ESC European Society of Cardiology

Cardiovascular Research (2025) **121**, 1146–1148
https://doi.org/10.1093/cvr/cvaf103

INVITED EDITORIAL

Cardiac regeneration beyond stem cells: harnessing sarcomere dynamics and endogenous repair mechanisms

Xiaoke Yin and Manuel Mayr *

National Heart and Lung Institute, Imperial College London, 86 Wood Lane, London W12 0BZ, UK

Online publish-ahead-of-print 3 June 2025

This editorial refers to 'Integrated proteomics identifies troponin I isoform switch as a regulator of a sarcomere-metabolism axis during cardiac regeneration' by T.J. Aballo *et al*., https://doi.org/10.1093/cvr/cvaf069.

Cardiac regeneration is still a key unmet clinical need, particularly after myocardial infarction (MI), when damaged cardiac tissue is replaced by a fibrotic scar.

Previous attempts at cardiac generation have focused on cell-based therapies. In the early 2000s, studies tested the intracoronary injection of bone marrow-derived cells, based on the hypothesis that endothelial progenitor cells in the bone marrow could differentiate into endothelial cells or even cardiomyocytes (CMs). However, it was later shown that the endothelial progenitor cells used in culture were not genuine stem cells, but macrophages contaminated with platelets, carrying many endothelial marker proteins.[1] Similarly, cardiac progenitor cells (CPCs) ultimately failed as a regenerative therapy for MI and heart failure,[2] despite initial studies suggesting that CPCs, such as c-kit$^+$ cells, cardiosphere-derived cells, and Sca-1$^+$ cells, had proliferative and regenerative potential in preclinical models. However, CPCs also did not differentiate efficiently into new CMs *in vivo*. While paracrine effects (i.e. the release of beneficial factors) were observed, these effects were transient and did not result in long-term functional improvements. Further undermining confidence in the field, several high-profile CPC studies were later retracted due to data fabrication.

Our previous proteomics study has compared neonatal mouse hearts and adult zebrafish hearts[3] to investigate cardiac regeneration. In both model organisms, CM proliferation contributes to the repair of the damaged heart tissue. In contrast, adult mammalian CMs have very limited, if any, regenerative potential. The decline in cardiac regenerative capacity during postnatal development is attributed to hypertrophic CM growth, cell cycle exit, mitochondrial maturation, and sarcomeric protein isoform switching.[4] Understanding these processes in detail and exploring ways to reverse them may help restore the regenerative ability in adult CMs.[5]

In the current issue, Aballo *et al*.[6] focus on the sarcomere isoform switch that occurs during postnatal development, specifically the transition from slow skeletal troponin I (ssTnI) to cardiac troponin I (cTnI).[7] TnI is the inhibitory subunit of the troponin complex, playing a key role in Ca^{2+} regulation of CM contraction and relaxation. During the postnatal development, ssTnI is gradually replaced by cTnI (*Figure 1A*). This transition coincides with the loss of regenerative capacity and the metabolic shift from glycolysis to oxidative phosphorylation. Overexpression of ssTnI has been shown to protect against ischaemia[8] and pressure overload injury,[9] by promoting glycolytic metabolism.

In this study, Aballo *et al*.[6] employed both top-down and bottom-up proteomics to better understand how sarcomere remodelling may influence the regenerative capacity of the heart. For conventional bottom-up proteomics, proteins are enzymatically digested to peptides before analysis by mass spectrometry (MS). In contrast, top-down proteomics is a MS-based approach that analyses intact proteins without prior digestion. Top-down proteomics is particularly useful when studying transitions of small, abundant proteins,[10] such as the ssTnI to cTnI switch, but necessitates a high-resolution instrument.

The authors compared left ventricular tissues from wild-type (WT) mice at postnatal Day 1 (P1)—when regeneration is still present, Day 8 (P8)—shortly after regenerative capacity is lost, and Day 28 (P28)—when regeneration is absent. Proteins enriched in P1 hearts were associated with development and mitosis. While cell cycle and developmental pathways were up-regulated in P1 hearts, P8 hearts showed an increase in fatty acid oxidation-related processes, marking the metabolic shift. A key finding was the isoform transition from ssTnI to cTnI, one of the most pronounced protein changes between P1 and P28. While ssTnI and cTnI were still present at similar levels at P8, ssTnI was fully replaced by cTnI at P28. This coincided with up-regulation of proteins related to lipid metabolism, the electron transport chain, and negative regulation of proteolysis in P28 hearts. These findings suggest that TnI isoform switching is closely linked to metabolic reprogramming, shifting from glycolysis to fatty acid oxidation, and aligns with the loss of regenerative capacity in neonatal mouse hearts (*Figure 1B*).

To assess the impact of ssTnI overexpression on postnatal heart development, Aballo *et al*.[6] used a Tnni1 transgenic (Tg) mouse model. Top-down proteomics confirmed that ssTnI levels remained high at P8 in Tnni1 Tg mice, while cTnI expression was absent at both P8 and P28 (*Figure 1B*). Global proteome analysis by bottom-up proteomics revealed that the baseline proteomic profiles of WT and ssTnI mice were highly similar. At P8, however, WT hearts exhibited higher levels of pyruvate dehydrogenase kinase 4, which inhibits pyruvate dehydrogenase, thereby reducing glycolysis and promoting fatty acid oxidation. Proteins related to mitochondrial metabolism were up-regulated at P8 in WT hearts. In contrast, ssTnI hearts showed increased levels of AMP-activated protein kinase (AMPK), a central metabolic regulator that balances glycolysis and fatty acid oxidation, alongside other glycolysis-related enzymes. These findings suggest that ssTnI overexpression may alter metabolic activity.

The opinions expressed in this article are not necessarily those of the Editors of *Cardiovascular Research* or of the European Society of Cardiology.

* Corresponding author. Tel: +44 20 7594 9909, E-mail: m.mayr@imperial.ac.uk

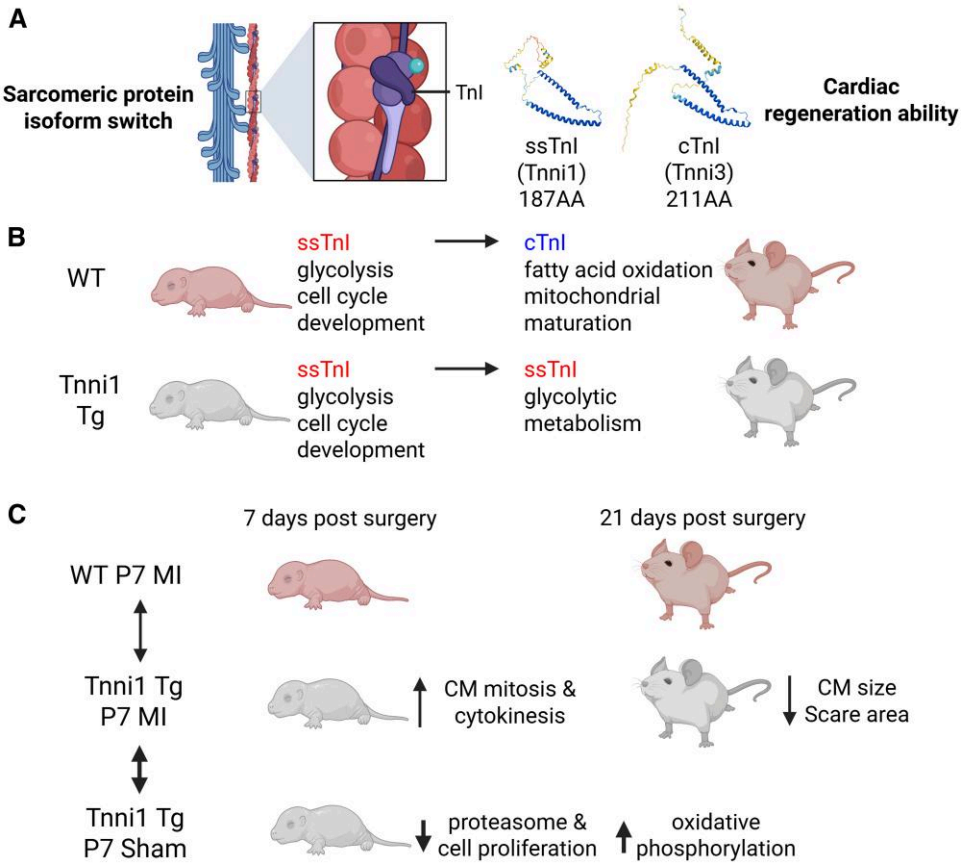

Figure 1 (*A*) Troponin I isoform switch linked to cardiac regeneration. During postnatal development in mice, slow skeletal troponin I (ssTnI) is gradually replaced by cardiac troponin I (cTnI). This transition coincides with the loss of cardiac regenerative capacity around postnatal Day 7 (P7). (*B*) Comparative -omics studies in transgenic mice at baseline. In wild-type (WT) mice, the isoform switch is accompanied by a metabolic transition from glycolysis to fatty acid oxidation, along with increased mitochondrial activity by postnatal Day 7 (P7). In contrast, in a cardiomyocyte-specific ssTnI transgenic (Tg) mouse model, ssTnI is retained throughout postnatal development period, with cTnI remaining undetectable. Glycolysis remains the dominant metabolic pathway in these Tg mice. (*C*) Comparative -omics studies in transgenic mice after myocardial infarction (MI). Tg mice exhibit a higher cardiomyocyte (CM) proliferation rate, smaller CM size, and reduced scar area compared to the WT mice following MI surgery at P7, indicating retained regenerative capacity in the presence of ssTnI. Additionally, Tg mice exhibit increased cell proliferation and decreased oxidative phosphorylation following MI compared to the sham-operated controls. The figure is generated using BioRender.

Next, the authors determined if the response to cardiac injury was altered in Tnni1 Tg mice. At P7, when WT hearts naturally scar rather than regenerate, MI was induced in both WT and Tnni1 Tg mice. CMs from Tnni1 Tg mice displayed increased mitosis and cytokinesis following injury. At 21 days post-surgery, WT CMs were significantly larger compared to CMs from Tnni1 Tg CMs, which is consistent with reduced hypertrophy and possibly a higher number of newly formed, smaller CMs post-MI. Remarkably, scar size was significantly reduced in Tnni1 Tg mice compared to WT mice at 21 days post-surgery (*Figure 1C*). Thus, the replacement of cTnI with ssTnI in the postnatal heart may enhance cardiac regeneration after P7 MI, potentially driven by CM proliferation and decreased reliance on fatty acid oxidation for energy production. While elevated AMPK expression was observed as baseline phenotype of the Tnni1 Tg mice, there was significant down-regulation of proteins related to oxidative phosphorylation (PDK4, NDUFS3, and ATP5PO) and significant up-regulation of proteins involved in the proteasome and cell proliferation following injury.

Overall, this study highlights the role of sarcomere structure in restricting CM proliferation, at least in mice. It remains to be seen whether similar results can be achieved in human CMs and offer therapeutic strategies for cardiac regeneration after MI.

Conflict of interest: none declared.

Funding

M.M. is a British Heart Foundation (BHF) Chair Holder (CH/16/3/32406) supported by a BHF programme grant (RG/F/21/110053) and the Imperial BHF Research Excellence Award (4) (RE/24/130023).

Data availability

There are no new data associated with this article.

References

1. Prokopi M, Mayr M. Proteomics: a reality-check for putative stem cells. *Circ Res* 2011;**108**: 499–511.
2. Lunde K, Solheim S, Aakhus S, Arnesen H, Abdelnoor M, Egeland T, Endresen K, Ilebekk A, Mangschau A, Fjeld JG, Smith HJ, Taraldsrud E, Grogaard HK, Bjornerheim R, Brekke M, Muller C, Hopp E, Ragnarsson A, Brinchmann JE, Forfang K. Intracoronary injection of mononuclear bone marrow cells in acute myocardial infarction. *N Engl J Med* 2006;**355**: 1199–1209.

3. Gomes RS, Skroblin P, Munster AB, Tomlins H, Langley SR, Zampetaki A, Yin X, Wardle FC, Mayr M. "Young at heart": regenerative potential linked to immature cardiac phenotypes. *J Mol Cell Cardiol* 2016;**92**:105–108.
4. Mahmoud AI. Metabolic switches during development and regeneration. *Development* 2023; **150**:dev202008.
5. Cardoso AC, Lam NT, Savla JJ, Nakada Y, Pereira AHM, Elnwasany A, Menendez-Montes I, Ensley EL, Petric UB, Sharma G, Sherry AD, Malloy CR, Khemtong C, Kinter MT, Tan WLW, Anene-Nzelu CG, Foo RS, Nguyen NUN, Li S, Ahmed MS, Elhelaly WM, Abdisalaam S, Asaithamby A, Xing C, Kanchwala M, Vale G, Eckert KM, Mitsche MA, McDonald JG, Hill JA, Huang L, Shaul PW, Szweda LI, Sadek HA. Mitochondrial substrate utilization regulates cardiomyocyte cell cycle progression. *Nat Metab* 2020;**2**:167–178.
6. Aballo TJ, Bae J, Paltzer WG, Chapman EA, Perciaccante AJ, Pergande MR, Salamon RJ, Nuttall DJ, Mann MW, Ge Y, Mahmoud AI. Integrated proteomics identifies troponin I isoform switch as a regulator of a sarcomere-metabolism axis during cardiac regeneration. *Cardiovasc Res* 2025;**121**:1240–1253.
7. Sasse S, Brand NJ, Kyprianou P, Dhoot GK, Wade R, Arai M, Periasamy M, Yacoub MH, Barton PJ. Troponin I gene expression during human cardiac development and in end-stage heart failure. *Circ Res* 1993;**72**:932–938.
8. Pound KM, Arteaga GM, Fasano M, Wilder T, Fischer SK, Warren CM, Wende AR, Farjah M, Abel ED, Solaro RJ. Expression of slow skeletal TnI in adult mouse hearts confers metabolic protection to ischemia. *J Mol Cell Cardiol* 2011;**51**:236–243.
9. Carley AN, Taglieri DM, Bi J, Solaro RJ, Lewandowski ED. Metabolic efficiency promotes protection from pressure overload in hearts expressing slow skeletal troponin I. *Circ Heart Fail* 2015;**8**:119–127.
10. Cai W, Tucholski TM, Gregorich ZR, Ge Y. Top-down proteomics: technology advancements and applications to heart diseases. *Expert Rev Proteomics* 2016;**13**:717–730.