by which pathogens can rewire host lipid metabolism, and their work indicates that the already dense thicket of reactions in the PIP metabolic network still has a few more branches to be uncovered. ❐

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Published online: 28 April 2022
https://doi.org/10.1038/s41556-022-00885-0

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Competing interests

The authors declare no competing interests.

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REGENERATION

Defining the pathways of heart regeneration

Although cardiac cell therapy has been intensely studied, the high expectations are still an unmet goal. A study now characterizes the translational potential and mode of action of human ventricular progenitors (HVPs) derived from embryonic stem cells, as a source for cardiac cell therapy.

Louk Theodoor Timmer and Eva van Rooij

Cardiac cell therapy, aiming to treat acute myocardial infarction or heart failure, has been an area of pre-clinical and clinical research for several decades. The first clinical application of cell therapy to treat the heart dates back to more than 20 years ago[1]. Since then, a multitude of clinical trials have been conducted, but given the inconsistent or at best moderately positive effects reported[2–4], the high expectations for cardiac cell therapy have so far not been met. These studies predominantly used bone marrow cells and while the lack of efficacy might simply be due to the inability of these cells to restore cardiac function, it might also be explained by the lack of fundamental understanding of the mode of action of the cells or a wrongful use in the trial itself. Whereas cell therapy was initially proposed to result in the remuscularization of the injured heart, it was later shown that the cell sources used had no intrinsic capability of (trans)differentiating into functional cardiomyocytes (CMs) themselves[5]. Nonetheless, beneficial effects were reproducibly present in pre-clinical studies[6] and these effects have been attributed to the fact that injection of bone marrow cells was shown to trigger an acute sterile inflammatory response that affected the activity of cardiac fibroblasts and enhanced the mechanical properties of the injured area[7]. Despite open-ended mechanistic questions, the incredible therapeutic potential of the method and room for improvement provide support to further explore the effect of cell therapy on heart repair.

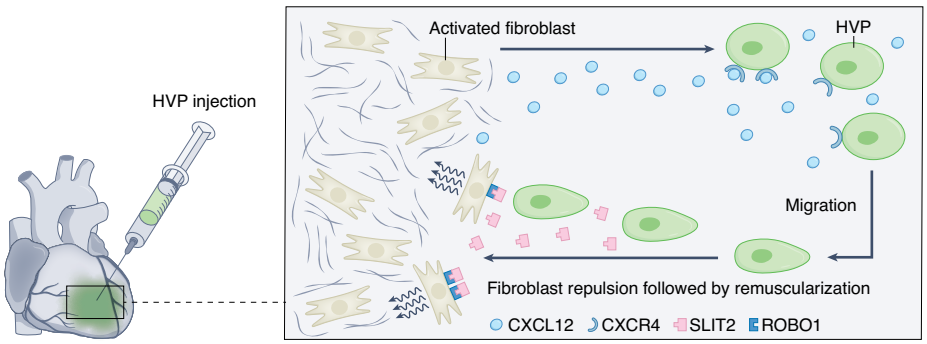

Fig. 1 | The proposed mode of action of HVP-mediated cardiac cell therapy. After injection, CXCL12 secreted by activated cardiac fibroblasts induces CXCR4-expressing HVPs to migrate towards the injury site. Next, HVPs induce the repulsion of cardiac fibroblasts in a SLIT2–ROBO1-dependent manner, followed by remuscularization of part of the injured area.

For cell therapy to be effective, a reproducible source of (engineered) cells capable of self-assembling into myocardium or a myocardium-like structure without causing arrhythmias or having tumorigenic potential is required. Human ventricular progenitors (HVPs) may fulfil these requirements[8]. HVPs are cardiac progenitor cells derived from human embryonic stem (ES) cells that have initiated the differentiation process towards cardiomyocytes. This subpopulation of cardiac progenitors is characterized by ISL1 expression, which is expressed after ES cells have adopted a mesodermal fate, but before they have committed to the CM cell fate. In vivo transplantation of HVPs results in their self-assembly and maturation into

a patch of ventricular-like myocardium[8]. As the same approach has been proven ineffective for the transplantation of cells that were at earlier (mesodermal state) or later (differentiated CMs) stages of development compared to HVPs, success appeared dependent on the differentiation state of the human ES cells. In continuation of the identification of HVPs as a potential cell source for cardiac cell therapy[8], in this issue of *Nature Cell Biology*, Poch et al. further characterized these HVPs in a more translational manner[9].

To gain mechanistical insight into the mode of action of HVPs, the authors used ex vivo heart slices of left ventricular tissue of adult non-human primates (NHP-LV), a species obviously evolutionarily close to humans to improve predictive value for potential translation to patients. Radiofrequency ablation (RFA) was used to induce a local injury site in this model. Seeding either eGFP-labelled HVPs or CMs on the opposite site of the tissue slice indicated a migratory capacity of the HVPs, which was absent in the CMs. The migration of HVPs initiated remuscularization in the injured area, which corresponded to a reduction in scar size and improved contractile function compared to the CM-treated tissue slices. This suggested that, in contrast to more differentiated CMs, HVPs contain an enhanced capacity to migrate into the injury area to restore the damage.

Poch et al.[9] noted a high abundance of activated cardiac fibroblasts (CFs) at the border zone of the injury site before the HVPs populated the site. This observation raised the question of whether intercellular communication between the existing CFs and added HVPs caused the migration of HVPs towards the site of injury. Potential ligand–receptor interactions between cell types important in regeneration can be identified using single-cell transcriptomics[10,11]. Single-cell transcriptomic analysis on migrating and injury-site-arriving HVPs in addition to resident NHP-LV cells suggested CXCL12 ligand expression from activated CFs stimulated CXCR4-expressing HVPs. The researchers confirmed that the CXCL12–CXCR4 axis was indeed involved in the migratory phenotype of HVPs in vitro. Next, CFs were isolated from NHP hearts and transduced to stably express dsRed. When RFA was performed on a monolayer of dsRed CFs in combination with the seeding of eGFP-labelled HVPs at the opposite site of the monolayer, it was shown that, during the migration to the injury site, HVPs caused CFs to repel, based on a SLIT2–ROBO1 interaction between the cell types (Fig. 1). These data may explain the reduced scar formation in the presence of HVPs.

To further translate their findings, Poch et al.[9] continued to assess the therapeutic potential of HVPs in a large animal model. Immunocompromised pigs received two RFA injuries, whereafter HVPs were injected ~1 cm from one RFA site to enable an intra-animal comparison. HVPs successfully migrated to the injury site, populating roughly one-fifth of the injury site two weeks post-transplantation. The migration of HVPs was practically prevented by a CXCR4 antagonist suggesting that, also in vivo, CXCR4 is required to attract HVPs to the site of injury where they self-assemble into a ventricular-like patch. In a porcine model of myocardial infarction, injecting HVPs into the border zone 3 weeks after a myocardial infarction resulted in several ventricular myocardium-like patches comprising 3 to 9.4% of the infarcted area 3 months post-injection. Even though mechanistic molecular studies were not performed on these tissues, the HVP-treated animals displayed a significant decrease in infarct volume compared to the control group. The global longitudinal strain, as a measure for left ventricular systolic function, only deteriorated in the control pigs, whereas it remained constant over the 3-month period for the HVP-treated animals.

This study complements a series of papers showing that embryonic or pluripotent stem-cell-derived (progenitor) cardiomyocytes can remuscularize parts of the infarcted heart and improve cardiac function in large animal models[12–14]. An aspect that received much attention in these earlier studies of cardiac cell therapy in non-human primates was the arrhythmic potential[12–14]. This remains an important aspect that will need to be studied for HVPs. It is also worth mentioning that although Poch et al.[9] suggest that HVPs may have some advantages over more differentiated CMs, no comparison to this cell type was made in their in vivo studies. As other studies showed the successful usage of CMs in non-human primates[13,14], a direct comparison would be needed to establish which source of cells has the highest potential for the clinic.

In line with current knowledge about other stem-cell populations, the positive effects of the HVPs involve intercellular signals in the setting of cardiac injury. While the paracrine effects of exogenous cells have been reported, this study suggested that host cells are signalling to the injected cells to support their migration into the injured area. This might reflect a currently understudied mechanism that could further explain the potential benefit of injected stem-cell populations.

Concluding, this study advances our understanding of mechanisms underlying successful cardiac cell therapy using HVPs and brings us one step closer to designing suitable strategies to test these applications in a clinical setting. Even though therapeutic feasibility remains to be proven, this study clearly supports the capability of HVPs to remuscularize parts of the infarcted heart, which could be of benefit for patients with ischaemic heart disease. ❐

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Published online: 12 May 2022
https://doi.org/10.1038/s41556-022-00914-y

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Competing interests

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