Basic Research in Cardiology (2021) 116:20
https://doi.org/10.1007/s00395-021-00857-9

EDITORIAL

Cardioprotection vs. regeneration: the case of extracellular vesicle-derived microRNAs

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Received: 5 March 2021 / Accepted: 5 March 2021 / Published online: 19 March 2021

Cardiomyocyte renewal in the adult mammalian heart occurs via proliferation of pre-existing cardiomyocytes, as opposed to differentiation from endogenous cardiomyogenic stem cells [7]. Unfortunately, the intrinsic renewal capacity of adult mammalian cardiomyocytes is limited, with similarly low rates reported for rodents and humans [3, 17]. Consequently, injuries associated with massive cardiomyocyte death, like an acute myocardial infarction (MI), promote scarring as opposed to noticeable tissue regeneration [14]. Stimulating cardiomyocyte proliferation may, however, provide a means to achieve myocardial regeneration after injury [2, 5, 6, 8, 13].

In this issue of *Basic Research in Cardiology*, Jung and colleagues propose a new microRNA-based strategy for ischemic heart repair [10]. They initially observed that human-induced pluripotent stem cell-derived cardiomyocytes (iCMs) release large amounts of extracellular vesicles (EVs) when cultured under hypoxic conditions. EVs are membrane-enclosed nanoscale particles that can transfer protein, lipid, RNA, and DNA cargo between cells through the extracellular space [12]. Three microRNAs (miR-20b, miR-92a, and miR-363), originating from a common pre-microRNA transcript, were highly enriched in hypoxic iCM-derived EVs. Treatment with iCM-derived EVs or directly overexpressing the three miRs protected iCMs from hypoxia-induced cell death. Conversely, downregulating the three miRs reduced iCM survival under hypoxic conditions, thus revealing a cell-autonomous protective mechanism [10].

Exploring therapeutic potential, the authors turned to a mouse model of acute MI induced by permanent coronary artery ligation. Injecting EVs or miR-20b-, miR-92a-, and miR-363-mimics encapsulated in a biodegradable polymer into the infarct border zone immediately after MI attenuated left ventricular dilatation and systolic dysfunction as assessed by magnetic resonance imaging (MRI) at 2 and 4 weeks. At 4 weeks, miR-treated mice had developed only very small scars (encompassing ~ 13% of the LV myocardium) compared with control animals (~ 38%). Mechanistically, the authors propose that the EVs, specifically the three miRs, stimulated cardiomyocyte proliferation in the infarct border zone leading to endogenous self-repair of the heart [10]. But is this a plausible explanation?

Supporting their case, the authors found that overexpressing the three miRs in hypoxic iCMs induced mRNA signatures related to cell cycle G1/S transition, DNA replication, and G2/M phase. As evidenced by EdU incorporation and Ki67 and Aurora B expression, these gene expression changes were associated with a modest increase in the number of iCMs progressing through the cell cycle. Using bioinformatic tools and miR-target validation, the authors identified Notch3 as a direct downstream target of the three miRs. Indeed, selectively targeting Notch3 using a small interfering RNA mimicked the effects of miR-overexpression in iCMs. Considering that iCMs provide a suitable platform to study cardiomyocyte proliferation in vitro [4], the authors regrettably do not report the effects of miR-transfection or Notch3 targeting on iCM cell numbers [10].

Quantitation of cell cycle and myofiber marker colocalization via confocal imaging of tissue sections suggested a high rate of cardiomyocyte cell cycle activity in miR-treated infarcted mice [10]. However, this approach is prone to

This comment refers to the article available at https://doi.org/10.1007/s00395-021-00858-8.

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misidentification of cardiomyocyte nuclei when employed in the absence of a cardiomyocyte-restricted nuclear marker or a cell membrane marker [1], as was the case here. Because miR-treatment also appeared to increase cell cycle activity in non-cardiomyocytes, nuclear misidentification would erroneously suggest increased cell cycle activity in the cardiomyocyte compartment of treated animals [18]. It was surprising that miR-mimics would elicit a similar induction of cell cycle activity in in vivo adult cardiomyocytes (which are exceedingly reluctant to proliferate) [3, 17] as compared to iCMs (a cell population which is highly responsive to mitogenic stimuli) [4]. It was also rather surprising that a mitotic marker would exhibit a larger labeling index than an S-phase marker [10], given that S-phase duration is much longer than M-phase duration [16]. Finally, no direct proof of cardiomyocyte cytokinesis is provided in the in vivo studies.

Permanent coronary artery ligation results in transmural necrosis, affecting cardiomyocytes and non-parenchymal cells alike. MRI suggested that the infarcted area was replaced by full-thickness, viable myocardium already 2 weeks after EV delivery. To curtail scar formation within this time frame would require exceptionally high rates of cardiomyocyte proliferation. Notably, miR-mimics were taken up not only by cardiomyocytes, but also vascular cells and fibroblasts in the infarct border zone. Microvessel density in the border zone was ~ 30% greater in miR-treated mice at 4 weeks [10]. Although, with small animal numbers, this potential treatment effect did not reach statistical significance, therapies promoting comparable improvements in border zone capillarization may very well reduce scarring after MI in mice [11, 15, 19]. MiR-mimics may have also reduced cell death after MI as suggested by their effects on hypoxic iCMs in vitro and their impact on the number of caspase 3^+ cells in the infarct border zone in vivo [10]. Serial histopathological examinations early after EV or miR-delivery would have been critical to understand the sequence of events and relative contributions of myocardial salvage, improved wound healing, and cardiomyocyte proliferation [5].

While the authors should be congratulated on defining a novel therapeutic approach to infarct repair, more evidence is needed to convince us, beyond a reasonable doubt, that miR-20b-, miR-92a-, and miR-363-mimics mediate their salubrious effects by stimulating myocardial regeneration, as opposed to promoting cardioprotection and wound healing [9, 14, 19].

Funding Open Access funding enabled and organized by Projekt DEAL.

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