JACC: BASIC TO TRANSLATIONAL SCIENCE VOL. 9, NO. 9, 2024

EDITORIAL COMMENT

TRIM55: An Enemy at the Post-MI Border?

TRIMming May Not Always Be Good

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"What happens, however, is that when the lack of oxygen and nutrient sustenance is not total, the cell enters into a state of hibernation within that universe that specialists call the ischemic penumbra or, even more poetically, turns into a sleeping beauty. The front line of the therapeutic struggle tries to reconstruct the permeability of the obstructed vessel, prolong this state of protective hibernation, and stabilize the membrane—a bit like sending reinforcements to the border police."

—Antonio Lobo Antunes[1]

Myocardial infarction (MI) encompasses ventricular muscle loss and a highly stepwise regulated scar tissue formation.[2] Conversely, the surviving myocardium in the adjacent border zone (BZ) undergoes a profound yet poorly understood morpho-functional remodeling, beginning with a narrow strip of fully perfused but hypo-contractile myocardium (**Figure 1**). Eventually, the BZ will expand to embrace additional contiguous myocardial areas, ushering in a progressive loss of contractile function as the heart remodels.[3] The BZ cardiomyocytes are still viable, but their fate depends on the ischemia, infiltrating immune cells, and fibroblasts in the neighboring infarct zone.[4] Hence, in addition to prompt revascularization ("*time is myocardium*"!), which aims to save more myocytes early after a significant ischemic attack, finding measures that patrol the BZ (ie, sentinels) and/or factors that account for its expansion (as it may occur from days to weeks after MI) should be primary grist for the cardiovascular research mill.

In humans, tripartite motif-containing protein 55 (TRIM55), a ubiquitin ligase of the E3 class, is encoded by the *TRIM55* gene and contains a RING zinc finger, a motif involved in protein-protein interactions. TRIM55 is associated transiently with microtubules, myosin, and titin during muscle sarcomere assembly, justifying its primary expression in the skeletal and heart muscles. As recently reported, TRIM55 is implicated in the pathogenesis of heart failure, although the impact of its genetic deletion appears to be heavily model dependent.[5] Indeed, *TRIM55* knockout mice develop more severe hypertrophy and early-onset systolic dysfunction. Conversely, in a rat model of spontaneous cardiac hypertrophy *TRIM55* expression negatively correlates with left ventricular (LV) mass. In human samples, *TRIM55* expression is lower in idiopathic dilated cardiomyopathy. Concerning myocardial ischemia, TRIM55, as a downstream target of miR-378a-5p, appears to be protective by reducing JNK1/2 activation.[6] Yet, the specific role played by TRIM55 in ischemic myocardial injury remains to be fully deciphered.

In this issue of *JACC: Basic to Translational Science*, Bu et al,[7] using gain-of-function and loss-of-function in vivo and in vitro approaches, set out to fill this gap. First, they found that TRIM55 is overexpressed in the myocardial BZ and that TRIM55$^{-/-}$ mice had markedly reduced infarct area and substitutive fibrosis, along with lower levels of Collagen I and transforming growth factor-β in the BZ at 28 days post-MI. Moreover, after MI, TRIM55$^{-/-}$ mice displayed a reduced Bax/Bcl-2 ratio and cleaved caspase-3 levels in the BZ and lower terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling-positive (TUNEL^{+}) cardiomyocytes, thus attesting to lower apoptosis. Accordingly, to determine if the protein had a direct or indirect role in aggravating the ischemic damage,

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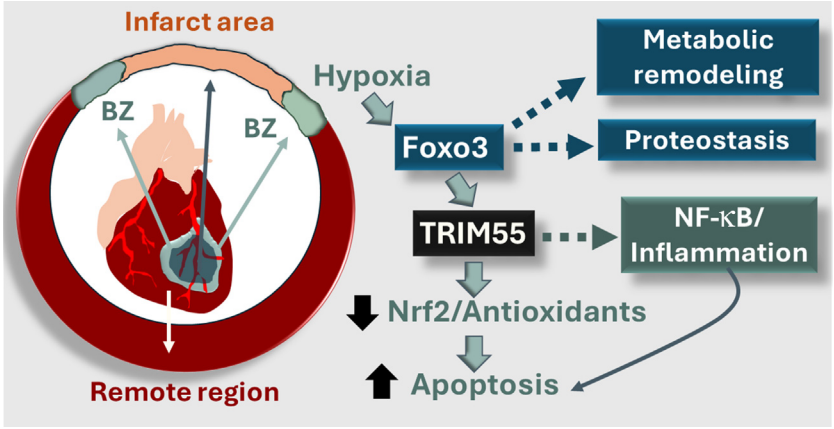
The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the Author Center.

ISSN 2452-302X https://doi.org/10.1016/j.jacbts.2024.06.009

JACC: BASIC TO TRANSLATIONAL SCIENCE VOL. 9, NO. 9, 2024
SEPTEMBER 2024:1123–1125

FIGURE 1 The Role of TRIM55 in the Myocardial Border Zone



Tripartite motif-containing protein 55 (TRIM55) is upregulated in the myocardial border zone (BZ) of mice with myocardial infarction, influencing primary antioxidant weapons, such as the nuclear factor erythroid 2-related factor 2/heme-oxygenase 1 (Nrf2/HO-1) axis. At the same time, however, the BZ showcases a marked upregulation of forkhead box transcription factor 3 (Foxo3) that may modulate additional pathways implicated, among other actions, in controlling the metabolic remodeling and proteostasis of the BZ and other myocardial zones. NF-κB = nuclear factor-κB.

the authors[7] generated TRIM55 overexpressing mice (using an adeno-associated virus approach), finding an expanded MI area and exacerbated LV dysfunction/fibrosis in these mice. In neonatal rat cardiomyocytes (NRCMs), they also overexpressed TRIM55 to show that such intervention conjured up with hypoxia (induced by $CoCl_2$), whereas knocking TRIM55 down produced the opposite effect. Mechanistically, the authors[7] observed that, at least in part, the pro-apoptotic attitude of TRIM55 owes to the inhibition of nuclear factor erythroid 2-related factor 2 (Nrf2), a key regulator of cellular resistance to oxidants; indeed, TRIM55 accelerated Nrf2 degradation by binding to it and through the proteasome pathway. The effects ultimately curtailed the expression of some Nrf2-dependent antioxidant weapons, such as heme-oxygenase 1, accounting for enhanced cardiomyocyte apoptosis. Next, when identifying factors eventually accounting for increased TRIM55 transcript levels, they observed a substantial upregulation of forkhead box transcription factor 3 (Foxo3) in hypoxia-challenged NRCMs. Such change was also evident in the MI BZ. They also demonstrated that Foxo3 overexpression in NRCMs augments TRIM55 protein/gene transcript levels, the opposite being true with its knockdown. Finally, by constructing a mutant TRIM55 promoter plasmid, the authors[7] showed that Foxo3 directly increases the TRIM55 transcriptional and protein expression after hypoxia.

The present study by Bu et al[7] is a trail-blazing one not only because it portends, for the first time, that TRIM55 has a possible "offender loitering at the border zone" of infarcted mice but also because, in cardiomyocytes, it connects TRIM55 to hypoxia. Accordingly, upregulating TRIM55 exacerbated the effects of $CoCl_2$-induced hypoxia on cell viability in neonatal rat cardiomyocytes. Although this evidence awaits validation in different, more adult cardiomyocytes, this finding aligns very well with recent studies—performed in an MI border-zone-on-a-chip model, demonstrating that the BZ is characterized by an O_2 gradient accounting for altered calcium handling and contractility, with gene expression of engineered cardiac tissues in a manner distinct from tissues exposed to global, uniform O_2 levels.[8] Hence, differentially oxygenated cells contribute to the infarct BZ's heterogeneous functional and transcriptional remodeling. Therefore, TRIM55 can perfectly fall into this well of factors triggered by the hypoxia-normoxia transition that characterizes the remote vs the BZ of the ischemic myocardium. As highlighted by the authors,[7] it remains to validate this new scenario in the post-ischemic human heart. Equally relevant, future studies should determine the exact source of possible harbingers of BZ expansion, such as TRIM55. Are cardiomyocytes the primary and only source of such factors? And from which ischemic zone are they coming from? From the healthy cells of the remote myocardium and/or from within the BZ? Answering

these questions is paramount because previous studies using conditioned media have highlighted that hypoxic cardiomyocytes can secrete factors, ultimately protecting germane myocyte cells from ischemic stress.[9]

Another exciting new fertile terrain of investigation (tightly linked to the outstanding questions highlighted previously) could emerge from present evidence showing the possible involvement of Foxo3. The forkhead family of transcription factors provides a balance between metabolic pathways and protein degradation.[10] In addition to regulating the transcription of the E3 ligases atrogin-1 and MuRF1 (encoded by the TRIM63 gene), FOXO can promote proteolysis through autophagy, which recaptures amino acids and fatty acids for ATP production and new protein translation. In cardiomyocytes, increased autophagy reduces apoptosis following ischemia-reperfusion. Conversely, acute cardiac-specific inhibition of autophagy causes LV dilation, decreased contractile function, and hypertrophy. Recent transcriptomic analysis reveals that BZ cells, besides structural alterations, bear a substantial upregulation of genes involved in mitochondrial activity/organization, energy metabolism (oxidative phosphorylation, fatty acid metabolism), glucose uptake, and metabolism, thus suggesting glucose metabolism preservation to some extent.[11] Therefore, the family is likely posited to rule the proteostasis and energetics of the ischemic myocardium. Yet, it would be essential to determine precisely where FOXO family members, such as Foxo3, are upregulated in ischemic myocardium and if Foxo could also exert beneficial effects distinct from adverse ones due to TRIM55 (**Figure 1**).

Although throwing TRIM55 into the fray of factors likely involved in the morpho-functional remodeling of specific MI areas, the present study portends TRIM55 as a novel (potentially) druggable target to avoid BZ expansion with time after MI. However, present and similar past studies call for an abiding pursuit of further elucidating the BZ composition. In the same vein, although undoubtedly efforts should be made to solidify the "frontier" discovering entities able to reinforce it, we should also strive to dig deeper into who the natural enemies are and "where" exactly they are coming from.

FUNDING SUPPORT AND AUTHOR DISCLOSURES

The authors have reported that they have no relationships relevant to the contents of this paper to disclose.

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KEY WORDS border zone, myocardial infarction, Nrf2, oxidative stress, TRIM55