Disruption of RIP1-FADD Complexes by MicroRNA-103/107 Provokes Necrotic Cardiac Cell Death

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The adult myocardium has a limited capacity for de novo regeneration. Hence, the loss of functional cardiac myocytes by apoptosis and necrosis is postulated as a central underlying mechanism for ventricular remodeling and heart failure. Although multiple signaling pathways have been proposed, a cogent explanation for how these highly conserved and intricate cell death pathways are regulated in the ischemic myocardium has not been resolved. Given that cardiac function is regulated by the number of viable cardiac myocytes, 1 unifying theme toward the ultimate therapeutic goal in preventing heart failure is to preserve the number of existing cardiac myocytes by suppressing cell death.

In the early days of understanding mechanisms of cell death signaling, much enthusiasm surrounded apoptosis in the pathogenesis of many human diseases, because at that time apoptosis was thought to be the only mode of cell death that was programmed or genetically regulated. Because necrosis has been viewed historically as an accidental or an unregulated form of death, it was largely overlooked or even ignored as a mode of cell death that could be manipulated therapeutically.2,3 During the past several years a greater appreciation and detailed understanding of the molecular signaling pathways that underlie apoptotic and necrotic signaling pathways, together with improved biochemical techniques, have sparked an insurgent interest in necrotic cell death with the resultant revelation that necrosis is indeed a regulated program. In fact, the concept that necrotic death is genetically programmed has tremendous implications for understanding the pathogenesis of cardiac diseases that were previously unexplored. Little is known of the signaling pathways that govern necrotic death in the heart.

Receptor interacting protein 1 (RIP1), a member of serine-threonine protein kinase family of RIPs, has been identified to play a central role in innate immunity, adaptive immunity, and induction of necrosis mediated by death receptor.4 Emerging evidences suggests a diverse role for RIP1 in multiple cell signaling pathways leading to necrosis, apoptosis, survival, or inflammation. RIP1 was first identified as an adaptor protein that coupled tumor necrosis factor-α receptor signaling to nuclear factor-κB activation.5 However, RIP1 was also found to be involved in necrosis induced by death receptor signaling. The dichotomous actions of RIP1 are related to its ability to form homo- and heterotypic complexes with other death fold domain proteins. Structurally, RIP1 comprised an N-terminal kinase domain, an intermediate domain and a carboxyl-terminal death domain. The intermediate domain is composed of 2 critical regions required for RIP1 function. Notably, a ubiquitination site for nuclear factor-κB activation and a receptor homology interacting motif is required for homotypic interactions with other receptor homology interacting motif-containing proteins for necrosis. Notably, the N-terminal kinase domain of RIP1 can be autophosphorylated and is also crucial for inducing necrosis, whereas the C-terminal death domain is required for protein–protein interactions with other death domain signaling proteins, such as fas-associated death domain (FADD), tumor necrosis factor-α receptor–associated death effector domain, and caspase 8.6 Cell fate largely depends on RIP1’s post-translational status, which influences its interaction with other proteins and presumably its ability to signal cell survival through nuclear factor-κB activation or necrosis. For example, K-63-linked polyubiquitination signals nuclear factor-κB activation, whereas K-48-linked polyubiquitination signals necrosis.4 Hence, the ubiquitination status of RIP1 by c-IAP1/2 or deubiquitination by cylindromatosis or A20 provide a key mechanism for the regulating RIP1 signaling. A role for RIP1 in necrotic cell death signaling was substantiated by studies in which pharmacological inhibition of RIP1 kinase activity with necrostatin-1 which inhibits the kinase activity of RIP-1 suppressed necrotic cell death without influencing apoptosis.8 The mechanism by which RIP1 kinase mediates necrosis is not well understood but is thought to involve its association via its receptor homology interacting motif domain with RIP3. Presumably the mitochondrial targeting of the RIP1/RIP3 complex is purported to influence mitochondrial metabolism and permeability transition pore opening on the inner mitochondrial membrane.5,8 Hence, although our understanding of the exact mechanism by which RIP1/RIP3 complexes trigger necrosis is not well understood, RIP1/RIP3 complex represents a key nodal point for regulation necrotic cell death in the heart.8

In this issue of Circulation Research, Wang et al9 provide new insight into the molecular signaling mechanisms that regulated RIP1/RIP3 complexes and necrosis in the ischemic heart. In elegant studies, the authors reveal a novel signaling platform involving the cytoplasmic adaptor protein FADD for

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miRNA 103/107 Regulates FADD and Necrosis

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Figure. Regulation of fas-associated death domain (FADD) and necrosis by lncRNA H19 and miR-103/107 in the heart during myocardial infarction (MI). Wang et al provide evidence that miR-103/107 provoke necrosis through regulation of FADD. Left. Under basal survival conditions long noncoding RNA H19 (lncRNA H19) activates FADD by inhibiting its negative regulator microRNAs 103 and 107 (miR-103/107). In the absence of miR-103/107, FADD forms complex with receptor-interacting protein-1 (RIP1) through its death domain (DD). Right. During ischemia reperfusion, miR-103/107 becomes upregulated from inactivation of lncRNA H19; activated miR-103/107 reduces expression and an association of FADD with RIP1. Subsequent to FADD’s dissociation, RIP3 forms complex with RIP1 through its RIP homotypic interaction motif (RHIM) domain and provokes necrosis. DED indicates death-effector domain; and KD, kinase domain.

suppressing RIP1/RIP3 complexes and necrotic cell death. Furthermore, the authors provide compelling evidence for a genetic link for the regulation of necrosis by miR-103/107 and long noncoding RNA (lncH19) through FADD.

Using a variety of in vitro and in vivo stress models, the authors systematically demonstrated that low-dose H$_2$O$_2$ (100 μmol/L) promoted apoptotic cell death, whereas high-dose H$_2$O$_2$ (500 μmol/L) promoted necrotic death that was accompanied by an unexpected loss of FADD expression. Importantly, gain of function of FADD in H9C2 cells was sufficient to prevent H$_2$O$_2$-induced necrosis, suggesting the involvement of FADD in the regulation of necrotic cell signaling. The authors further showed that inactivation of either RIP1 or RIP3 was sufficient to prevent H$_2$O$_2$-induced necrosis. These novel observations pointed toward a crucial role for RIP1 and RIP3 in executing necrosis in the heart. The authors next showed that H$_2$O$_2$-induced necrotic death was increased further on knockdown of FADD but was prevented by inactivation of either RIP1 or RIP3 proteins, suggesting a putative link between FADD, RIP1, and RIP3 for necrotic cell death signaling. Perhaps most compelling, the authors discovered that under basal conditions, FADD binds to RIP1 and prevents RIP1 from associating with RIP3; however, after H$_2$O$_2$ treatment, the loss of FADD activity freed RIP1 to interact with RIP3 and trigger necrosis. Interestingly, restoring FADD activity suppressed RIP1/RIP3 interaction and necrotic cell death. These data are highly suggestive of a model in which FADD displaces RIP3 from RIP1 to suppress necrosis, with the loss of FADD resulting in RIP1/RIP3 complexes and necrosis (Figure). To begin to address the mechanisms by which FADD is downregulated during cardiac stress, interestingly the authors discovered seed elements for the miRNA103/107 within the FADD coding sequence, suggesting that FADD may be regulated by miRNA103/107. This observation, together with the finding that miRNA103/107 are expressed at low levels under basal conditions but highly induced in response to H$_2$O$_2$ or myocardial infarction, raised the interestingly possibility that FADD and subsequently FADD–RIP–1 complexes are regulated by miRNA103/107. To test this possibility, and how FADD is regulated under basal and stress conditions, the authors systematically overexpressed miR103 and miR107 in cells. This resulted in a dramatic reduction in FADD expression consistent with the idea that miRNA103 and107 regulate FADD activity. Conversely, knockdown of miR-103/107 in cells upregulated FADD and suppressed H$_2$O$_2$-induced necrotic cell death in vitro and in vivo. These studies support the notion that miRNA103/107 play a crucial role in regulating FADD expression and necrosis signaling. Perhaps one of the most interesting aspects of the study was the demonstration that miRNA103/107 was suppressed by lncH19 under basal conditions. In fact, the loss of lnc H19 in response to H$_2$O$_2$ or myocardial infarction corresponded with an increase in mirRNA103/107 expression, loss of FADD activity, increased RIP1/RIP3 complexes, and necrosis. Hence, the findings of the present study reveal a novel signaling axis that functionally couples FADD regulation by noncoding RNA lnc19, miRNA103/107s to necrotic cell death via RIP1/RIP3 signaling.

Although this study provides new important insight in underlying mechanisms of necrotic cell death signaling in the heart, there are several questions that remain unanswered. In particular, although the authors have convincingly demonstrated the importance of FADD–RIP1 complexes for suppressing necrosis, it remains to be proven where these interactions take place within the cell. Moreover, the temporal activation of RIP1–RIP3 complexes leading to necrosis is unknown. For example, it remains undetermined whether RIP1/RIP3 alter mitochondrial metabolism? Respiration? Or influence other death effectors that impact mitochondrial permeability transition pore? Interestingly, the authors found that knockdown of miR-103/107 prevented necrosis induced by high dose of H$_2$O$_2$, presumably by increasing or stabilizing FADD; however, knockdown of miRNA103/107 did not prevent apoptosis induced by low doses of H$_2$O$_2$; this observation suggests the existence of 2 mutually independent pathways for apoptosis and necrosis signaling, where necrosis and not apoptosis is dependent on miR-103/107 and FADD. Alternatively, it is possible, that miRNA103/107 target or influence the expression of other proteins that regulate necrotic cell death. For this
reason, it would be important to understand the molecular switches that determine the cells’ fate and decision for activating 1 cell death pathway over the other. For instance, it is unknown whether the ability of FADD/RIP1 to suppress necrosis is a universally considered feature of postmitotic organs such as the heart or has a more global impact on cell survival. Therefore, it would also be important to know whether the ubiquitination status of RIP1, that is, K-63 versus K-48, influences FADD complexes and necrosis in other cell types. Finally, whether other adapter proteins such as TRAF2 (tumor necrosis factor receptor-associated factor), tumor necrosis factor-α receptor-associated death effector domain, or TAK1 (transforming growth factor beta-activated kinase 1) are influenced directly or indirectly by IncH19-miR-103/107 will be important to workout. Nevertheless, the authors provide compelling new evidence that necrosis signaling in cardiac myocytes is regulated by miRNA 103/107 via FADD–RIP1/RIP3 complexes.

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References


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