polymorphonuclear leukocytes in the infarct area.15 GDF-15 was shown to reduce polymorphonuclear leukocyte recruitment through direct modulation of chemokine signaling and integrin activation, promoting the resolution of inflammation. Collectively, GDF-15 functions as a stress-inducible cardiokine that protects against the pathological myocardial remodeling in response to ischemia or pressure overload, indicating that GDF-15 represents a potential target for various heart diseases.

Clinically, circulating GDF-15 levels are elevated in patients with acute coronary syndromes (ACS), and high circulating GDF-15 concentrations are associated with increased risks of mortality, recurrent myocardial infarction, and adverse events in patients with ACS.16–18 Circulating GDF-15 levels are also predictive of adverse outcomes of invasive procedures in ACS patients.18 Measurement of GDF-15 enhances the predictive value of the Global Registry

Circulation is available at http://circ.ahajournals.org
DOI: 10.1161/CIRCULATIONAHA.112.150656

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(Circulation. 2012;126:e327-e332.)

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of Acute Coronary Events (GRACE) score for death and myocardial infarction in ACS patients. An increased level of GDF-15 is also associated with an increased risk of death in patients with heart failure. Therefore, GDF-15 acts as a useful prognostic biomarker of heart diseases, and measurement of GDF-15 levels provides information to monitor disease severity and to predict cardiovascular risk.

Recently, Widera and coworkers performed a systematic survey of secreted proteins that activate GDF-15 expression. In this approach, they used a promoter region fragment of the GDF-15 gene fused to a luciferase reporter gene to screen an infarcted mouse heart cDNA expression library. This procedure led to the identification of a cDNA encoding the secreted glycoprotein follistatin-like 1 (Fstl1; discussed later in greater detail). Studies with transgenic and knockout mouse models revealed that Fstl1 is essential and sufficient for GDF-15 expression in mouse heart. It was also shown that, like GDF-15, FSTL1 is prognostic for cardiovascular death in ACS and that serum levels of FSTL1 and GDF-15 are independently and closely associated with one another in this patient population. Collectively, these data identify GDF-15 and FSTL1 as components of a “biomarker network” and underscore the potential importance of this biological pathway in cardiovascular disease.

**Myostatin as a Mediator of Cachexia in Heart Failure**

Myostatin, also known as GDF8, is a member of the transforming growth factor-β superfamily proteins that classically acts as an inhibitor of skeletal muscle growth. Myostatin is abundantly expressed in skeletal muscle, and systemic overexpression of myostatin in mice can induce cachexia such as body and muscle wasting. In this regard, cachexia is associated with an increased risk for mortality in heart failure, and it has been reported that myostatin is also expressed in the heart and upregulated in failing human hearts.

A recent report has identified myostatin as a cardiokine that can mediate skeletal muscle wasting during heart failure. In this mouse study, cardiac myocyte–specific deletion of the myostatin gene has no effects on baseline heart or skeletal muscle size. Circulating myostatin levels were found to be elevated in response to pressure overload in wild-type mice but not in cardiac myocyte–specific myostatin knockout mice, indicating that the injured heart is a major source of circulating myostatin under these conditions. Of importance, cardiac myocyte–specific ablation of myostatin was reported to prevent cachexic changes, including muscle wasting and atrophy in heart failure, without alterations in cardiac function and hypertrophy. Conversely, cardiac myocyte–specific overexpression of myostatin was shown to promote muscle wasting in mice. The injection of a myostatin-blocking antibody in preexisting heart failure maintained muscle mass. Collectively, these data indicate that myostatin is a potential target molecule for the prevention or treatment of cachexia during heart failure.

**Role of Activin A and Fstl3 in Cardiac Injury and Growth**

Like GDF-15 and myostatin, activin A is a member of the transforming growth factor-β superfamily that promotes muscle wasting. Activin A binds to the activin IIB receptor at the cell membrane, leading to the activation of SMAD2/3 signaling. Both follistatin and Fstl3 bind to activin A and antagonize its function. Activin-A and Fstl3 but not follistatin show marked upregulation in heart in response to ischemic injury on pressure-overload hypertrophy in mice. Activin A protects cardiac myocytes from apoptosis under conditions of hypoxia/reoxygenation in vitro, and systemic delivery of activin A prevents cardiac injury after ischemia/reperfusion in vivo. Overexpression of Fstl3 abolishes the ability of activin A to activate SMAD2/3 and to promote cardiomyocyte survival, whereas cardiac-specific ablation of Fstl3 protects against myocardial ischemia/reperfusion injury in mice. In a model of myocardial pressure overload, cardiac-specific Fstl3-deficient mice exhibit attenuated cardiac hypertrophy after pressure overload. Corroborating these findings in mice, it was shown that activin A suppresses hypertrophic responses in cultured cardiac myocytes and that this effect is reversed by overexpression of Fstl3. It has also been shown that myocyte-derived Fstl3 affects cardiac fibrosis by activating fibroblast proliferation and collagen production. Collectively, these findings indicate that both activin A and Fstl3 are cardiokines with opposing actions that function to regulate myocardial growth, fibrosis, and the response to ischemic stress. Furthermore, because heterozygous activin A–deficient mice have increased skeletal muscle mass, it is conceivable that heart-derived activin A may influence the cardiac cachexic states by acting on skeletal muscle in an endocrine manner. Further experiments are required to explore this hypothesis.

**Protective Role of Fstl1 in the Cardiovascular System**

Fstl1, also referred to as TSC-36, is a secreted glycoprotein that belongs to the follistatin family of proteins. However, it has low relative homology to follistatin and Fstl3, suggesting that its ability to modulate transforming growth factor-β superfamily proteins may be minimal relative to other family members. Fstl1 was identified as a secreted cardiac protein that is upregulated during the physiological phase of heart growth in cardiac-specific inducible Akt1 transgenic mice. Fstl1 expression is also increased in murine heart in response to various cardiac stresses, including myocardial pressure overload and ischemia, and these cardiac stresses lead to elevations in the level of circulating Fstl1. Systemic delivery of an adenoviral vector encoding murine Fstl1 attenuates myocardial infarct size and apoptosis in a mouse model of ischemia/reperfusion injury. Consistent with these observations, Fstl1 was found to protect cultured cardiac myocytes from hypoxia/reoxygenation-induced apoptosis via the immediate activation of PI3K-Akt signaling, whereas knockdown of Fstl1 leads to an increase in cardiomyocyte apoptosis in vitro. Fstl1 activates Akt signaling in vascular endothelial cells and cardiac myocytes through interaction with a putative receptor, Disco-interacting protein 2 homolog.
A, on the cell surface. Thus, Fstl1 appears to function as an injury-induced cardiokine that protects against myocardial ischemic injury via autocrine/paracrine signaling.

It has been shown that Fstl1 can function to reduce pressure overload–induced cardiac hypertrophy. Cardiac myocyte–specific Fstl1-deficient mice exhibit exacerbation of cardiac growth and dysfunction after pressure overload, whereas systemic delivery of Fstl1 or overexpression of Fstl1 results in diminished cardiac hypertrophic responses and improved function. The beneficial actions of Fstl1 on the hypertrophic responses appear to be mediated through its ability to promote AMP-activated protein kinase (AMPK) signaling in cardiac myocytes. It has also been shown that Fstl1 stimulates endothelial cell growth in response to tissue ischemia in muscle; the actions of Fstl1 on the vasculature may contribute to its ability to protect the heart from pressure overload and ischemic injuries. Recently, it was reported that Fstl1 is a positive regulator of GDF-15 expression in cultured myocytes and murine heart, raising the possibility that these factors function in a cardiokine network to protect the heart from stress. Taken together, these findings indicate that Fstl1 can function as a cardiokine that exerts antiapoptotic, antihypertrophic, and proangiogenic actions on the myocardium.

Recently, recombinant human FSTL1 protein has been shown to protect the heart from ischemia/reperfusion injury in both murine and porcine models. This cardioprotection was attributed to a suppression of myocyte apoptosis through upregulation of AMPK and a suppression of myocardial inflammation. Mechanistic studies showed that Fstl1 inhibits inflammation by blocking the ability of bone morphogenic protein 4 to activate proinflammatory signaling. These studies suggest that FSTL1 may have utility in the treatment of cardiac remodeling after a myocardial infarct.

Clinically, FSTL1 is expressed in myocytes in human heart, and its expression is elevated in failing myocardium. Plasma levels of FSTL1 positively correlate with left ventricular hypertrophy in patients with heart failure, and increased FSTL1 levels are related to a higher risk of mortality. Elevated levels of FSTL1 are also predictive of all-cause and cardiovascular mortality in ACS patients. The identification of FSTL1 as an upstream regulator of GDF-15, another ACS biomarker, suggests that these proteins function as components of an interactive network that functions to control cardiac remodeling in response to stress. In this regard, FSTL1 and GDF-15 appear to reflect overlapping disease pathways because they are most closely associated with each other when considering various clinical, biochemical, and angiographic parameters. Although these 2 proteins provide equivalent information about patient outcome in ACS, the identification of FSTL1 and GDF-15 as orthogonal biomarkers serves to highlight the importance of their molecular pathways in cardiovascular pathology. Thus, further experimental studies to understand the functional interplay between FSTL1 and GDF-15 are warranted.

Role of Macrophage Migration Inhibitory Factor in Cardiac Ischemia

Migration inhibitory factor (MIF) is a macrophage cytokine that regulates inflammatory and immune responses. Several reports have demonstrated that MIF acts as a cardioprotective cardiokine. MIF is expressed by cardiomyocytes in the heart, and its secretion from the heart is increased during ischemia/reperfusion. MIF stimulates AMPK activation through interaction with the cell surface receptor CD74, promoting glucose uptake in the heart. MIF deficiency leads to reductions in AMPK signaling and glucose uptake during ischemia, contributing to cardiac damage after ischemia/reperfusion. Aged hearts show impaired AMPK activation during ischemia and reduced MIF expression, whereas exogenous MIF enhances ischemia-induced AMPK activation and improves cardiac function in these hearts. MIF deficiency has also been shown to contribute to increases in myocardial dysfunction, apoptosis, and Jun N-terminal kinase activation after ischemia/reperfusion. Thus, MIF functions as a cardiokine that protects the heart from ischemia/reperfusion injury through at least 2 mechanisms: enhancement of AMPK activation during ischemia and suppression of Jun N-terminal kinase activation during reperfusion. A recent report also demonstrated that the posttranslational S-nitrosylation modification of MIF at cysteine 81 residue participates in the protective actions of this factor on cardiac damage. Taken together, these findings indicate that MIF protects the heart from ischemic injury and that manipulation of MIF-dependent signaling pathways during ischemia may represent a strategy for the prevention of cardiac damage.

Mesencephalic Astrocyte-Derived Neurotrophic Factor as an Endoplasmic Reticulum Stress Response Cardiokine

Mesencephalic astrocyte-derived neurotrophic factor (MANF) is an endoplasmic reticulum stress-inducible cardiokine that attenuates cardiac hypertrophy and myocardial ischemic injury. MANF, also known as ARMET (arginine rich mutated in early stages of tumors), is a secreted factor originally identified as a neurotrophic factor that protects rat dopaminergic neurons in vitro and prevents the degeneration of dopaminergic neurons involved in Parkinson disease. MANF was also identified as an endoplasmic reticulum stress response gene in the heart in a mouse model expressing ATF6, a regulator of the unfolded protein response, in cardiac myocytes. Myocardial infarction also leads to the induction of MANF expression in both myocytes and nonmyocyte cells in mouse hearts. Cell culture experiments demonstrated that MANF expression and secretion are stimulated by endoplasmic reticulum stress inducers in an ATF6-dependent manner. Consistent with these findings, MANF expression is increased in several cell lines in response to endoplasmic reticulum stress and by cerebral ischemia in vivo. Furthermore, treatment of cardiomyocytes with MANF protein reduces cell death in response to ischemia/reperfusion, whereas knockdown of MANF enhances cardiomyocyte death under conditions of ischemia/reperfusion. A recent study has shown that systemic administration of recombinant MANF protein prevents cardiac damage in mice after ischemia/reperfusion. MANF also suppresses agonist-stimulated hypertrophic responses in cultured cardiac myocytes.
Protease Inhibitor 16 as an Antihypertrophic Cardiokine

Protease inhibitor 16 (PI16) is a cardiokine that acts in an autocrine/paracrine manner to inhibit cardiac hypertrophy. PI16 was identified as a cardiokine via a secretion trap screening strategy using a murine cardiac cDNA library. PI16 is secreted from cultured cardiac myocytes, and its expression is markedly upregulated in failing myocardium in both mice and clinical specimens. Overexpression of PI16 inhibits agonist-stimulated hypertrophic responses in cultured cardiac myocytes, whereas knockdown of PI16 increases cardiomyocyte hypertrophy. Furthermore, cardiac-specific PI16 transgenic mice display smaller hearts and cardiomyocytes but have normal cardiac structure and function.

Interleukin-33 as a Mediator of Fibroblast-Myocyte Communication

Interleukin (IL)-33, a member of the IL-1 family, is a ligand for the ST2 receptor. IL-33 is reported to activate nuclear factor-κB and mitogen-activated protein kinases via ST2, thereby modulating inflammatory responses. A number of studies have demonstrated that IL-33 plays a role in various heart diseases. IL-33 is produced predominantly by cardiac fibroblasts, and its expression and secretion are promoted by mechanical strain. IL-33 suppresses agonist-stimulated hypertrophic responses in cultured cardiac myocytes. IL-33 administration also attenuates cardiac hypertrophy and fibrosis after pressure overload in wild-type mice but not in ST2-deficient mice. Furthermore, in mice, IL-33 treatment attenuates cardiomyocyte apoptosis and protects against cardiac dysfunction after myocardial infarction through ST2-dependent mechanisms. These observations indicate that the IL-33–ST2 signaling axis represents a cardioprotective paracrine system between fibroblasts and cardiomyocytes. In contrast, a recent report showed that IL-33 promotes eosinophilic pericarditis and cardiac dysfunction in a mouse model of autoimmune myocarditis. Thus, the actions of IL-33 on the heart may be either protective or detrimental, depending on the disease models.

Secreted Frizzled-Related Protein as a Modulator of Cardiac Fibrosis

Secreted frizzled-related protein (Sfrp) 2 expression is markedly upregulated in the rodent heart after myocardial infarction, suggesting that Sfrp2 acts as a stress-inducible cardiokine. Sfrp2 belongs to the family of Sfrps, which function mainly to bind Wnt proteins and to antagonize their functions. In this regard, it has been shown that Sfrp2 protects cardiomyocytes from apoptosis via suppression of canonical Wnt signaling, but recent evidence indicates that Sfrp2 also functions to modulate cardiac fibrosis in the infarcted heart independently of Wnt signaling. The cells expressing Sfrp2 in the infarcted heart exhibit a fibroblastic appearance, indicating that the cardiac fibroblast is a major source of this protein. It was demonstrated that Sfrp2 directly enhances the procollagen C-proteinase activity of bone morphogenetic protein 1/Tolloid-like metalloproteinases, thereby leading to an increase in collagen deposition. In this regard, Sfrp2 knockout mice exhibit a reduction of collagen content and fibrosis and an improvement in cardiac function after myocardial infarction. In contrast, another study showed that Sfrp2 exhibits a biphasic effect on bone morphogenetic protein 1 activity. A high dose of Sfrp2 inhibits bone morphogenetic protein 1 activity in vitro, whereas Sfrp2 at low concentrations increases bone morphogenetic protein 1 activity. In the same study, the direct injection of Sfrp2 into the infarcted hearts 2 days after myocardial infarction reduced cardiac fibrosis and improved cardiac function. Furthermore, peri-infarct intramyocardial injection of mesenchymal stem cells into mice leads to enhanced vascular density, reduced infarct size, and improved cardiac function after myocardial infarction, and these beneficial actions are attributed to the paracrine actions of Sfrp2.

Neuregulin as an Endothelium-Derived Cardiokine

The inhibition of vascular endothelial growth factor–mediated angiogenesis during cardiac hypertrophy leads to impaired cardiac growth and contractile dysfunction. These studies also provided support for the notion that complex mechanisms, likely involving as-yet unidentified cardiokines, participate in the communication between cardiac myocytes and vascular cells. In this regard, neuregulins play important roles in heart development and cardiac disease. Neuregulin is expressed in human cardiac endothelial cells, and its secretion is stimulated in response to ischemia/reperfusion. Coculture experiments demonstrate that neuregulins derived from endothelial cells protect cardiac myocytes from hypoxia/reoxygenation-induced apoptosis. Furthermore, deletion of neuregulins from vascular endothelial cells leads to increased infarct size and apoptosis after myocardial ischemia/reperfusion. Thus, neuregulins appear to act as an endothelial cell–derived cardiokine that protects the heart from ischemia/reperfusion damage.

Concluding Remarks

Recent findings reinforce the concept that the heart functions as a secretory organ, producing a variety of cardiokines that can affect the function of various cell types. Cardiokines can have protective and detrimental activities in the heart, and an imbalance in cardiokine production during cardiac remodeling may contribute to disease outcome. Cardiokines may also participate in cachectic processes by acting on distal metabolic tissues and influencing whole-body homeostasis. The further identification and characterization of cardiokines will lead to a better understanding of how cell types communicate within the heart and may enable the development of new therapeutic and diagnostic agents for heart disease.

Sources of Funding

This work was funded by US National Institutes of Health grants HL102874, AG34972, AG15052, and HL68758 to Dr Walsh; a Grant-in-Aid for Scientific Research to Dr Ouchi; and Grant-in-Aid for Young Scientists B23790844 to Dr Shimano.

Disclosures

None.
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Key Words: follistatin-related proteins ▪ growth differentiation factor 15 ▪ protease inhibitor 16, mouse ▪ interleukin-33, mouse ▪ mesencephalic astrocyte-derived neurotrophic factor, mouse (MANF protein, mouse) ▪ migration inhibitory factor, mouse (MIF protein, mouse) ▪ myostatin ▪ secreted frizzled-related protein (Sfrp) ▪ neuregulins

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Circulation. 2012;126:e327-e332
doi: 10.1161/CIRCULATIONAHA.112.150656
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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