Cardiometabolic diseases comprise a spectrum of interconnected pathological alterations in metabolic organs and in the cardiovascular system that occur alone or simultaneously.1 Although great efforts have been undertaken to develop therapeutic agents (eg, statins and tissue-type plasminogen activator) for these unfavorable conditions, epidemiological data demonstrate that the prevalence of cardiometabolic diseases is continuously increasing and constitutes the heaviest burden on global health and economic development.2 An ambiguous understanding of the latent pathogenesis of cardiometabolic diseases might be responsible for their consistently high morbidity and mortality. In recent years, the discovery of a close interaction between the inflammatory response and the various cardiometabolic disorders has provided novel insight into the cause of these pathological conditions. Chronic low-grade inflammation has been identified as an important mediator of various cardiometabolic dysfunctions and has been shown to be involved in the entire process of lipid profile disturbance and the resultant hepatic steatosis, obesity, and hyperlipidemia, leading to severe cardiovascular disease.3–5 However, the inhibition of inflammation unfortunately failed to block the long-term development of cardiometabolic diseases and rendered the host susceptible to infection,6 suggesting that inflammation might only represent an outcome or a regulator of these diseases rather than the principal pathogenic factor. Therefore, what is the actual cause of cardiometabolic diseases?

Innate immunity, an evolutionarily ancient and universal response of the host to defend against infection and injury, underlies inflammatory responses that is triggered by innate immune molecular cascades.7 Most recently, these immune signaling cascades have been not only found to extend beyond the purview of the immune system but also involved in or even play a central mechanistic role in broader physiological functions, including the function in the cardiometabolic system. A clear understanding of the mechanistic basis for the link between innate immunity and cardiometabolic diseases might provide a promising strategy for the treatment of these pathological conditions. However, it is difficult to fully elucidate the interactions between these 2 complex systems. The good news is that recent studies of members in the family of interferon (IFN) regulatory factors (IRFs), key regulators of the innate immune system, have enabled the understanding of this complex interaction. Originally identified as transcriptional regulators of type I IFNs, IRFs consist of 9 members, IRF1 to IRF9, in mammals.8 Most recently, studies by our research group and others demonstrated that IRFs are susceptible sensors of cardiometabolic stress and function as powerful mediators of related diseases.9,10 More meaningfully, the concept of IRF signaling was gradually highlighted by investigations on whether and how IRFs regulate cardiometabolic conditions, which further supported the implication that the immune system plays a role in the development of cardiometabolic diseases. Thus, the emergence of IRF signaling represents a potentially crucial mechanism underlying the pathogenesis of cardiometabolic diseases, and thus suggests a promising therapeutic strategy. In this review, we summarize and update the impacts of IRFs on cardiometabolic diseases and further discuss the potential involvement of IRF signaling in these pathological conditions, including metabolic disorder, vascular injury, cardiac hypertrophy, and various ischemia/reperfusion (I/R)–induced injuries to tissues.

Background of the IRF Family

The mammalian IRF family of transcription factors comprises 9 members: IRF1, IRF2, IRF3, IRF4 (PIP, LSIRF, or ICSAT), IRF5, IRF6, IRF7, IRF8 (ICSBP), and IRF9 (ISGF3γ). IRF10 has been identified in chickens.8,11 First characterized as transcriptional regulators of type I IFN and IFN-inducible genes, IRFs play critical roles in regulating innate and adaptive immunity.4 The functions of IRFs in immune cell development and maturation, as well as in tumor growth, have been well characterized in several excellent previous reviews.8,11 Herein, we briefly summarize the background of each IRF and describe the recent findings.

Structure of IRF Members

All IRF proteins contain 2 major domains: an N-terminal DNA-binding domain and a C-terminal IRF association domain (IAD).4 The well-conserved DNA-binding domain is characterized by a helix–turn–helix domain containing 5 tryptophan-rich repeats that recognize DNA sequences approximately corresponding to the IFN-stimulated response element (ISRE; 5′/GNGAAANGAAACT). In contrast, the C-terminal regions of IRFs are less conserved and mediate interactions with their
cofactors or other transcription factors. Except for IRF1 and IRF2, IRFs share an IAD1 that is structurally similar to the Mad homology 2 domains of the Smad family; alternatively, IRF1 and IRF2 share an IAD2. The different structures of these C-terminal IADs determine the distinct mechanisms by which these IRFs regulate downstream events and contribute to the diverse or even opposing functions of IRFs under certain pathological conditions. On the basis of their specific and overlapping structures, it was found that IRF1, IRF3, and IRF7 can form a large complex termed the IFN-β enhanceosome/DRAF1, which includes nuclear factor-kB (NF-kB), activating protein 1, and CREB-binding protein/p300, to activate IFN-β transcription.17 IRF4 exhibits low affinity to the ISRE but much higher affinity to PU.1 and SPI-1 or with heterodimers of JUN and basic leucine zipper transcription factor, activating transcription factor-like.13,14 For IRF5, a homodimer or a heterodimer with IRF3 or IRF7 could be formed after activation. Interestingly, the binding of IRF5 to IRF3 synergistically increases downstream IFN-α production,15 whereas the IRF5/IRF7 heterodimer masks the DNA-binding domain and suppresses the activity of IFN-α.16 Similar to IRF4, the IRF8–PU.1 complex might be generated and acts as an activator of DNA elements comprising IRF- and E-26–binding sites, whereas an IRF8–IRF1 complex suppresses ISRE activity.9,10 IRF9 can associate with signal transducer and activator of transcription 1 (STAT1) and STAT2 to form a heterotrimeric complex called interferon-stimulated gene factor 3 (ISGF3) in response to type I IFN and activate ISREs transcription.17 More recently, our studies have demonstrated that dependent on diverse interacting partners, IRFs also exert critical roles in regulating nonimmune cells function in cardiometabolic diseases.9,10

**Interferon Regulatory Factor 1**

IRF1 is the first member of the IRF family that is characterized by its affinity to IFN-β regulatory DNA elements.18 In the physiological states, IRF1 is extensively expressed at a low level in various cell types with the major localization in the nucleus; however, after stimulation with viral infection or immune factors, the expression of IRF1 is dramatically upregulated under mediation by STAT and NF-kB transcription factors.19 The changed expression of IRF1 exerts crucial roles in regulating the development and differentiation of inflammatory and immune cells, including B cells, T help 1 (Th1) cells, and dendritic cells (DCs).8 Except for its influence on immune functions, IRF1 also exerts a potent antiproliferative and proapoptotic effect on various mammalian cell lines, suggesting its potentially clinical application for the treatment of proliferative diseases.20 In 1993, Matsuyama et al21 established the first IRF1 knockout (KO) mouse strain, which is susceptible to bacterial and virus infections, whereas the loss expression and function of IRF1 may promote the development of leukemia.

**Interferon Regulatory Factor 2**

IRF2, possessing a similar structure to IRF1, was first discovered as a transcriptional repressor that antagonizes IRF1-mediated transcriptional activation. Similar to IRF1, IRF2 is constitutively expressed in various cells and organs and is inducible by type I IFN and virus infection but to a lesser extent than that of IRF1.18 However, phosphorylated IRF2 represses both IRF-E and ISRE-regulated transcription activation, thereby weakening IRF1-induced IFN-α/β signaling.22 Functionally, IRF2 promotes the reprogramming of myeloid progenitor toward the megakaryocytic lineage but abrogates granulocyte colony-stimulating factor (G-CSF)–induced granulocytic differentiation.1 In addition to the influence on cell differentiation, IRF2 is essential for the development and maturation of various immune cells. Because of the deficiency of its potent regulation on immune cells, IRF2−/− mice exhibit impaired hematopoiesis and B lymphopoiesis, disturbed Th1 immunity, blunted maturation of CD11b+CD5+ NK cells, and lost splenic CD4+/CD8+ DCs.21

**Interferon Regulatory Factor 3**

IRF3 is ubiquitously expressed in a variety of cells and presents in an inactive form in the cytoplasm, but could be immediately phosphorylated and activated on viral infection.8 The sensibility of IRF3 in the innate immune system allows its tight involvement in immune cell development and function. In murine B lymphocytes, IRF3 is required for IgG2a production after CpG-B stimulation, whereas in T cells, IRF3 directly interacts with RAR-related orphan receptor (ROR)-γt and thus represses interleukin (IL)-17 expression and Th17 cell differentiation. In addition, the generation of Treg cells from naive CD4+ lymphocytes could be markedly blocked by IRF3.23,24 On the basis of its predominant function in immune cells, IRF3 functions as a profound regulator during bacteria or virus infection and largely participates in autoimmune diseases, eg, systemic lupus erythematosus.11,25 Loss-of-function approaches have demonstrated that IRF3 ablation in macrophages leads to a failure to undergo lipopolysaccharide-induced apoptosis in the presence of a p38 inhibitor or on Salmonella typhimurium infection.26 Mice with IRF3 deficiency exhibit impaired IFN-α/β production and reduced resistance to viral infection.27

**Interferon Regulatory Factor 4**

IRF4 was originally identified as a nuclear factor that was specifically expressed in lymphocytes and was associated with the E-26 family transcription factor PU.1.27 Unlike other members of the IRF family, IRF4 is more often induced by diverse mitogenic stimuli, eg, antigen receptor engagement, lipopolysaccharide stimulation, and CD40 signaling, rather than by IFNs.14,28 Among the 9 IRFs, IRF4 is the most frequently studied member for their regulation on the development of immune cells. Various developmental stages of B cells, including pre-B-cell differentiation, receptor editing, germinal center reaction, and plasma cell generation, are mediated by IRF4.29,30 In T cells, IRF4 deficiency leads to the differentiation or the dysfunction of Th2, Th9, Th17, CXCR5/ICOS/CD44 follicular Th and Treg cells, resulting in the development of autoimmune diseases.28 Aside from the functions of B and T cells, the development of DCs and the polarization of macrophages have also been found to be regulated by IRF4 expression and activation.31

**Interferon Regulatory Factor 5**

IRF5, which was originally cloned from a human DC library, is expressed in numerous immune cell types, eg, macrophages, DCs, B cells, and monocytes.32 Similar to IRF1, resulting from the presence of 2 functional nuclear localization
sequences (NLSs), the expression of IRF5 is often detected in the nucleus of unstimulated cells.\textsuperscript{15} By suppressing Blimp-1 expression, a master regulator of plasma cell differentiation, IRF5 promotes B-cell maturation, and B cells with IRF5 deficiency showed diminished cytokine-induced class switching to IgG2a/c.\textsuperscript{13} Studies of the immune regulation of IRF5 further identified its essential function in the development of Th1 cells and the differentiation of macrophages.\textsuperscript{8} Clinical investigations revealed a close correlation between IRF5 and diverse autoimmune diseases, such as systemic lupus erythematosus, inflammatory bowel disease, and rheumatoid arthritis.\textsuperscript{35} The extensive and dominant roles of IRF5 in multiple immune cell compartments during inflammation have been validated in IRF5\textsuperscript{+/−} mice, which exhibited reduced death and dramatically decreased expression of proinflammatory cytokines mediated by endotoxin-induced shock.\textsuperscript{35}

**Interferon Regulatory Factor 6**

Unlike other IRF members, functional studies of IRF6 have focused on its regulation in epidermal development and differentiation, whereas its impacts on immune cells remain largely unknown.\textsuperscript{36} There are 2 major IRF6-deficient mouse models: a knockin of the most common IRF6 protein mutation (R84C), which is found in patients with popliteal pterygium syndrome,\textsuperscript{37} and a complete loss-of-function allele.\textsuperscript{38} Either the partial or complete deficiency of IRF6 function results in severe defects in limb and skin development and in the compromised differentiation and proliferation of keratinocytes in the interfollicular epidermis.\textsuperscript{36,37} In addition to popliteal pterygium syndrome, the mutation of IRF6 has also been associated with another autosomal-dominant genetic disorder, Van der Woude syndrome, which is characterized by combinations of cleft lip, syndactyly, skin folds, and genital anomalies.\textsuperscript{38} In these syndromes, an interaction between the DeltaNp63 isoform of p63 and an enhancer of IRF6 that positively activates IRF6 expression has been established, leading to the negative regulation of the p63 levels and the subsequent inhibition on the proliferation of keratinocytes.\textsuperscript{39}

**Interferon Regulatory Factor 7**

IRF7, which shares considerable homology with IRF3, was initially cloned in the biological context of Epstein–Barr virus latency. IRF7 is constitutively expressed in various immune and nonimmune cells and at a relatively high level in plasmacytoid DCs.\textsuperscript{40} Similar to IRF3, IRF7 can be activated by viral infection, IFNs, lipopolysaccharide, and various chemical reagents, such as sodium butyrate and phorbol ester (TPA).\textsuperscript{41} The activated IRF7 triggers the expression of IFN-β, which binds to its receptor, and in turn, induces IRF7 expression to form a positive feedback loop.\textsuperscript{31} Accordingly, splenic plasmacytoid DCs isolated from IRF7-deficient mice exhibit impaired type I IFN gene induction on treatment with single-stranded RNA or CpG-A.\textsuperscript{42} Although sharing similar structures and immune regulations, the functions of IRF3 and IRF7 are not redundant; IRF3 plays a major role in the early phase of the induction of IFNs, whereas IRF7 is particularly critical in the late phase.\textsuperscript{42} In addition to the regulation of IFN production, IRF7 is a key modulator of monocyte differentiation into macrophages. In response to stimulation of plasmoidum berghei ANKA infection or nonlethal plasmoidum chabaudi chabaudi AS infection, the upregulation of IRF7 suppressed Th1 responses. In addition, IRF7 is essential for the proliferation or activation of DCs, CD4+, CD8+, and NK cells. The available evidence supporting the molecular and cellular regulation of IRF7 suggests that this IRF family member largely participates in autoimmune diseases, as further validated by the close association of IRF7 expression with systemic sclerosis.\textsuperscript{43}

**Interferon Regulatory Factor 8**

IRF8 was previously considered to be selectively expressed in the lymphoid and myeloid lineages of the immune system, a property that shares with its closely related family member IRF4.\textsuperscript{4} Via a γ activation sequence element in its promoter, IRF8 could be induced by IFNs in various inflammatory and immune cells.\textsuperscript{44} Similar to IRF4, IRF8 is best known for its regulation on immune cell development and maturation. IRF8 stimulates myeloid progenitor cells differentiating into macrophages but potently inhibits granulocytic differentiation.\textsuperscript{45} In B cells, IRF8 orchestrates the transition from large pre-B to small pre-B cells and the entry of germinal center B cells into the plasma cell differentiation program.\textsuperscript{46} In T cells, IRF8 suppresses the Th17 response by interacting with ROR-γt and inactivating IL-17 transcription, and the depletion of IRF8 abrogates the differentiation of naïve CD8 T cells into effector cells.\textsuperscript{47} In addition to its regulation of B- and T-cell differentiation, IRF8 is essential for DC development and cytokine secretion. When compared with IRF4, which is expressed in CD4+ DCs, IRF8 is expressed in CD8α+ DCs and is essential for their development. Accordingly, IRF8\textsuperscript{−/−} mice display a marked reduction of CD8α+ DCs, and CD8β+ DCs lacking IRF8 are unable to undergo complete phenotypic activation in response to maturation-inducing stimuli.\textsuperscript{48} A recent genetic association study has strongly implicated a variant near the IRF8 gene in systemic lupus erythematosus susceptibility and multiple sclerosis.\textsuperscript{49}

**Interferon Regulatory Factor 9**

IRF9 is a DNA-binding subunit of the transcription factor ISGF3 and is constitutively expressed in various cells and organs.\textsuperscript{49} In response to IFN stimulation, IFN receptors are activated to phosphorylate STAT1 and STAT2, leading to their heterodimerization and interaction with IRF9 to form the ISGF3 complex, which translocates to the nucleus, where it regulates the production of IFN-regulated genes.\textsuperscript{50} Thus, because of the disruption of immune regulatory function, IRF9\textsuperscript{−/−} mice exhibit severely impaired production of IFN-α and IFN-β induced by viral infection and enhanced susceptibility to infection with the viruses encephalomyocarditis virus, vesicular stomatitis virus, and herpes simplex virus.\textsuperscript{50} In contrast to the other IRFs, the details of how IRF9 regulates immune cell development have not been well investigated. Taken together, previous studies of IRFs identified their comprehensive and potent impacts on immune responses and related diseases that are mediated by the complex network of IRF signaling.

**IRF Signaling**

In the innate immune system, in response to stimuli, eg, viruses, bacteria, and cytokines, pathogen-associated molecular patterns and danger-associated molecular patterns are
released and recognized by corresponding receptors, which subsequently signal to IRFs via various adaptors and kinases, facilitating the modification of IRF structures to alter their binding capacity, concomitant with the activation or suppression of downstream targets of IRFs. Accumulating studies have indicated that IRF signaling in the immune system largely overlaps with the pattern recognition receptor (PRR) and IFN pathways (Figure 1).

**PRR-Mediated IRF Signaling**

PRRs consist of 2 classes: transmembrane PRRs, which are represented by Toll-like receptors (TLRs) and C-type lectin receptors and cytosolic PRRs, including retinoic acid-inducible gene-I–like receptors (RLRs), nucleotide-binding oligomerization domain (NOD)–like receptors (NLRs), and cytosolic DNA sensors. Different IRF members respond to different PRRs, primarily TLRs, depending on the type and location of the invading pathogen and the upstream cascades to induce the transcription of type I IFNs and the production of proinflammatory cytokines and chemokines, contributing to coordination of the elimination of pathogens and infected cells. 

**Transmembrane PRR-Mediated IRF Signaling**

The TLR family, as major transmembrane PRRs, comprises 13 members in mammals (10 in humans), each of which processes a specific reorganization pattern. All TLR members contain intracellular Toll-interleukin-1 receptor (TIR) domains, which transmit downstream signals by recruiting TIR-containing adaptor proteins, eg, myeloid differentiation primary response protein 88 (MyD88), TIR-associated protein, TIR domain–containing adaptor–inducing IFN (TRIF), and Toll-receptor–associated molecule. According to the adaptor proteins activated, TLR-mediated IRF signaling can be divided into a MyD88-dependent pathway and a TRIF-dependent (or MyD88-independent) pathway. 

---

**Figure 1.** Interferon (IFN) regulatory factor (IRF) signaling in the innate immune system. On stimuli of microbial infections or the ligands of pattern recognition receptors or IFN receptors, IRF signalings are activated to regulate immune responses. CARD indicates caspase recruitment domain family; dsRNA, double-stranded RNA; IKK, inhibitor of nuclear factor kappa-B kinase; IRAK, interleukin-1 receptor–associated kinase; ISGF, interferon-stimulated gene factor; ISRE, IFN-stimulated response element; MDA, melanoma differentiation-associated gene; MyD88, myeloid differentiation primary response protein 88; NOD, nucleotide-binding oligomerization domain; PAMPs, pathogen-associated molecular patterns; RIG, retinoic acid-inducible gene; SIKE, suppressor of IKK; STAT, signal transducer and activator of transcription; STING, stimulator of interferon genes; ssRNA, single-stranded RNA; TBK1, TANK-binding kinase 1; TLR, Toll-like receptor; TRAF, Tumor necrosis factor (TNF) receptor–associated factor; TRAM, Toll-receptor–associated molecule; and TRIF, TIR domain–containing adaptor–inducing IFN.
Within the TLR family, TLR2, TLR7, and TLR9 are the major members that are involved in the MyD88-dependent IRF signaling. Recent studies by Kwa et al. have indicated that within the IFR family, IRF1 and IRF6 are involved in TLR2-regulated chemokine gene expression in epithelial cells, which is modulated by interleukin-1 receptor-associated kinase 1 (IRAK1) and requires the presence of MyD88. In addition, IRF1 is effectively activated by MyD88 on TLR6 or TLR9 stimulation. On TLR9 activation, IRF3 is activated by interacting with the middle region of MyD88, facilitating the nuclear translocation of IRF3 to activate downstream cytokine gene transcription. Similar to IRF1, IRF5 interacts with the central region of MyD88 and translocates to the nucleus to trigger type I IFN gene induction after the activation of TLR7 and TLR9. Tumor necrosis factor (TNF) receptor–associated factor 6 (TRAF6) positively regulates the TLR7/9–MyD88–IRF5 signaling response via its K63-linked ubiquitination capacity. After the activation of TLR7 or TLR9 by their ligands, MyD88 also directly interacts with IRF7 via its death domain to form a complex with TRAF6 in the cytoplasm. The IRAK4–IRAK1–inhibitor of nuclear factor kappa-B kinase alpha (IKKα) caspase functions as a signal transducer between MyD88 and TRAF6 that is required for the phosphorylation of IRF7 and the subsequent activation of IFN-dependent promoters. Although the activation of IRF8 is induced by TLR9, IRF8 does not interact with MyD88 but rather binds to TRAF6 and enhances TRAF6 ubiquitination, potentially contributing to the crosstalk between IFNs and IRF signaling. In contrast to other IRFs, IRF4 acts as a negative feedback regulator of TLR-induced IRF activation. IRF4 mRNA is induced by TLR7 and TLR9 activation and competes with IRF5 for interaction with MyD88, thereby suppressing the IRF5 nuclear translocation and IRF5-mediated ISRE activation.

TLR3 and TLR4 are the major TLR members that modulate the modifications of IRFs, especially IRF3 and IRF7, in a MyD88-dependent, but TRIF- and TANK-binding kinase 1 (TBK1)-dependent manner. Like TLR7 and TLR9, TLR3 is localized to the membranes of endosomes and phagosomes. In response to double-stranded RNA or other pathogens, TLR3 directly binds to TRIF and subsequently activates the IRF kinase TBK1, which promotes the phosphorylation, dimerization, and nuclear translocation of IRF3 and IFN7. TRIF-deficient mice are defective in TRIM3-mediated IFN-β expression and IRF3 activation. Similarly, after its activation, TLR4 forms a complex with Toll-receptor–associated molecule and TRIF and then recruits TRAF3 and TBK1 to activate IRF3/7 for the further induction of type I IFN gene expression. The activation of the TLR–MyD88/TRIF–IRF cascade is influenced by various factors. For instance, suppressor of IKKe was identified to act as a negative regulator of IRF signaling by interacting with IKKe and TBK1 and by disrupting their interactions with TRIF to preferentially influence the TLR3-induced IRF3 activation pathway. In addition, A20, an NF-κB–inducible zinc finger protein, interacts with TBK1 and IKKe or IKKe to inhibit TLR3- or virus-induced IRF3 dimerization and ISRE-dependent transcriptional activation. Mindin (also known as spondin 2), a member of the Mindin-F-spondin family of secreted extracellular matrix (ECM) proteins, acts as a pattern recognition molecule that is crucial for triggering the activation of TLR–TRAF–IRF signaling.

Similar to TLRs, C-type lectin receptor are localized to the plasma membrane and are involved in the induction of pathogen-specific gene expression profiles. Carbohydrate, protein, and lipid components that are specific to both pathogens and self-antigens function as C-type lectin receptor ligands. Dectin-1, a C-type lectin receptor, recruits Syk and CARD9, activates IRF5, and induces IFN-β production in response to Curdlan and Zymosan stimulation in vitro or to C. albicans infection in vivo.

Cytosolic PRR-Mediated IRF Signaling

The RLR and NLR families are essential cytosolic receptors for the detection of RNAs, especially uncapped 3′-phosphate RNA, double-stranded RNA and single-stranded RNA. The RLR family comprises melanoma differentiation-associated gene 5 and retinoic acid-inducible gene-I. On stimulation, melanoma differentiation-associated gene 5 or retinoic acid-inducible gene-I interacts with the N-terminal CARD-containing adapter IFN-β-promoter stimulator 1 via its CARD–CARD domain and transmits a signal to TBK1 or IKKe, which induces the phosphorylation of IRF3 and IRF7. A20 inhibits the retinoic acid-inducible gene-I–mediated activation of IRF3 and IRF7, as well as downstream IFN promoter activity. In addition to the activation of IRF3 and IRF7, RLR showed a potential capacity to regulate the expression and function of IRF5 and IRF8. However, the precise mechanism by which RLR mediates IRF5 and IRF8 activation remains unknown. Within the NLR family, which consists of 3 subfamilies (NODs [NOD1-5 and CIITA], NLRPs [NLRP1–14], and IPAF), NOD2 activates IRF3 via the adaptor protein IFN-β-promoter stimulator 1 and induces IFN-β expression on single-stranded RNA stimulation, primarily via the downstream kinase receptor–interacting protein 2, and functions as an effective activator of IRF5.

In addition to these cytosolic RNA-sensing mechanisms, the cytosolic DNA-sensing system is involved in IRF signaling activation for the recognition of and response to microbial and host DNA stimuli. In response to cytosolic dsDNA, direct DNA sensors, such as IFN-γ-inducible 16 and DEAD-box-polypeptide 41, bind to specific DNA motifs, leading to the activation of STING, a candidate DNA sensor that is localized to the endoplasmic reticulum. The tail of the STING C-terminal domain then acts as a scaffold for the assembly of IRF3 near TBK1, thereby promoting type I IFN gene expression. However, other IRFs can be activated by cytosolic PRRs remains largely unknown.

IFN-Mediated IRF Signaling

Aside from PRRs, ligand-induced IFN receptors, including the type I IFN (IFN-α and IFN-β) receptor and the type II IFN (IFN-γ) receptor, have been identified as classical activators of IRF signaling in response to IFN stimuli. In response to IFN-α/β stimulation, type I IFN (IFN-α and IFN-β) receptors activate tyrosine kinase 2 and JAK1, leading to the phosphorylation of STAT1 and STAT2, which interact with IRF9 and form ISGF3. This heterotrimeric complex subsequently translocates to the nucleus and binds to ISRE or IRF-E, leading to the expression of IFN-inducible genes, including IRF1. Similar to type I IFN (IFN-α and IFN-β) receptors, type II IFN (IFN-γ) receptors activate IRF signaling by forming...
a complex of STAT1/STAT2/IRF9 or STAT1/STAT1/IRF9 and by promoting the production of IFN-inducible genes. In addition, the homodimeric STAT1 complex exhibits transcriptional capacity and binds to the γ activation sequence domain in the promoter of IRF1 to enhance IRF1 downstream processes.25 Interestingly, IRF2 attenuates ISRE- or IRF-E–regulated transcriptional activation, and thus inhibits IFN-induced IRF1 activation.8 During the accumulating investigations of the complex IRF signaling-formed innate immune network, various proteins have been identified to be involved in this sophisticated molecular events. The tripartite motif, an E3 ligase family containing 70 members in humans, has been highlighted for its extensive regulation on multiple layers of IRF signaling, including receptors, adaptors, kinases, and transcription factors.77

Most recently, our group revealed that aside from immune stimuli, various cardiometabolic stresses modulate the expression and activation of IRF, which, in turn, impacts the progression of cardiometabolic diseases. The influences of IRF signaling on a series of cardiometabolic diseases, including metabolic disorder, vascular injury, cardiac remodeling, and I/R injury, are summarized in this review (Table), and the potential underlying mechanisms that are involved in these diseases are further discussed.

**IRFs in Cardiometabolic Diseases**

As discussed above, since the discovery of the first IRF member, related studies have focused on the immunomodulatory functions of the IRF family and the underlying molecular events, which have been well summarized in previous reviews.8 Recently, emerging investigations have revealed the powerful effects of IRFs and the associated factors on innate immune signaling processes as prominent regulators of the cardiometabolic system. The deregulation of IRFs led to the development of metabolic disorder and severe cardiovascular diseases, eg, vascular intimal hyperplasia, cardiac remodeling, and stroke (Table; Figure 2). Thus, the specific counterbalancing or safe artificial modulation of the IRF levels might significantly contribute to the management of the initiation and progression of cardiometabolic diseases.

**IRFs in Obesity, Insulin Resistance, and Hepatic Steatosis**

Metabolic diseases, which are characterized by obesity, insulin resistance, and hepatic steatosis, are associated with multifaceted disturbances in systemic homeostasis and pose a threat to an array of cardiovascular diseases.106 In this pathological state, excessive fat intake and synthesis induce obesity and liver dysfunction, representing a first assault on the body.107 Because of adipose and hepatic tissue–generated cytokines, oxidative species, or endotoxins from the intestinal lumen, chronic inflammation occurs and intimately interacts with insulin resistance.108 Under physiological conditions, the homeostasis of lipids and carbohydrates is under the sophisticated control of integrated cellular and molecular programs that enable the rapid and effective adjustment to dynamic metabolic changes.108 However, in response to continuous pathological stimuli, eg, a high-fat diet (HFD), the population of cells and their related molecular events undergo compositional, structural, and procedural modifications and thus cause phenotypic and functional changes in corresponding tissues, especially hepatic and adipose tissue.109

Among the extensive and complex biological events, peroxisome proliferator-activated receptors (PPARs, including PPARα, PPARγ, and PPARδ) and their associated pathways have been underlined for their irreplaceable regulation of lipid metabolism and energy balance. In terms of metabolic diseases, PPARα enhances the catabolism of fatty acids in the liver110; PPARγ is essential for mediating adipocyte differentiation and insulin sensitivity,111 and PPARδ regulates the triglyceride levels.112 Given that the appropriate management of lipid metabolism and inflammation is interdependent,1 the deregulation of classical inflammation-related signaling pathways, eg, the IKKβ/NF-κB and JNK1/activating protein-1 pathways, during the progression of metabolic diseases has been considered as a consensus phenotype in previous studies.113 Considering the high integration of the inflammatory response and metabolic regulation, it is not surprising that immune regulators such as IRFs are comprehensively and influentially involved in obesity, insulin resistance, and hepatic steatosis. In addition to inflammatory events, alterations in other metabolic pathways, such as compromised AMP kinase and endoplasmic reticulum stress signaling,114,115 have been found to be involved in IRF-mediated metabolic diseases.

**Interferon Regulatory Factor 1**

In recent years, Eguchi et al116 reported a systematic study of the function of IRFs in the metabolic system based on a high-throughput DNase hypersensitivity analysis. Interestingly, all 9 of these IRFs exhibited a top-scoring binding activity and displayed regulatory effects on adipogenesis in cultured fat cells. Importantly, direct evidence for the linkage of IRFs to metabolic diseases has emerged from experiments on animals subjected to artificial IRF gene disruption. Nakazawa et al78 reported that IRF1-KO completely suppressed spontaneous insulinitis and diabetes mellitus in a nonobese diabetic type I diabetes mellitus mouse model. Further investigation demonstrated that the negative regulatory effect of IRF1 deficiency on diabetes mellitus in these mice benefited from its immune function, as indicated by the increase of CD4+ and Mac-1+ splenic cells and the decreases of CD3+ and CD8+ cells, as well as the decreased IFN-γ/IL-10 ratio in IRF1+/- mice.

**Interferon Regulatory Factor 3**

IRF3 is constitutively expressed in various tissues, including liver and white adipose tissue.8 Most recently, our research group found that the mRNA and protein expressions of IRF3 were significantly reduced in the liver of ob/ob mice that were fed normal chow and of C57BL/6J mice that were subjected to HFD administration for 26 weeks.117 Intriguingly, in white adipose tissue, the expression of IRF3 was upregulated in HFD-treated mice.118 The alteration of IRF3 expression suggested the potential participation of IRF3 in both spontaneous and inducible obesity. The ameliorating effects of IRF3 on abnormal lipid and insulin metabolism were validated by the observations that IRF3 overexpression dramatically decreased the HFD-induced dysfunction of insulin sensitivity and lipid homeostasis, and that IRF3-KO mice showed accelerated and exacerbated adiposity, hepatic steatosis, and insulin resistance compared with...
Table. Summary of IRF Members and Their Functions in the Cardiometabolic System

<table>
<thead>
<tr>
<th>IRF</th>
<th>Expression</th>
<th>Cardiometabolic Functions</th>
<th>Targets</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>IRF1</td>
<td>Constitutive in both immune and nonimmune cells; brain, heart, liver, lung, spleen, thymus, kidney, muscle, intestine, adipose, vessel</td>
<td>Promotes T1DM; protects against neointima formation; proatherosclerosis; induces cell cycle arrest and apoptosis; accelerates cardiac remodeling; and exacerbates ischemic stroke and I/R–induced liver damage</td>
<td>IFN-γ, IL-10, INOS, IL-4, IL-12, IL-15, CD40, PPARγ</td>
<td>78–82</td>
</tr>
<tr>
<td>IRF2</td>
<td>Like IRF1</td>
<td>Reduce hepatic I/R injury</td>
<td>IRF1, INOS</td>
<td>18, 83</td>
</tr>
<tr>
<td>IRF3</td>
<td>Most cell types; spleen, thymus, prostate, testis, ovary, small intestine, colon, peripheral blood leukocytes</td>
<td>Maintains insulin sensitivity and lipid homeostasis; blunts inflammation; inhibits neointimal formation; regulates pulmonary hypertension; promotes atherosclerosis; reduces cardiac hypertrophy; indispensable for TLR ligands pretreatment-induced tolerance to I/R injury; and increases hepatic I/R injury</td>
<td>IKKβ, PPARγ, ERK2, IL-23/IL-17</td>
<td>84–87</td>
</tr>
<tr>
<td>IRF4</td>
<td>Constitutive in cardiomyocytes, neurons, and immune cells; adipose tissue, heart, brain, liver, spleen, lung, kidney and skeletal muscle</td>
<td>Blunts obesity; keeps activation of insulin activation; suppresses neointima formation; participates in pulmonary hypertension; and exacerbates cardiac remodeling; improves stroke outcomes</td>
<td>PGC-1α, IRF5, IL-4, CREB, SRF</td>
<td>29, 88–91</td>
</tr>
<tr>
<td>IRF5</td>
<td>Mainly in lymphocytes, monocytes, DCs: lymphoid tissue, skeletal muscle, prostate, heart</td>
<td>Participates in cardiac remodeling, insulin resistance, and obesity</td>
<td>TGFβ1</td>
<td>92–94</td>
</tr>
<tr>
<td>IRF6</td>
<td>Constitutive in skin, eye, liver, lung, placenta, testes, longue, brain, heart, and spleen</td>
<td>Unknown</td>
<td>Unknown</td>
<td>38</td>
</tr>
<tr>
<td>IRF7</td>
<td>Most cell types and organs; liver, adipose, muscle tissue, spleen, thymus, prostate, testes, uterus, small intestine, colon (mucosal lining), and peripheral blood leukocytes</td>
<td>Enhances obesity, hepatic steatosis, and the related inflammation; inhibits neointimal formation; suppresses cardiac remodeling; and required for TLR ligands pretreatment-elicited neuroprotection</td>
<td>AMPK, ATF3, IKKβ, IFN-α, IFN-β</td>
<td>95–99</td>
</tr>
<tr>
<td>IRF8</td>
<td>Expressed in cardiomyocytes, neurons, and lymphocytes; heart, brain and immune organs</td>
<td>Accelerates vascular hyperplasia; alleviates cardiac hypertrophy; and reduces stroke-induced infarct lesions</td>
<td>SRF, NFATc1, apoptosis-related genes</td>
<td>100–102</td>
</tr>
<tr>
<td>IRF9</td>
<td>Broadly in various cells and organs (like IRF1)</td>
<td>Inhibits obesity, insulin resistance, and hepatic steatosis; augments intimal hyperplasia; anti-atherosclerosis; anti-hypertrophy; promotes myocardial I/R responses; and aggravates I/R–induced organ damage</td>
<td>PPARγ, Sirt1, SRF</td>
<td>74, 103–105</td>
</tr>
</tbody>
</table>

AMPK indicates AMP kinase; ATF, activating transcription factor; CREB, cAMP response element–binding protein; ERK, extracellular signal regulated kinase; I/R, ischemia/reperfusion; IFN, interferon; IKK, inhibitor of nuclear factor kappa-B kinase; IL, interleukin; IRF, IFN regulatory factor; INOS, inducible nitric oxide synthase; NFATc1, nuclear factor of activated T cell c1; PGC-1α, peroxisome proliferator-activated receptor gamma, coactivator 1 alpha; PPAR, peroxisome proliferator-activated receptor; SRF, serum response factor; T1DM, type 1 diabetes mellitus; TGF, transforming growth factor; and TLR, Toll-like receptor.

wild-type (WT) controls. The protective effect of IRF3 on metabolic disorder is closely associated with its capacity on inflammatory inhibition. The infiltration of macrophages in the white adipose tissue of IRF3-KO mice was much higher than that of WT controls. Alternatively, IRF3 deficiency dramatically enhanced the activation of IKKβ/NF-κB signaling in the liver. Applying multiple biological approaches, the interaction between the IAD of IRF3 and the N-terminal domain of IKKβ has been identified to be largely responsible for the metabolic counterbalance property of IRF3. A recent pharmacological study indicated that the downregulation of IRF3 in adipose tissue is involved in the antiobesity effects of resveratrol in HFD-stimulated mice, suggesting a promising strategy of targeting IRF3 for the treatment of metabolic diseases.

Interferon Regulatory Factor 4

IRF4 was originally found to be expressed exclusively in lymphocytes; however, Eguchi et al. reported that IRF4 was highly expressed in adipose tissue macrophages and regulated the polarization of adipose tissue macrophages during the development of HFD-induced obesity. The deletion of IRF4 in macrophages led to enhanced mitogen-activated protein kinase (MAPK) signaling, inactivated Akt signaling, and increased proinflammatory cytokine production, resulting in impaired insulin sensitivity of cocultured adipocytes. Consistently, the specific KO of IRF4 in myeloid cells deteriorated the HFD-induced chronic inflammatory response and downregulation of insulin signaling in hepatic, adipose, and skeletal muscle tissue. In addition, Kong et al. recently reported that IRF4 functions as a dominant transcriptional regulator of thermogenesis via its direct interaction with peroxisome proliferator-activated receptor gamma, coactivator 1 alpha; PPAR, peroxisome proliferator-activated receptor; SRF, serum response factor; T1DM, type 1 diabetes mellitus; TGF, transforming growth factor; and TLR, Toll-like receptor.

Interferon Regulatory Factor 5

A most recently published study reported a close correlation of IRF5 expression with the insulin sensitivity and with collagen deposition in human obese adipose tissue. On the basis of an HFD-induced obesity model, Dalmases et al. unveiled that the global and myeloid cell–specific, but not the adipocyte-specific, IRF5 deficiency imposed a beneficial effect on...
adipose tissue remodeling, insulin sensitivity, and alternatively activated macrophage accumulation when compared with the corresponding WT controls. The loss-of-function approach indicated that the regulatory capacity of IRF5 on adipose tissue remodeling and insulin resistance might be largely dependent on its influence in the polarization of macrophage and the formation of collagen in adipose tissue. A further genome-wide analysis performed on the adipose tissue macrophage highlighted the transforming growth factor \( \beta_1 \), the transcriptional activity of which can be directly inhibited by IRF5.122

**Interferon Regulatory Factor 7**

In lipid metabolism, AMP kinase acts as crucial cellular energy sensor and increases catabolism once the AMP/ATP ratio is higher than the normal level.114 In our recent study, the increased expression of IRF7 was observed in the hepatic, adipose, and gastrocnemius muscle tissues of mice that were subjected to an HFD and of genetically obese (\( \text{ob/ob} \)) mice. IRF7 deficiency clearly increased the levels of phosphorylated AMP kinase-\( \alpha \) and AMP kinase-\( \beta \) and thereby promoted steatolysis in an HFD-induced obese mouse model.95 In addition, IRF7 powerfully regulated endoplasmic reticulum stress and inflammatory signaling. Resulting from the multifaceted influence of IRF7 on various aspects of metabolism, \( \text{IRF7-KO} \) mice maintained relatively intact glucose and lipid homeostasis, attenuated endoplasmic reticulum stress, and a reduced inflammatory response compared with their lean counterparts.95 Thus, targeting IRF7 might represent a promising strategy for the clinical therapy of obesity and related metabolic disorders.

**Interferon Regulatory Factor 9**

Because IRF9 is a key regulator of type I IFN signaling, mice carrying an \( \text{IRF9} \) deletion are susceptible to infection and various other immune diseases.123 Interestingly, the indispensable role of IRF9 has also been validated in the metabolic system. When compared with nontransgenic controls, \( \text{IRF9-overexpressing mice} \) are more tolerant to HFD-induced and genetically related obesity, hepatic steatosis, inflammation, and insulin resistance, whereas \( \text{IRF9-KO mice} \) exhibit more severe metabolic abnormalities than their \( \text{IRF9}\text{+/+ littermates} \).103 Drawing evidence from yeast 2-hybrid screening, the interaction of IRF9 with PPAR\( \alpha \) contributes to the antidiabetic role of IRF9.103 Three domains in PPAR\( \alpha \) provide binding sites for IRF9, which facilitates the activation of PPAR\( \alpha \) target genes. Importantly, specific PPAR\( \alpha \) overexpression in

---

**Figure 2.** Interferon (IFN) regulatory factors (IRFs) in cardiometabolic diseases. Under pathological cardiometabolic conditions, IRFs sense cardiometabolic stimuli and are subjected to structural and functional alterations to regulate the cellular behaviors involved in cardiometabolic diseases in immune-dependent or immune-independent manners. The regulations of IRF2, IRF5, and IRF6 on cardiometabolic diseases remain poorly understood. ATF indicates activating transcription factor; Bcl-2, B-cell CLL/lymphoma 2; CREB, cAMP response element–binding protein; ERK, extracellular signal regulated kinase; IKK, inhibitor of nuclear factor kappa-B kinase; iNOS, inducible nitric oxide synthase; MAPK, mitogen-activated protein kinase; NF-\( \kappa \)B, nuclear factor-\( \kappa \)B; NFATc, nuclear factor of activated T cell c; PDGF-BB, platelet-derived growth factor-BB; PGC-1\( \alpha \), peroxisome proliferator-activated receptor gamma, coactivator 1 alpha; PPAR, peroxisome proliferator-activated receptor; SIRT1, sirtuin 1; SRF, serum response factor; and TGF, transforming growth factor.
the liver reverses the IRF9-KO–induced exacerbation of metabolic disorder, suggesting that IRF9 performs its antidiabetic function in a PPARα-dependent manner. Taken together, previous investigations by our research group and others have demonstrated the critically important roles of IRF1, IRF3, IRF4, IRF5, IRF7, and IRF9 in the initiation and progression of metabolic diseases. The impacts of the other IRF family members on the metabolic system and the corresponding mechanisms need to be further elucidated.

**IRFs in Vascular Injury**

Vascular injury is a clinically detrimental condition that is prevalent during the initiation, progression, treatment, and prognosis of various vascular diseases involving multiple pathological processes, including endothelial dysfunction, ECM degradation, vascular proliferation, and inflammatory cell infiltration.124,125 Neointima formation is among the most common stages of this complex process. The phenotypic and functional deregulation of a highly differentiated cell type, vascular smooth muscle cells (VSMCs), is considered to be an essentially causative factor in the formation of intimal hyperplasia.126,127 Immediately after a biomechanical and biochemical insult, phenotypic plasticity facilitates the switching of VSMCs from a differentiated (also termed contractile) phenotype to a dedifferentiated (also termed synthetic) state under the regulation of intricate intracellular and intercellular interactions.128 Although the initial phenotypic switching of VSMCs occurs to promote vascular repair, VSMCs exhibiting a synthetic phenotype are particularly sensitive to pathological stimuli and display a markedly increased probability of proliferation, migration, and ECM production, thereby contributing to the morbidity of clinical events, including atherosclerosis, abdominal aortic aneurysm, in-stent restenosis, transplant vasculopathy, and other pathological vascular conditions.128,129 The participation of immune mechanisms in vascular injury has been demonstrated in various clinical events and experimental models.130 In addition to immune signalings, nonimmune-related molecular events, eg, the Smad, platelet-derived growth factor (PDGF), transforming growth factor, and kruppel-like factor (KLF) signaling pathways, regulate the phenotype and function of VSMCs and other cell types, thereby resulting in the suppression or promotion of vascular remodeling.131 Notably, in response to pathological stimuli, IRFs mediate neointima formation and related diseases via these immune-independent mechanisms in addition to immune-dependent programs.

**Interferon Regulatory Factor 1**

Immune homeostasis is important for preserving the architectural and functional integrity of the blood vessel wall; the deficiency or overactivation of the immune system can cause rambunctious intimal thickening after vascular injury.132 The disturbance of IRF1 expression is implicated in immunity-regulated vascular remodeling. Dimayuga et al133 indicated that in an immune-deficient Rag-1-KO mouse model, the protein expression of IRF1 is dramatically increased in response to the intrinsic presence of IFN-γ, promoting intimal thickening, which was further confirmed in an in vitro smooth muscle cell proliferation experiment and in a balloon injury rat model.134,135 The direct relationship between IRF1 and neointima formation was reported by Li136 and Wessel et al137 10 years ago. Mice carrying an IRF1 deletion exhibited significantly higher susceptibility to neointima formation compared with C57BL/6J controls. Mechanistic studies indicated that the upregulation of antiproliferative and proapoptotic genes, AT2 receptor, interleukin-1 beta-converting enzyme (ICE), and inducible nitric oxide synthase (iNOS), rendered the suppressive effect of IRF1 on vascular remodeling.136,137 In addition to biomechanical stress, a diet that induces hypertriglyceridemia imposes higher risk for developing vascular disease, the best known of which is atherosclerosis.138 Using pro- and antiatherogenic triglyceride-rich lipoproteins, Sun et al139 reported that a diet enriched in proatherogenic triglyceride-rich lipoprotein promoted the production of proinflammatory cytokines and chemokines, whereas a diet enriched in antiatherogenic triglyceride-rich lipoprotein ameliorated the inflammatory response during atherosclerosis formation. The deregulation of IRF1 expression and its binding to cofactors recapitulated the distinct influences of these different triglyceride-rich lipoprotein on atherosclerosis. However, unlike the biomechanical stress-induced exacerbation of vascular injury, IRF1 downregulation markedly reduced the level of TNF-α–triggered vascular cell adhesion molecule-1 production, which might help to alleviate atherosclerosis.140 Consistently, our unpublished data confirmed the proatherosclerotic property of IRF1 because of its positive regulation of inflammation. The paradoxically phenotypic influence of IRF1 on vascular remodeling induced by different stresses exhibited a complex balance in the pleiotropic modulation of IRF1 on vascular dysfunction. The in-depth mechanism underlying these effects of IRF1 requires further investigation.

**Interferon Regulatory Factor 3**

PDGF-BB is a widely used mitogen and chemoattractant for the proliferation and migration of VSMCs to mimic the process of neointima formation in vitro.141 After PDGF-BB stimulation in vitro or carotid wire injury in vivo, the expression of IRF3 was significantly decreased in VSMCs than that in the untreated controls.142 The artificially SMC-specific overexpression of IRF3 reduced VSMC proliferation genes and elevated the expression of differentiation markers, thereby leading to a significant decrease in the intima/media ratio and to the amelioration of intimal hyperplasia.143 Mechanistically, in the nucleus of VSMCs, IRF3 binds to the aortic banding (AB) domain of PPARγ, a negative mediator of SMC proliferation, enhances the transactivation of PPARγ and correspondingly decreases the expression of proliferation cell nuclear antigen. Using a PPARγ-TG/IRF3-KO mouse model, our research group reported that the overexpression of PPARγ largely negated the proproliferative effect of IRF3 deficiency.144 Interestingly, in contrast to the deterioration on wire injury–induced neointima formation by IRF3 deletion, our recent data delineated a significant retardation–derived by IRF3-KO on HFD-induced atherosclerotic plaque formation in ApoE−/− mice (unpublished data). The antiproliferative capacity on VSMCs might partially explain the opposing effects of IRF3 in different vascular injury models. In addition to VSMCs, endothelial cells, which form the barrier between the
intravascular and the extravascular environments, play critical roles in maintaining the homeostasis of blood vessels. IRF3 is required for dsDNA-stimulated inflammatory responses in endothelial cells, suggesting the multidimensional regulation of cellular and molecular programs by IRF3 during the progression of vascular diseases. In addition, resulting from the dysfunction of endothelial cells, especially cell migration and proliferation occurred in pulmonary artery, pulmonary hypertension could be initiated and promoted. Using human pulmonary artery endothelial cell, Bauer et al observed a inhibitory function of high mobility group box 1 (HMGB1) on the migration of endothelial cells, which could be abolished by IRF3 knockdown, suggesting that a critical regulation of IRF3 in the progression of pulmonary hypertension.

**Interferon Regulatory Factor 4**

The regulation of vascular diseases by IRF4 has yet to be reported to the best of our knowledge. Our ongoing studies have indicated that compared with that in WT mice, wire injury–induced neointima formation in IRF4-KO mice was significantly increased and was accompanied by the elevated expression of the proliferative factors proliferation cell nuclear antigen and Cyclin D1, as well as dramatically reduced expression of the differentiation markers SMA, SM22α, and smoothelin. However, IRF4-overexpressing mice with exhibited completely contrasting phenotypes (unpublished data). In the ApoE−/− background, IRF4-KO mice exhibited an increase in HFD-induced arterial lesions from 40.9% to 48.55%, along with clearly exacerbated macrophage infiltration into the vessel wall. A recent study also suggested the role of IRF4 in pulmonary arterial hypertension, evidenced by the fact that the IRF4 expression is significantly decreased in PBMCs from patients with the limited cutaneous systemic sclerosis, a disease always with the complication of pulmonary hypertension. However, the mechanisms underlying these effects remain under investigation.

**Interferon Regulatory Factor 7**

Because of the structural similarity of IRF7 to IRF3, IRF7 and IRF3 gene-disturbed mice always exhibit analogous immune system phenotypes in response to certain detrimental stimuli. In response to carotid artery wire injury, similar to IRF3-overexpressing mice, SMC-specific IRF7-TG mice showed alleviated intimal hyperplasia and inhibited VSMC proliferation, whereas vascular remodeling was significantly potentiated in mice carrying a global IRF7 deficiency. The suppression of hyperproliferative vascular disorders by IRF7 depends on its direct interaction with activating transcription factor 3, as evidenced by the observation that the double TG or double KO of IRF7 and activating transcription factor-3 greatly abolished the negative or positive regulatory effects of IRF7-TG or IRF7-KO alone, respectively. Our recent studies have revealed that during HFD-induced atherosclerosis in ApoE−/− mice, the expression of IRF7 was significantly reduced compared with that in normal chow–fed controls. The global KO of IRF7 led to a reduced susceptibility to HFD-induced plaque formation and chronic inflammatory responses (unpublished data).

**Interferon Regulatory Factor 8**

The expression of IRF8 was initially considered to be limited in the cells that are responsible for immune responses. The essential regulation of IRF8 in immune-related cells enables the acceleration of atherosclerosis in ApoE−/− mice carrying a hematopoietic IRF8 deficiency. However, in recent years, IRF8 expression was observed in VSMCs and was greatly increased after PDGF-BB administration in vitro or after wire injury in vivo. On the basis of gain- and loss-of-function approaches, suppressed phenotypic switching of VSMCs was found in IRF8-deficient mice after vascular injury, whereas IRF8 overexpression promoted the transformation of VSMCs from a contractile phenotype to a synthetic phenotype compared with WT controls. Further molecular biological studies elucidated the potential mechanism underlying the IRF8-mediated exacerbation of mechanical stress-induced vascular lesions. During the development and maintenance of VSMCs, myocardin acts as a prominent regulator by interacting with its coactivator serum response factor (SRF) and by modulating the activation of the SRF/CArG axis. IRF8 directly binds to myocardin by occupying the binding site for the recruitment of the acetyltransferase p300. The interaction between IRF8 and myocardin reduced SRF/myocardin transactivation, facilitating SMC phenotypic switching and neointima formation.

**Interferon Regulatory Factor 9**

Previous studies of vascular diseases indicated a vital role of SIRT1 in protecting against vascular pathologies, including injury-induced neointima formation. Our most recent study identified an implication of the IRF9–SIRT1 axis in the development of vascular remodeling via its regulation on the proliferation, phenotypic switching, and migration of VSMCs. IRF9 in VSMCs was activated in response to arterial injury in vivo or PDGF-BB stress in vitro and underwent translocation into the nucleus, where IRF9 bound to SIRT1, resulting in the relief of SIRT1-suppressed activating protein-1 transactivation and the downstream proliferative effectors. Thus, the ablation of IRF9 in mice led to the prohibition of vascular injury-induced intimal hyperplasia. Unlike the nonimmune-related mechanism underlying wire injury–induced neointima formation, IRF9 potentially restrained the development of HFD-induced atherosclerosis via its regulation on chronic inflammation (unpublished data).

Abdominal aortic aneurysm is a common vascular degenerative and remodeling disorder that is characterized by a chronic inflammatory response and ECM degradation. Ongoing experiments from our research group have suggested a powerful influence of IRF family members on the incidence and progression of abdominal aortic aneurysm. The deterministically protective or detrimental effects of IRFs and the corresponding cellular and molecular events remain under investigation.

**IRFs in Cardiac Hypertrophy and Hypertension**

Cardiac hypertrophy is a response of the heart to extrinsic and intrinsic stress. During cardiac development and physiological adaptation, a complex but coordinated signaling system mediates cardiomyocyte enlargement and drives energy production to meet the demands of an increased workload. In response to pathological stimuli, continuous pathogenic signals may cause the development of compensatory hypertrophy
to a maladaptive level, together with a reduction in cardiac output and a dramatic elevation of the risk of heart failure, arrhythmia, and sudden death. The mechanical load and humoral factors, eg, angiotensin II (Ang II), endothelin-1, and norepinephrine, are the most common inductors of pathological cardiac hypertrophy, which is always accompanied by hypertension. The orchestrated signal transduction events recognize injurious stimuli, induce the structural and functional changes in regulators, and ultimately reactivate fetal gene, promote protein synthesis, and increase cell size. Pathological hypertrophy typically involves the reprogramming of a myriad of signaling events, such as calci-neurin–nuclear factor of activated T cells, insulin-like growth factor-I–phosphatidylinositol 3-kinase–AKT, and MAPK signaling. In addition, microRNAs (miRNAs) participate in the development of cardiac hypertrophy, heart failure, and hypertension. In recent years, members of the IRF family have been reported to be involved in the etiopathogenesis of cardiac hypertrophy via their regulation of the signaling events mediating cardiomyocyte enlargement rather than immune-related activities.

**Interferon Regulatory Factor 1**

In failing human hearts, IRF1 expression was significantly reduced in cardiomyocytes. Consistently, reduced IRF1 expression was observed in mice subjected to pressure overload–induced severe cardiac hypertrophy. A loss-of-function experiment indicated that reduced IRF1 expression is associated with the retardation of AB-induced cardiac hypertrophy, whereas the cardiac-specific overexpression of *IRF1* leads to accelerated hypertrophic response of ventricular dilation and cardiac dysfunction caused by the direct activation of iNOS. The ablation of iNOS largely abolished this exacerbation of cardiac dysfunction caused by the direct activation of iNOS.

**Interferon Regulatory Factor 3**

As mentioned above, Ang II is a critical hormonal intermediate in the development of cardiac hypertrophy, fibrosis, and subsequent heart failure. *Tsushima et al* reported that fibrosis, but not hypertrophy, may be regulated by IRF3 during Ang II–induced hypertension. When compared with WT mice, IRF3–/– mice exhibited blunted formation of perivascular and interstitial fibrosis and suppressed heart failure. However, recent studies by our research group indicated that both hypertrophy and fibrosis are mediated by *IRF3* overexpression specifically in cardiomyocytes. Moreover, *IRF3* deficiency dramatically exacerbated, not suppressed, pressure overload–induced cardiac hypertrophy in vivo and isoproterenol-induced cardiomyocyte enlargement in vitro. The inconsistent influence of IRF3 on cardiac remodeling might occur as a result of the different models used and corresponding mechanisms involved. In the pressure overload–induced cardiac remodeling model, IRF3 interacted with extracellular signal regulated kinase (ERK2) to inhibit ERK1/2 signaling. Ang II–induced IRF3 activation has also been linked to the activation of ERK1/2, but located upstream of IRF3. Comprehensively considering the paradoxical effects of IRF3 on cardiac remodeling in diverse models, IRF3 might act as a sensitive sensor of distinct pathological stimuli and induce disparate phenotypes. The response of IRF3 to prohypertrophic factors and the underlying mechanism remains to be fully elucidated.

**Interferon Regulatory Factor 4**

IRF4, which is highly expressed in human and murine hearts in the physiological state, is involved in both human dilated cardiomyopathy and chronic AB-induced mouse heart failure. In human and mouse hypertrophic heart samples, the expression of IRF4 is significantly decreased, which is accompanied by the reactivation of fetal genes. In mice carrying an *IRF4* gene deletion, pressure overload–induced cardiac hypertrophy, fibrosis, and dysfunction were dramatically ameliorated in a manner that depended on the decreased expression and activation of cAMP response element–binding protein, a required activator of the fetal gene program.

**Interferon Regulatory Factor 5**

The tight skin (*Tsk*+/−) mouse is an extreme autoimmune disease model that exhibits spontaneous cardiac dysfunction. After treatment with the ApoAI mimetic 4F, the phosphoserine levels of IRF5 were significantly decreased, but the total ubiquitination level of IRF5 was increased in the heart of these mice. Additional studies have indicated that the alteration of IRF5 expression in response to 4F administration is closely associated with the interaction of 4F with IRF5, and that this interaction is at least partly responsible for the protective effect of 4F on cardiac remodeling. Evidence that IRF5 is activated and translocated to the nuclei of myocardial cells indicates that IRF5–related cardioprotection might originate from the direct function of cardiomyocytes rather than their immune regulatory capacity.

**Interferon Regulatory Factor 7**

NF-κB signaling is a controversial molecular events for its unclear function during pathological cardiac hypertrophy. Previous studies have reported that cardiac-specific NF-κB inhibition leads to attenuated Ang II infusion–induced cardiac remodeling, but that cardiac-specific *IKKβ*–deficient exhibited exacerbated cardiac hypertrophy and dysfunction. In a recent study by our group, we demonstrated that dependent on the direct binding of IRF7 to *IKKβ* in the cytoplasm, NF-κB signaling was greatly inactivated, resulting in the suppression of AB-induced cardiac hypertrophy. Thus, the overexpression of IRF7 significantly suppressed chronic pressure overload–induced heart failure. Further investigation is required to elucidate the multiple divergent effects of the *IKKβ–IkB–NFκB* pathway and the potential application of IRF7 as a therapeutic target for the treatment of heart failure.

**Interferon Regulatory Factor 8**

In heart samples from humans with dilated cardiomyopathy or hypertrophic cardiomyopathy, the protein expression of IRF8 is strikingly decreased when compared with heart samples from healthy controls, suggesting the important involvement of IRF8 in the progression of heart failure. On the basis of
gene disruption experiments, we identified IRF8 as a bona fide suppressor of pressure overload– and Ang II–induced cardiomyocyte enlargement. Mechanistic studies have indicated that IRF8 directly interacts with nuclear factor of activated T cell c1 to prevent its translocation, thereby inhibiting the hypertrophic response.101 The constitutive activation of nuclear factor of activated T cell c1 in IRF8-KO mice largely nullified the IRF8 deficiency–induced increase in cardiac dysfunction.101

**Interferon Regulatory Factor 9**

In addition to the regulatory functions in VSMC differentiation, the SRF/myocardin complex participates in the progression of cardiac hypertrophy. In response to prohypertrophic pressure overload, IRF9 binds to the transcription activation domain of myocardin to suppress its transcriptional activity. Correspondingly, the overexpression of IRF9 in the heart resulted in reduced cardiomyocyte size, decreased fibrosis, and increased cardiac function.100 Taken together, studies of the regulation of cardiac hypertrophy by IRF family members have revealed their clinical applicability to cardiac disease treatment and have indicated the necessity to better understand the complexity of heart failure.

**IRFs in I/R injury**

Ischemia contributes to parenchymal cell death in various organs, especially the brain, the heart, and the liver, caused by glycogen consumption, the lack of an oxygen supply, and ATP depletion.166 Although the restoration of normoxic conditions to the ischemic region is helpful for the prevention of ischemia-induced cellular injury, reperfusion introduces profound oxidative and inflammatory immune responses, leading to the exacerbation of cell death and a resultant adverse impact on tissue structure and function.187 Thus, extensive experimental and clinical efforts have focused on the suppression of oxidative stress, the inflammatory response, and cell death to ameliorate the extent of I/R injury–related tissue damage.188 Ischemic preconditioning is a promising strategy for improving the outcomes of I/R injury.189 During the initiation, progression, or prevention of I/R injury, IRFs exert a profound influence on stroke, myocardial I/R, and hepatic I/R injury, deriving from their potent roles in the immune response. However, interestingly, the effects of IRFs on I/R injury extend beyond their regulation of the inflammatory response.

**Interferon Regulatory Factor 1**

The relationship between IRFs and I/R injury was first investigated in 1998.170 Paschen et al170 observed that after transient cerebral ischemia, the mRNA level of IRF1 in rat brain tissue was dramatically upregulated and that upregulation was maintained for up to 24 hours after reperfusion. Gene disruption studies have indicated that compared with that in WT controls, the cerebral I/R injury–induced necrotic volume is greatly reduced and neurological function is improved in both IRF1+/− and IRF1−/− mice.171 The immunoreactivity of IRF1 in neutrophils indicated that the cerebroprotective effect of IRF1 deficiency might result from its regulation on I/R–induced inflammatory responses.80 Similar to its altered expression in ischemic stroke, the level of IRF1 in the liver is significantly increased at the early stage of postischemic stimulation, and its expression linearly correlates to I/R injury–induced or IFN-α–exacerbated liver damage.81,172 Compared with their WT littermates, mice carrying an IRF1 deficiency displayed significantly reduced production of proinflammatory cytokines and chemokines, suppressed MAPK activation, and decreased iNOS mRNA expression after hepatic I/R injury.81 The detrimental effect of IRF1 overexpression on liver damage has been confirmed in an orthotopic liver transplantation mouse model.173,174 Rats that were pretreated with AdIRF1 exhibited increased susceptibility to liver damage, along with enhanced early apoptosis and immune responses in the transplanted grafts; however, IRF1 deficiency in the mouse liver grafts dramatically reduced liver injury and improved the survival rate of these mice.174 The mechanisms underlying the deteriorated effects of IRF1 on hepatic I/R injury–induced liver damage might include the alteration of the inflammatory and apoptotic responses by mediating MAPK and TLR signaling.81,173

**Interferon Regulatory Factor 2**

Given that IRF2 suppresses the transcriptional activation of IRF1 in the immune system,175 the upregulation of IRF2 might provide a possible strategy to prevent the IRF1–elicited exacerbation of I/R injury. Studies by Klune et al183 validated this speculation. After hepatic warm I/R injury, the overexpression of IRF2 limited the production of IRF1 induction-triggered expression of proinflammatory genes and iNOS. Orthotopic liver transplantation using heterozygotic IRF2-KO donor grafts significantly worsened liver injury after a cold hepatic I/R procedure compared with orthotopic liver transplantation using WT control donor grafts.83 The crosstalk between IRF1 and IRF2, as well as other potential mechanisms underlying the protective effect of IRF2 on hepatic I/R–related liver damage, remains to be further studied.

**Interferon Regulatory Factor 3**

In an effort to ameliorate I/R–induced tissue damage, multiple preconditioning stimuli have been developed.176–178 Chloroquine, a suppressor of transient global cerebral ischemia, displays a potent property of enhancing the learning and memory capacity of rats that are subjected to I/R injury caused by its inhibition of the TLR3/IRF3–IFN-β signaling pathway.178 In addition, mice pretreated with a TLR4 ligand (lipopolysaccharide) or a TLR9 ligand (unmethylated CpG oligodeoxynucleotides) exhibited significant neuroprotection against ischemic injury compared with non preconditioned control mice. An analysis of the genomic profiles of the indicated groups revealed that the presence of IRF3 and IRF7 is necessary for TLR preconditioning-related neuroprotection against cerebral I/R injury.177,178 Unexpectedly, although IRF3 is necessary for TLR ligand preconditioning–derived cerebroprotection, no significant difference in brain damage has been observed between IRF3-KO mice and their WT controls.178 However, interestingly, an improvement of stroke outcome has been found in IRF3-KO rats compared with WT rats in our recent studies (unpublished data). The pleiotropic regulation of cerebral I/R injury by IRF3 and its possible mechanism are not fully understood. In response to hepatic I/R injury, IRF3 ablation markedly increased the liver necrotic area, the serum alanine aminotransferase (ALT) and aspartate transaminase (AST) levels,
and the extent of neutrophil infiltration into the ischemic tissue compared with WT. The upregulation of the IL-23/IL-17 axis recapitulated the role of IRF3 deficiency in the effects of hepatic ischemic stimuli.86

**Interferon Regulatory Factor 4**

In middle cerebral artery occlusion–challenged brain tissue in vivo and oxygen glucose deprivation–treated primary cortical neurons in vitro, the expression of IRF4 was significantly increased when compared with the corresponding untreated controls.29 Using genetic approaches, Guo et al29 reported the role of IRF4 as a negative regulator in stroke. Additional studies of the underlying mechanisms of IRF4 function demonstrated that the reduced infarct area in IRF4-TG mice depended on its interaction with SRF to facilitate the transcription of SRF,29 suggesting a novel potential strategy for promoting neuronal survival by targeting the IRF4–SRF axis in stroke.

**Interferon Regulatory Factor 5**

Macrophages, consisting of the M1 and M2 types, are critically important cells that regulate the extent of inflammatory events during the development of ischemia-induced infarctions. The continued dominance of M1 macrophages may accelerate and exacerbate ischemic infarction.179 Courties et al93 found that silencing IRF5 in M1 macrophages led to a strikingly suppressed progression of myocardial ischemia–induced necrosis and cardiac dysfunction caused by the downregulation of proinflammatory responses. These sophisticated molecular processes must be fully clarified.

**Interferon Regulatory Factor 7**

Similar to TLR4 and TLR9 ligand preconditioning-induced cerebroprotection, pretreatment with the TLR7 agonist Gardiquimod significantly reduced the infarct percentage and functional deficits in the brain of WT mice post middle cerebral artery occlusion injury.98 However, the neuroprotective effect of Gardiquimod was completely abolished in IRF7−/− mice, indicating that the TLR7 agonist preconditioning–induced attenuation of cerebral damage requires the expression of IRF7.98 The mechanistic role of IRF7 in I/R injury is poorly characterized.

**Interferon Regulatory Factor 8**

The impact of IRF8 on I/R injury has only been investigated in the middle cerebral artery occlusion–induced mouse model in vivo and in an oxygen glucose deprivation–induced neuronal apoptosis model in vitro.102 After cerebral ischemia, the expression of IRF8 gradually decreased in neurons in a time-dependent manner. As a negative regulator of stroke progression, IRF8 overexpression clearly suppressed I/R–induced inflammatory cell infiltration, neuronal apoptosis, and oxidative injury post middle cerebral artery occlusion challenge.102

**Interferon Regulatory Factor 9**

Recent studies by our group have demonstrated the deleterious effects of IRF9 on various I/R injuries.180–182 During the process of ischemic injury, Sirt1 has been distinguished for its ability to deacetylate numerous transcription factors, including the proapoptotic factor p53.183 In IRF9-overexpressing mice, the level of Sirt1 expression was significantly reduced post ischemia, concomitant with increased p53 acetylation. The IRF9–activated p53, in turn, enhanced the expression and activation of IRF9, forming a positive feedback loop. Thus, depending on the negative regulation of the Sirt1–p53 axis, IRF9−/− mice showed more severe tissue damage after I/R injury, whereas the dysfunction of the corresponding tissue induced by I/R was markedly mitigated by IRF9 ablation.180–182

Considering the sensitivity of IRFs to various cardiometabolic stimuli and their extensive and potent regulatory effects on the initiation and progression of these diseases, IRFs might serve as promising novel clinical targets for the treatment of cardiometabolic diseases. However, 1 question must be answered before the clinical translation of this target: What occurs after cardiometabolic stress that causes the structural and functional alteration of IRFs?

**Upstream IRF Signaling in Cardiometabolic Diseases**

Under the conditions of cardiometabolic diseases, receptors recognize pathological stimuli and transmit causative signals to target factors via upstream cascades. The profound and perplexing functions of IRF family members on cardiometabolic conditions prompted our research group and others to investigate the upstream events contributing to the modification, activation, or suppression of IRFs and the corresponding downstream responses. Previous studies of the signaling events influencing IRF functions in cardiometabolic diseases focused on the upstream factors of IRF in the immune system. This review summarizes the functional regulation of cardiometabolic diseases by the classical upstream factors of IRFs, including TLR, TRAF, A20, Mindin, Tollip, and CARD (Figure 3).

**Upstream IRF Signaling in Obesity, Insulin Resistance, and Hepatic Steatosis**

TLRs, which are well-characterized immune receptors, are the upstream factors that are predominantly responsible for the activation of IRFs in the immune system.51 Substantial previous studies indicated that TLRs recognize pathogen-associated molecular patterns derived from various microbes in response to infections.184,185 Apart from the exogenous molecules, TLR ligands have been found including a much broader range of endogenous components, eg, lipopolysaccharide, HMGB1, danger-associated molecular patterns, heat shock proteins, and lipopeptides, which are released from injured/inflamed tissues and dying cells.186,187 On HFD challenge, TLRs mediated obesity, insulin resistance, and hepatic steatosis through increased free fatty acids, lipid derivatives, and glucose from adipocytes, liver, or the skeletal muscles.188 The alteration of gut microbiota is also involved in TLRs-regulated insulin resistance and hepatic steatosis through increasing the TLR ligand, lipopolysaccharide, and level in the plasma.189 Recently, Kim et al190 reported that in both HFD- and leptin deficiency–induced metabolic diseases, the expression of TLRs, including TLR1 to TLR9 and TLR11 to TLR13, was upregulated in murine adipose tissue, leading to the activation of their downstream IRFs. Among these TLR family members, TLR2 and TLR4 have been studied most extensively for their regulation of metabolic disorders. Ehres et al191 and Himes et al192 reported significantly reduced adiposity and hepatic steatosis, increased insulin sensitivity, and improved...
glucose tolerance in TLR2-KO mice in an HFD-induced obesity background compared with WT controls. Similar to mice carrying a TLR2 deficiency, reduced metabolic dysfunction and inflammatory responses have been observed in C3H/HeJ mice carrying the functional ablation of TLR4 in models of metabolic dysfunction induced by various stimuli. In contrast to TLR2- or TLR4-KO mice, in which the restoration of metabolism is observed, mice that are genetically deficient in TLR5 are prone to obesity, hyperlipidemia, and insulin resistance. In a recent study, Wang et al reported that the activation of TLR9 induced the expression of Foxp3 and thereby promoted Treg development, contributing to the progression of fulminant type 1 diabetes mellitus; alternatively, the down-regulation of IRF7 significantly blocked the enhancing effect of TLR9 on fulminant type 1 diabetes mellitus. Thus, TLR9 to IRF7 could be deduced as a positive axis in the development of metabolic diseases. However, whether TLR–IRF signaling extensively exists during metabolic disorder is unknown.

TRAFs are key adaptor molecules that regulate TLR-activated IRFs and related immune signaling effectors. Previous investigations have indicated that liver-specific TLR2-KO mice exhibited attenuated diet-induced hyperglycemia compared with controls through the inactivation of glucagon signaling; however, the deficiency of TLR2 in the liver was not sufficient to significantly influence the HFD-induced inflammatory response or insulin sensitivity. Most recently, Chen et al reported that TRAF3 in myeloid cells is essential for the development of metabolic disorders, as evidenced by the finding that the myeloid cell–specific deficiency of TRAF3 dramatically reduced genetic (ob/ob) or HFD-induced insulin resistance, glucose intolerance, hepatic steatosis, and obesity resulting from its powerful capacity to regulate inflammatory responses. Although TRAFs are positive regulators of IRF activation, to the best of our knowledge, there is no evidence about the involvement of IRFs in TRAF-regulated metabolic dysfunction.
A20, a protein that was originally identified as an inducible gene on TNF treatment, is a dominant gatekeeper for maintaining homeostasis.\textsuperscript{201} In addition to its negative regulation of NF-κB and apoptotic signaling, A20 has been recently shown to mediate IRF signaling via its interaction with TBK1 and IKKs.\textsuperscript{202} In obese human subjects, the expression of A20 in adipose tissue negatively correlates with insulin sensitivity. However, interestingly, the A20 level is significantly elevated during bariatric surgery-induced weight loss, suggesting that A20 represents a potential target for mitigating obesity-related pathology.\textsuperscript{203} The underlying mechanism and detailed interaction between IRFs and A20 in metabolic diseases are largely unknown.

In the immune system, the ECM protein Mindin is a crucial pattern recognition molecule that triggers the activation of TLR/TRAF/IRF signaling.\textsuperscript{205} Our recent study displayed decreased Mindin expression in the livers of HFD- and in ob/ob-induced obese mice.\textsuperscript{206} The extent of diet- or genetically induced obesity, hepatic steatosis and insulin resistance in Mindin-KO mice is markedly increased compared with WT controls.\textsuperscript{204} A microarray assay revealed that Mindin-affected genes were closely associated with PPARα; however, IRFs were not screened out. The requirement of an interaction between Mindin and PPARα for Mindin-regulated metabolic diseases was further confirmed by the observation that mice overexpressing Mindin containing a mutated PPARα binding domain failed to retard HFD-induced pathological phenotypes.\textsuperscript{204}

In IRF-mediated immune signaling, CARD3, an adaptor protein that contains an N-terminal serine/threonine kinase domain and a C-terminal CARD, interacts with NOD and TRAF to regulate downstream molecular programs.\textsuperscript{208} The function of CARD3 in inflammation has been observed in the HFD-induced obesity model. CARD3-KO mice exhibited increased production of inflammatory cytokines and impaired M1/M2 macrophage homeostasis compared with their WT controls in response to HFD stimulation; these results might be partially explained the CARD3 deficiency–induced promotion of obesity, hepatic steatosis, and insulin resistance after HFD feeding.\textsuperscript{206} Considering the location of IRFs downstream of the CARD/TRAF-mediated inflammatory response in the immune system and the involvement of IRFs in HFD-induced metabolic disorder, it is possible that the CARD/IRF axis is involved in the inflammatory response regulated by CARD3 during obesity and hepatic steatosis. However, this hypothesis requires further evaluation.

**Upstream IRF Signaling in Vascular Injury**

Previous studies have indicated that IRFs exacerbate or ameliorate vascular injury in an immune-dependent or immune-independent manner. In terms of atherosclerosis, chronic low-grade inflammation has been identified as the major characteristic contributing to the initiation and promotion of this pathological condition.\textsuperscript{207} Therefore, upstream IRF signaling during atherosclerosis might overlap with the immune pathways. TLRs are among the most well-known immune factors that are localized to the cell membrane. In the wire-induced vascular injury, HMGB1 functions as one of crucial endogenous activators of TLRs and participates in the progression of neointima formation,\textsuperscript{208} whereas during formation of atherosclerotic plaque, oxidized low-density lipoprotein, amyloid-β, and peptidoglycan can trigger TLR signaling and are closely associated with plaque vulnerability.\textsuperscript{186,209}

The stimulation of TLRs might ultimately induce the activation of IRFs via multistage factors, eg, MyD88, tripartite motif, TRAF, A20, and IKKs, in various cell types, including macrophages, DCs, endothelial cells, and SMCs, that participate in the progression of atherosclerosis.\textsuperscript{210} Accumulating studies using murine models focusing on the regulation of atherosclerosis by TLRs have deduced a phenomenon in which extracellular TLRs, including TLR1,\textsuperscript{211} TLR2,\textsuperscript{209} TLR4,\textsuperscript{212} and TLR6,\textsuperscript{211} mediate proatherogenic signaling programs, whereas endosomal TLRs, including TLR3\textsuperscript{213} and TLR7,\textsuperscript{214} suppress the progression of atherosclerosis. Studies by our laboratory have indicated that, as the downstream factors of TLR4, IRF3 and IRF7 potently promote the development of atherosclerosis (unpublished data), raising the possibility that TLR4 might be involved in IRF3/7-regulated vascular injury as an upstream effector. However, interestingly, although TLR3 positively mediates the activation of IRF3 and IRF7, gene deficiency experiments have demonstrated a contrasting influence of TLR3 and IRF3/7 on the pathogenesis of atheroma. Thus, the upstream signaling of IRF-involved vascular diseases might exert distinct responses to different stimuli, and additional studies exploring the actual molecular events upstream of IRF that are responsible for the regulation of IRFs in vascular diseases are warranted.

Several lines of evidence have demonstrated the participation of TRAFs, crucial adaptor proteins involved in signaling upstream of IRFs, in the development of vascular injury, especially in atherosclerosis.\textsuperscript{215} Early studies of the impact of TRAFs on atherogenesis have indicated that the expression levels of TRAF1, TRAF2, TRAF3, and TRAF6 were significantly increased by the activation of CD40 signaling,\textsuperscript{216} a molecular program that is significantly involved in atherosclerosis.\textsuperscript{215} Additional studies revealed that the downregulation of TRAF1, TRAF3, or TRAF6 markedly increased the CD40 ligand-induced inflammatory response, whereas the silencing of TRAF2 and TRAF3 retarded vascular endothelial inflammation.\textsuperscript{216,217} Together, these previous studies indicated that the role of TRAFs in vascular injury primarily depends on their regulation on CD40-related signaling. However, whether TRAFs act as upstream factors of IRF signaling during vascular diseases remains unclear.

As discussed in the above sections, after vascular injury, neointima formation occurs via an inflammatory and proliferative process.\textsuperscript{130} A20, a TNF-inducible gene that is a key factor negatively regulating the expression of IFN-γ–induced genes, prevents vascular wall thickening by inhibiting endothelial cell injury, inflammatory cell responses, and SMC proliferation.\textsuperscript{218,219} In addition, benefiting from the inhibitory effect of A20 on NF-κB signaling and oxidized low-density lipoprotein insults, A20 overexpression resulted in the potent suppression of the formation of atherogenic lesions.\textsuperscript{210,221} In a partial carotid artery ligation-induced focal arterial stenosis model, mice carrying an A20 hybridization showed significantly elevated intimal hyperplasia, which depended on the increased induction of IFN-dependent genes, including IRFs.\textsuperscript{222} The regulatory functions of A20 in vascular injury combined with its capacity...
to block the activation of IRFs promise the A20/IRFs axis as potential candidate target for the treatment of vascular diseases. A most recent study from our group demonstrated that, in response to PDGF-BB stimuli in vitro or wire injury in vivo, the expression of Mindin in VSMCs was significantly reduced. On the basis of gain- and loss-of-function experiments, we showed that Mindin has a strong inhibitory ability on the proliferation and migration of VSMCs post vascular injury, thereby suppressing intimal hyperplasia. Our further mechanistic studies suggested that the antiproliferative capacity of Mindin dependent on the inactivation of AKT–GSK3β/mTOR–FOXO3A–FOXO1 signaling axis during intimal thickening. Whether IRFs involved in the Mindin-regulated neointima formation is unknown.

CARD8, similar to CARD3 mentioned above, belongs to the CARD family, which is involved in innate immunity based on its interaction with the NF-kB pathway, leading to the suppression of inflammatory activation. Paramel et al reported that the expression of CARD8 mRNA was much higher in human atherosclerotic plaques than that in the transplant donor vessel. However, whether enhanced CARD8 expression is indispensable for the development of atherosclerosis and whether the underlying mechanisms are associated with the activation or suppression of IRFs remain unknown. Taken together, although extensive studies have explored the profound influence of IRFs on various vascular injury conditions, the events preceding the structural and functional alteration of IRFs are far from fully understood. IRF signaling in the innate immune system provides one of multiple possible directions for future research.

Upstream IRF Signaling in Cardiac Remodeling

As summarized in the above sections of this review, IRFs regulate the progression of cardiac hypertrophy primarily, but not exclusively, via immune-independent pathways. Similar to IRFs, TLRs are involved in noninfectious cardiac injury, apart from their abilities to initiate and shape innate and adaptive immune responses to infectious pathogens. A loss-of-function experimental approach indicated that the AB-induced increases in cardiomyocyte size and heart weight in WT mice were significantly blocked by TLR4 ablation, which might be because of the suppression of phosphatidylinositol 3-kinase/Akt/mTOR signaling and NF-kB–binding activity in TLR4-KO mice compared with their WT controls. A TLR4 downregulation-mediated cardioprotective effect has also been observed in Sprague–Dawley rats that were subjected to Ang II infusion. Studies of the relation between TLRs and cardiac remodeling also revealed a close correlation of TLR8 with dilated cardiomyopathy and the resultant heart failure. In addition, cardiac myosin has been found serving as a direct endogenous ligand of TLR2 and TLR8 to trigger production of proinflammatory cytokines, and thereby promote inflammatory response in the myocardium and enhance cardiac remodeling. In the pressure overload– or Ang II–induced cardiac hypertrophy, however, the exact ligands of TLRs have not been clearly identified. Furthermore, the mechanisms underlying TLR-regulated cardiac dysfunction and whether TLRs are located upstream of IRF signaling during the related cardiac diseases are poorly characterized.

As in metabolic and vascular diseases, NF-kB activation is involved in hypertrophy-induced cardiac remodeling. Both phenylephrine- or endothelin-1-stimulated cardiomyocyte enlargement in vitro and AB-induced cardiac hypertrophy in vivo have identified the bona fide suppressive function of A20 on hypertrophic responses based on its negative regulation of NF-kB and the transforming growth factor-beta (TGF-β)-activated kinase 1 (TAK1)–dependent JNK/p38 signaling cascade. Suzuki et al reported that A20 overexpression markedly induced the activation of IRF3 in a TAK1-dependent manner that was negatively regulated by IRF4 in response to stimulation with human T-cell lymphotropic virus. These studies suggested that A20/TAK1/IRFs might form a crucial signaling cascade to regulate cardiac remodeling; however, this hypothesis must be further confirmed.

During the physiological and pathological cardiac remodeling, ECM not only provides structural and mechanical substrates but also regulates extracellular and intercellular signals. Investigations by our laboratory have provided functional evidence that an ECM protein, Mindin, affects the IFN-inducible GTPases. Most recently, Li et al revealed that the specific overexpression of CARD6 in the cardiomyocytes alleviated pressure overload–induced hypertrophic and fibrotic responses, whereas CARD6 deficiency accelerated and exaggerated the pathological cardiac remodeling. The blunted MEK1–MEK–ERK1/2 and JNK1/2 signaling, cardiac remodeling induced by neuroendocrine factors or an ill-defined mechanical stretch was dramatically exacerbated in Mindin-KO mice. Although Mindin has been identified as an upstream factor of IRFs in the immune system, the activity of this signaling axis has not been observed in the events of cardiac remodeling.

Tollip is a dominant negative regulator of TLR-mediated signaling via the MyD88–Tollip–IRAK complex. In both pressure overload– and IL-1β–induced cardiomyocyte enlargement, the association of Tollip with IRAK1 was weakened, leading to increased NF-kB–binding activity and the enhanced activation of p38. The overexpression of Tollip significantly reduced the AB-induced cardiac hypertrophic and fibrotic responses, thereby resulting in a marked improvement in cardiac function compared with the WT controls. Previous studies have indicated that 2 IRF members, IRF4 and IRF5, compete for interaction with MyD88, potentially resulting in the alteration of Tollip activation. However, further investigation is required to determine whether the binding pattern between IRFs and Tollip or other upstream factors in the immune system is altered in cardiac diseases.

CARD6 is a CARD family member that originally identified as an immune regulator structurally and functionally relating to the IFN-inducible GTPases. Most recently, Li et al revealed that the specific overexpression of CARD6 in the cardiomyocytes alleviated pressure overload–induced hypertrophic and fibrotic responses, whereas CARD6 deficiency accelerated and exaggerated the pathological cardiac remodeling. The blunted MEK1–MEK–ERK1/2 and JNK1/2 signaling is responsible for the ameliorating effect on cardiac dysfunction by CARD6. However, the relationship of IRFs to CARD-regulated cardiac hypertrophy has not been well understood.

Upstream IRF Signaling in I/R Injury

Numerous lines of evidence have demonstrated that the molecular cascades that are responsible for apoptosis and...
inflammation constitute the central events underlying the pathogenesis of I/R injury–induced tissue damage. The role of the major upstream factors of IRFs, TLRs, has been intensely investigated in the field of I/R injury. Members of this family are capable of regulating tissue damage after both infectious and noninfectious stresses, including ischemic insult. Unlike other pathological cardiometabolic conditions, one marked characteristic of I/R injury is the massive cell death and the resultant secretion of abundant danger-associated molecular patterns and HMGB1, leading to the activation of TLRs and exacerbation of cell damage. Previous studies have indicated that the influence of TLRs, especially TLR2 and TLR4, on I/R injury seems to be a double-edged sword. Both TLR2 and TLR4 are expressed in neurons and are upregulated post ischemia. When compared with WT mice, TLR4-KO mice exhibited a decreased brain infarct size and maintained neurological function because of the suppression of NF-κB activation, whereas in TLR2-KO mice, the pro-survival Akt and ERK cascades were significantly inhibited, thereby decreasing neurological function and increasing the brain infarct size. Interestingly, the activation of TLR2, TLR4, or TLR9 is closely associated with the improvement of the cerebral I/R outcome. Preconditioning with Pam3Cyskk, lipopolysaccharide, or CpG oligodeoxynucleotides induces the upregulation of TLR2, TLR4, or TLR9, respectively, which significantly reduces the brain infarct size and improves neurological function. Notably, IRF3 is required for the preactivation of TLR4-regulated cerebroprotective function. An investigation of the relevance of other TLR family members to stroke indicated that the expression of TLR7 and TLR8 was associated with poor outcome and enhanced inflammation in acute ischemic stroke but that mice carrying a TLR3 or TLR9 ablation exhibited comparable brain damage after cerebral I/R injury with WT controls. As in stroke insults, postischemic ischemia, the chronic pharmacological or genetic inhibition of TLR4 accounts for the improved survival and decreased liver pathology via suppression of the inflammatory response, apoptosis, oxidative stress, and endothelial overactivation. The TLR2 mRNA level was increased in the ischemic lobes of mice that underwent partial hepatic I/R, and this increase was associated with an increase in TNF-α expression but was reversely accompanied with the alleviation of liver damage. In addition, Bamboat et al. reported evidence that the inhibition of TLR9 conferred degraded inflammatory cytokine production to protect against hepatic ischemic insults.

In a myocardial I/R–induced cardiac dysfunction model, TLR2 or TLR4 deficiency or downregulation closely correlated with the amelioration of heart damage compared with the WT controls. However, although TLR2 ablation resulted in transient protection against ischemic injury, left ventricular dilation after I/R injury was observed in TLR2−/− mice. In addition to the failure of long-term cardioprotection, mice carrying a TLR2 deficiency failed to benefit from the ischemic preconditioning–induced suppression of myocardial I/R injury. Most recently, researchers reported that TLR3, the major TLR member that is responsible for the activation of IRF3 and IRF7, acted as a promoter of I/R–induced cardiac injury. Wang et al. also reported that the inactivation of TLR4/NF-κB and Keap-1/NRF-2 (nuclear factor, erythroid derived 2) signalings involved in liver X receptor–regulated cardiac repair and functional improvement post MI injury. According to immune signaling, the activation of TLRs may recruit adaptor proteins and may subsequently activate downstream factors to regulate the expression and function of IRFs, resulting in the induction of inflammatory and apoptotic responses. Notably, during hepatic I/R injury–induced liver damage, the nuclear upregulation of IRF1 has been linked to the expression of functional TLR4. However, whether and how other molecular signaling pathways are involved in IRF-related I/R injury require further investigation.

In terms of cerebral or hepatic I/R–induced tissue damage, the expression of TRAF1 was markedly induced in the brain or liver, respectively. The artificial genetic downregulation of TRAF1 significantly reduced I/R–induced cytotoxicity in vitro and in vivo. Mechanistic studies demonstrated that TRAF1 directly interacts with ASK1 to positively regulate proapoptotic JNK signaling and negatively regulate the pro-survival Akt pathway. Similar to TRAF1, TRAF5 was expressed at a higher level in ischemic brain caused by the suppression of Akt/FoxO1 signaling. Consequently, TRAF5 deficiency in mice dramatically alleviated cerebral I/R–mediated infarction and resulted in an improved outcome of ischemic stroke. Although the activation of IRFs was modulated by the TRAF family in the immune system, the influence of TRAFs on IRFs has not been examined with respect to I/R injury; understanding this issue will facilitate our understanding of IRF signalings in ischemic injury.

The NF-κB–inhibitory protein A20 exhibits synergistic properties, including anti-inflammation, antiapoptosis, and proproliferation, in response to I/R injury. Both the mRNA and the protein expression of A20 were upregulated in hepatocytes that were subjected to hypoxia and reperfusion. The specific overexpression of A20 in the liver significantly suppressed toxic hepatitis and enhanced hepatic dysfunction in a lethal radical hepatectomy mouse model, which was dependent on the enhanced activation of PPARα. However, interestingly, using a warm hepatic I/R injury model, Yu et al. reported that liver-specific A20 upregulation exacerbated liver damage, as demonstrated by enlarged hepatocellular necrosis and inflammatory cell infiltration. The inconsistent consequences of previous studies suggested that the influence of A20 on hepatic I/R injury might depend on the differences in the liver damage model and in the animal background. Although A20 has been defined as an upstream factor of IRF signaling during its regulation of the immune response, mechanistic investigations of I/R injury emphasized that PPARα, rather than IRFs, is located downstream of A20-regulated tissue damage, indicating that other more important upstream factors might regulate IRF-related I/R injury remain to be identified.

Tollip, a negative regulator of TLR signaling, has been found dramatically upregulated after ischemic challenge in both the infarcted human and the mice hearts. Although the influence of TLRs in MI is controversial, a recent study from our research group unambiguously demonstrated that cardiac-specific overexpression of Tollip significantly exacerbated MI-induced mortality, cardiac dysfunction, and infarct
area compared with WT controls, resulting from the enhanced inflammatory cell infiltration, cytokines production, and myocardial apoptosis. The inhibition of Tollip on Akt signaling has been found closely related to its detrimental effects on MI injury.\textsuperscript{22} Collectively, like IRFs, their upstream regulators in the innate immune network could exhibit critical modulation on cardiometabolic diseases. However, in most cases, an IRF-dependent manner has not been found during these regulators-mediated progression of cardiometabolic disease. Instead, these factors regulate the development of these pathologic conditions through other immune downstream, or even through an immune-independent manner. Thus, the IRF signaling might be much broader than we have thought, and the authentic upstream regulators of IRFs under cardiometabolic conditions remain to be fully understood.

**Expanding IRF Signaling: An Immune-Independent Program**

Since the discovery of first IRF as an IFN transcription factor in 1988, studies on members of this family mostly limited to their influence in immune responses for the next 20 years.\textsuperscript{8,74} However, it should be noted that IRFs have long been exist in metazoa, even before IFNs. So, is it true that IRFs mainly function as IFN regulators and only participate in the immune response? Actually, serial investigations from our laboratory and others support an evidence on the participation of IRF signalings in cardiometabolic diseases, including obesity, insulin resistance, hepatic steatosis, vascular injury, cardiac remodeling, and I/R insults.\textsuperscript{9,10} Comprehensively considering these findings, it is not difficult to conclude that the expressionional and functional profiles of different IRF members under certain cardiometabolic conditions are different in the presence of exogenous stimuli when compared with the physiological state. In addition, differences have also been observed in the functional roles (promotion or suppression) of a specific IRF member in response to distinct pathological insults. It is interesting to investigate why sharing identical or similar domains, different IRFs hold different, or even reverse, functions in cardiometabolic diseases.

In terms of structure, the similarities and differences of IRFs might partially explain their unique expression and functional properties under diverse cardiometabolic conditions. Furthermore, given that a myriad of cascades exist between pathological stimuli and the structural alteration of IRFs, as well as between the functional changes of IRFs and the response of cellular behaviors, a major contributor to the outcome of IRF alteration is the upstream and downstream signaling, which determines when and where IRFs are turned on or turned off and what occurs after the alteration of IRFs under specific conditions. Studies on the mechanisms underlying regulatory effects on cardiometabolic diseases by IRFs indicated that different downstream factors of IRFs might directly contribute to the variance of their regulatory capacity on cardiometabolic conditions. Importantly, although IRFs could regulate inflammatory or immune responses during cardiometabolic diseases, in more cases, IRFs promote or suppress immune-unrelated cellular behaviors of parenchymal cells through immune-independent downstream mechanisms, such as PPAR\textgamma-related lipogenesis, activating transcription factor-3-associated VSMC proliferation, nuclear factor of activated T cell c1-regulated cardiomyocytes hypertrophy, and SRF-mediated neuronal survival.\textsuperscript{11} About the potential upstream regulators, IRF members correspond to specific upstream factors under certain conditions. The impacts of IRFs on certain cardiometabolic stress were indeed associated with their classical upstream factors in the immune system, eg, TLRs, TRAFs, A20, Mindin, and Tollip; however, most classic IRF upstream factor-modulated cardiometabolic diseases are not dependent on IRFs, suggesting that upstream IRF signaling is much broader than these immune factors. The classic cardiometabolic conditions regulators (eg, GPCR, AMP kinase, and Akt) and other immune upstream factors (eg, carabim, suppressor of IKKr, tripartite motif, NRF, and responsive to centrifugal force and shear stress gene 1 [RECS1]) provide possible candidates to the further investigations. Therefore, much effort is required to determine how IRFs regulate or are regulated by transient and prolonged cardiometabolic stresses via upstream and downstream signal transduction. If additional IRF upstream regulators can be identified, what is the relationship between these factors and the classic immune upstream regulators of IRFs? Besides, the conditions under which immune-dependent or immune-independent IRF signaling is activated remain to be determined.

Collectively, although IRFs were originally considered as key immune regulators, the mechanisms by which IRFs function in parenchymal cells indicated that additional IRF signaling that are independent of PRRs and IFN, and even independent of immunity, have coevolved to regulate cellular fates in response to cardiometabolic stresses. Thus, the name of interferon regulatory factors may just represents that the first member of this family, IRF1, was discovered as a transcription factor specifically binding to the regulatory elements of IFN-\beta gene,\textsuperscript{82} but not their authentic functions. On the basis of these serial discoveries, a new conception can be introduced: the IRF signaling is much broader than an immune regulatory event but also an immune-independent program. Moreover, we wonder whether it is a general phenomenon for IRFs possessing an immune-independent regulatory manner or just a special case in certain cardiometabolic diseases?

To answer this question, we should consider from the aspect of evolution. A phylogenetic analysis by Nehyba et al\textsuperscript{276} suggested that IRFs has emerged long before their discovery and exist in all principal metazoan groups, including porifera, placozoa, ctenophore, cnidiania, and bilateria, but not in the signal-cell choanoflagellata, and that the IRFs in the most ancient multicellular organism, sponges, already possess the major defined IRF features in vertebrates.\textsuperscript{276} Accordingly, IRFs could be considered as factors carrying a modern architecture in an ancient life. However, intriguingly, IFN genes did not appear until vertebrate.\textsuperscript{277} So, what is the function of IRFs before IFN discovery? Comparing the development of single-cell choanoflagellata and multicellular metazoan, embryogenesis has been highlighted among functions of the multicellular organism-specific transcription factors. Reasonably, it could be deduced that IRFs are possibly involved in the progress of embryo formation and the related cellular events, for instance, proliferation, differentiation, maturity, and apoptosis. Thus, it might be a
consequent phenomenon, but not a special case, that IRFs can regulate the development of cardiometabolic diseases in an immune-independent manner. An extensive investigation on the essential roles of IFR signalings in the life will be helpful for our further confirmation and fully understanding on the relationship between IRF signaling and cardiometabolic conditions. In addition, considering the key regulatory of IRFs in immune system and the immune-independent regulatory manner of IRFs, several important questions need to be answered: how about other factors in innate immune signaling? Whether an immune-independent manner also exist in regulatory mechanisms of other innate immune factors? What is the essential function of innate immune network? Only limited to immunity or a much broader range? To answer these questions, comprehensively in-depth studies merit to be performed.

Conclusions
It is inspiring that the multiple functions of IRF signaling factors permit them acting as essential sensors and powerful regulators of cardiometabolic diseases, representing an important area of research to better understand the role of the innate immune system in the pathogenesis of cardiometabolic diseases. However, it seems that the deeper we investigate these roles, the more puzzling they become. Among the 9 members of the IRF family, IRF1, IRF3, IRF4, IRF7, IRF8, and IRF9 have been extensively and thoroughly investigated for their cardiometabolic regulatory functions and mechanisms, whereas the effects of IRF2, IRF5, and IRF6 must be further investigated. Concluded from previous information, the underlying mechanisms of IRFs functions in cardiometabolic diseases suggested that as key innate immune regulators, IRFs can influence initiation and progression of pathological cardiometabolic conditions in both immune-dependent and immune-independent manners, and that the upstream factors of IRF signaling might not exclusively originate from the innate immune network.

Several barriers to the translation of IRFs as therapeutic targets to clinical application still remain. First, it is important to note that these activated IRF signaling processes exhibit diverse context-specific responses across different models, organ systems, and cell types. In addition, the consequences of activating or blocking a given IRF signal may differ at different stages of disease development. Therefore, the clarification of the crosstalk between different IRFs and between different IRF signaling pathways under certain conditions will be helpful for the further application of IRF signalings to the treatment of cardiometabolic diseases, and the targeting of the appropriate tissues, cells, and signaling factors at the right time will be necessary to avoid latent side effects. In addition, as transcription factors, it is difficult to use IRFs as direct therapeutic targets; therefore, upstream factors and the underlying mechanisms of IRFs should be elucidated. Furthermore, considerations should be placed on the issue that the role of IRFs in human diseases might be limited when compared with that in preclinical murine models. Moving forward, clinical gene or pharmacology-related evidence is necessary to support the applications of IRFs in preventing and treating pathological cardiometabolic conditions.

Sources of Funding
This work was supported by grants from the National Science Fund for Distinguished Young Scholars (No 81425005), the National Natural Science Foundation of China (No. 81170086), the National Science and Technology Support Project (Nos 2011BAI15B02, 2012BAI39B05, 2013YQ030923-05, 2014BAI02B01, and 2015BA080801), the Key Project of the National Natural Science Foundation (No 81330005), the National Basic Research Program of China (No 2011CB503902), and the Natural Science Foundation of Hubei Province (2013CBF239).

Disclosures
None.

References


496. doi: 10.1038/nij2.


Zhang et al. IRF Signaling in Cardiometabolic Diseases 243


IRF Signalings in Cardiometabolic Diseases

Zhang et al.


193. Zhang et al. IRF Signalings in Cardiometabolic Diseases 245


Interferon Regulatory Factor Signalings in Cardiometabolic Diseases
Xiao-Jing Zhang, Peng Zhang and Hongliang Li

Hypertension. 2015;66:222-247; originally published online June 15, 2015;
doi: 10.1161/HYPERTENSIONAHA.115.04898

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2015 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://hyper.ahajournals.org/content/66/2/222

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published
in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial
Office. Once the online version of the published article for which permission is being requested is located,
click Request Permissions in the middle column of the Web page under Services. Further information about
this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/