

# Wnt is coming: A role for serum Wnt2 and 4 in fibrotic remodeling following acute myocardial infarction



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Myocardial infarction (MI) remains a common and morbid manifestation of cardiovascular disease, and principally occurs following atherosclerotic plaque rupture resulting in atherothrombosis and epicardial coronary artery stenosis.<sup>1</sup> Following MI, the heart undergoes remodeling characterized by cardiomyocyte death, ventricular hypertrophy/dilation, and excessive deposition of extracellular matrix (ECM), resulting in myocardial fibrosis. In the short term, fibrosis is an appropriate compensatory response, replacing cells lost to necrosis, stabilizing the remaining tissue and preventing myocardial rupture and death. However, following scar maturation, continued collagen production increases tensile strength and reduces ventricular compliance. This results in a feedforward loop whereby fibrosis begets a further fibrotic response, culminating in diastolic dysfunction, systolic dysfunction and the development of heart failure (HF), along with providing a substrate for the development of cardiac arrhythmias.<sup>2</sup> As such, understanding key molecular drivers of fibrosis remains essential to restoring a compensated response to injury and improving the prognosis of MI patients.

The Wnt protein family are highly-conserved, secreted glycoproteins that bind the Frizzled (Fzd) family of transmembrane receptors. Engagement between Wnt ligands and Fzd receptors prompts the formation of a complex that includes the low-density-lipoprotein-receptor-related proteins (LRP)–5 and LRP6 which can activate canonical Wnt/  $\beta$ -catenin signalling or numerous  $\beta$ -catenin-independent cascades. Recent literature has implicated a role for Wnt signalling in promoting cardiac fibrosis through a complex feedback loop that includes transforming growth factor (TGF  $\beta$ ),  $\beta$ -catenin, and Akt.<sup>3</sup> In previous work, experimental MI in mice has triggered an upregulation of several Wnt

proteins including Wnt2 and Wnt4, however the significance of serum Wnt2 and Wnt4 in acute MI patients and their impact on cardiac fibrosis remains nebulous.<sup>4</sup>

In volume 74 of *EBioMedicine*, Yin and colleagues<sup>5</sup> investigated the impact of Wnt2 and Wnt4 on outcomes in acute MI patients and further characterized the physiological and molecular consequences of Wnt2 and Wnt4 modulation with *in vivo* and *in vitro* rodent models.

In patients following acute MI, Yin et al. demonstrated a positive correlation between both serum Wnt2 and Wnt4 levels and major adverse cardiovascular events defined as hospital readmission for unstable angina or MI, HF, stroke, and all cause death over a 1-year follow-up period. These findings were corroborated in experimental MI induced by left anterior descending artery (LAD) ligation in C57BL/6 male mice, wherein Wnt2 and Wnt4 serum protein levels increased approximately 3-fold 3 days following surgery and were maintained until 28 days post-MI. Interestingly, cardiac Wnt2 and Wnt4 showed a similarly significant increase 3 days post-MI but a decrease to below-baseline levels at day 7, with a recovery to baseline at day 14 and day 28 post-MI.

To explore the molecular underpinnings of Wnt2 and Wnt4 action in cardiac cells, Yin and colleagues used genetic or pharmacological means to modulate Wnt signalling constituents in both their experimental MI model and in neonatal rat cardiomyocytes (NRCMs) or neonatal rat cardiac fibroblasts (NRCFs) subjected to hypoxia. Similar to the LAD ligation model, hypoxia prompted the increased expression and secretion of Wnt2 and Wnt4 from both NRCMs and NRCFs with an accompanying increase in expression of  $\beta$ -catenin, Fzd2, Fzd4, and NF- $\kappa$ B-p65 phosphorylation. Adeno-associated virus 9 (AAV9)-mediated knockdown (KD) of Wnt2/4 in LAD-ligated hearts significantly decreased fibrosis and cardiac dysfunction compared to control MI hearts. Similarly, knockdown of Wnt2/4 in NRCFs decreased pro-fibrotic gene programming in response to hypoxia; Yin and colleagues further conducted elegant genetic (lentivirus-mediated transduction of shRNA or siRNA) or pharmacological (ICG-001, a Wnt/ $\beta$ -catenin inhibitor, or JSH23 and Neferine, two NF- $\kappa$ B inhibitors) experiments that demonstrated a link between Wnt2 and Wnt4,  $\beta$ -catenin/NF- $\kappa$ B activation, and pro-fibrotic signalling in cardiac fibroblasts.

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Yin and colleagues concluded that Wnt2/4 play critical early roles in the activation of the fibrotic response to ischemic injury. Although the experimental models do identify a role for serum Wnt2/4 upregulation early after MI, the interesting lack of correlation in time-dependent changes in cardiac tissue Wnt2/4 expression and serum Wnt2/4 remains unexplained. Furthermore, the reduction in infarct size and fibrosis in response to Wnt2/4 KD may be an epiphenomenon secondary to cell types not explored in this study such as macrophages, which have been implicated in post-MI remodeling via Wnt-dependent mechanisms.<sup>6</sup>

The AAV9-mediated KD of Wnt2/4 does not precisely recapitulate the patient scenario because KD of Wnt2/4 was performed 3-weeks prior to MI. Although the clinical data in this study identified elevated Wnt2/4 in the serum of acute MI patients, the exact temporospatial relationships of Wnt2/4 and remodeling remain difficult to determine. Therefore, both *in vivo* and *in vitro* experiments wherein KD of Wnt2 and Wnt4 is performed at numerous time points following MI would better link the clinical and rodent data outlined in this work.

Yin and colleagues have presented a robust mechanistic framework that outlined a clear link between Wnt2 and Wnt4,  $\beta$ -catenin/NF- $\kappa$ B activation, and pro-fibrotic signalling in cardiac fibroblasts. The group further demonstrated that Wnt2 and Wnt4 are novel markers of adverse outcomes following an acute MI. This study therefore opens the door to the investigation of Wnt2/4 as both biomarkers and therapeutic targets in HF. Despite significant research attention, therapies targeting fibrosis have not been successful in human clinical studies.<sup>7</sup> Given that HF remains one of the most expensive diagnoses with a dismal prognosis, the clinical importance of new biomarkers and therapeutic targets as presented in this publication, cannot be overstated.

## Contributors

XAL, PBM, and KAC contributed equally to this commentary.

## Declaration of interests

The authors declare no conflicts of interest related to this work.

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