

Cardiac stromal cells on stage: From dull filler to specialized actors

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Key words: Cardiac fibroblasts, Cardiac stroma, Cardiac repair, Cardiac remodeling

Abstract: Cardiac stromal cells have faced through the years a significant evolution in their definitions concerning their phenotypes, markers, and functions. They are surging to key roles in physiopathology, becoming important targets to be exploited for cardiac repair. In this perspective, we briefly discuss their role in novel therapeutic strategies for enhancing cardiac repair and regeneration.

A New Profile for the Old Stroma

The stromal compartment has been long considered as an unspecialized space filled with extracellular matrix and support cells. These were basically all classified as fibroblasts, whose only duty was to produce and remodel the extracellular matrix (ECM), both under homeostatic and pathological conditions (Borg and Baudino, 2011; Ceausu *et al.*, 2021). This has been the vision for the stroma of many organs and tissues, including the heart, and cardiac fibroblasts have long been considered as naïve cells with the only ability to respond to injury by producing scar tissue. Now we understand that cardiac stromal cells (CSCs) present many different phenotypes and profiles, involved in advanced and complex functions, such as vascular support and crosstalk with the immune system (Forte *et al.*, 2018; Plikus *et al.*, 2021). In fact, a striking change of perspective has arisen from single cell sequencing studies on whole dissociated organs, where transcriptomic evidence has defined the identity of many different cardiac stromal populations, some even able to go against fibrotic progression (Farbehi *et al.*, 2019; Forte *et al.*, 2020). This is creating a new identikit far from the dull ECM-producing cell type, which creates fibrosis in response to any kind of injury, paradoxically going against the interests of its own microenvironment.

Recent evidence suggests a co-causative role of fibroblasts electrical coupling to cardiomyocytes in the pathogenesis of arrhythmias (Giacomelli *et al.*, 2020), as a sort of revelation against the dominant and much more noble functional role

that has always been attributed to cardiomyocytes. Thus, if you somehow take a direct role in the organ's function *par excellence*, are you really "stromal"? It seems to be somehow a game changer even in the classical definition of roles for parenchymal vs. stromal cells; even more so, if you consider that now stromal cells can be directly reprogrammed into parenchymal cells, i.e., into induced cardiomyocytes (iCMs) (Tani *et al.*, 2018), therefore further challenging their functional and ontogenetic gaps.

Research in cardiac physiopathology and therapy in the last 20 years has evidenced remarkable potential in targeting and/or exploiting CSC abilities, in spite of everything else. Many different strategies have been explored that can fit in two main approaches (Fig. 1): 1) reducing the impact of the classical fibrotic function of activated stromal cells (i.e., fibroblasts and myofibroblasts) by either positively selecting beneficial phenotypes, or conversely by depleting the pro-fibrotic fraction; or 2) partially resetting the ontogenetic program and converting the stroma into parenchyma (in their more classical meanings).

Supporting Balanced Repair Mechanisms

Myocardial healing after injury requires an ideal balance among inflammatory and repair signals (Forte *et al.*, 2018). In the typical condition of a cardiovascular patient (e.g., with advanced age, comorbidities, multiple risk factors) this balance is strongly pushed towards excessive inflammatory signaling, cell death, and activation of fibroblasts towards ECM deposition, with consequent extensive scar tissue formation and progressive functional impairment of the organ (Schirone *et al.*, 2017). Multiple studies have highlighted how *ex vivo* selection and subsequent transplantation into the injured organ of specific

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Received: 09 November 2021; Accepted: 13 December 2021



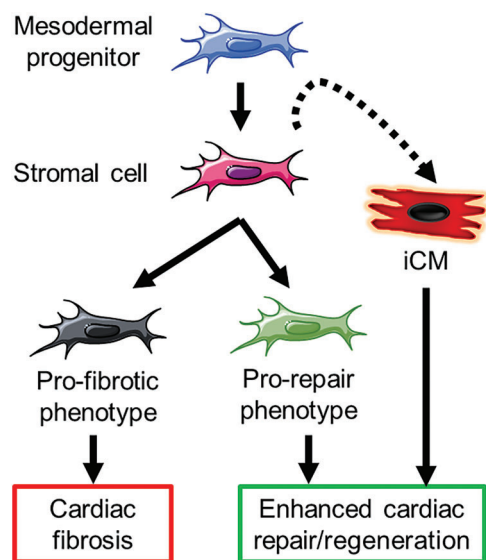


FIGURE 1. An overview of possible mechanisms to be exploited by targeting cardiac stromal cells for enhanced cardiac repair. iCM: induced cardiomyocyte.

resident subpopulations of the heart (described by different authors with multiple terms, such as mesenchymal/stromal, primitive/progenitor, or non-activated cells) can exert positive conditioning on tissue repair by multiple mechanisms, including anti-apoptotic, pro-angiogenic, anti-inflammatory, and anti-fibrotic effects (de Couto *et al.*, 2015; Pagano *et al.*, 2018; Tseliou *et al.*, 2015; Cencioni *et al.*, 2017). Isolating, expanding, and re-infusing endogenous cell populations boosted in this beneficial phenotype has been the strategy of cardiac cell therapy protocols, that have yielded, at least to some extent, beneficial effects, including positive myocardial conditioning and reduction of scar tissue at both pre-clinical and clinical level (Fernandez-Aviles *et al.*, 2017; Malliaras *et al.*, 2014; Ostovaneh *et al.*, 2021; Zwetsloot *et al.*, 2016).

As an alternative to actively increasing the number of pro-repair cells by transplantation, many groups have worked on depleting stromal populations with enhanced pro-fibrotic drive *in situ*. This negative selection can be achieved by pharmacological targeting of fibrotic cells (e.g., by inhibitors of the renin-angiotensin-aldosterone system, or of β -adrenergic signaling, both implicated in cardiac fibrosis progression) (Chimenti *et al.*, 2016; Fang *et al.*, 2017; Kong *et al.*, 2014), or by the use of monoclonal antibodies against specific pro-fibrotic cytokine pathways, such as TGF- β 1 (Warisara *et al.*, 2020). Alternatively, novel immune-therapy based strategies have been shown to be feasible in the heart, such as the generation of chimeric antigen receptor (CAR)-T cells against activated pro-fibrotic fibroblasts (Haig *et al.*, 2019). Overall, providing an advantage to the stromal players that drive a balanced and cardioprotective healing process, or conversely penalizing cell populations that sustain a fibrotic override, both represent efficient (and possibly synergistic) strategies to improve tissue healing after acute injury, or in chronic disease conditions.

Converting Stromal Cells into (Classical) Parenchymal Cells

The exploitation of induced pluripotency as an effective way of producing differentiated cells starting from adult somatic cells

has been considered for cardiac cell therapy applications, possibly allowing the easy “refill” of lost cardiomyocytes. The risk of cumulative somatic and acquired genetic aberrations, though, represents a serious obstacle to its large-scale application (Ben-David and Benvenisty, 2011). Instead, the discovery of direct cardiac reprogramming (Ieda *et al.*, 2010; Tani *et al.*, 2018) has provided many advantages compared to fully induced pluripotency, particularly for the risk of tumor formation. Therefore, its applicability appears much more feasible. This strategy is adaptable also to direct *in situ* and *in vivo* strategies, where the targeted delivery of reprogramming molecules by carriers of different nature may convert CSCs (possibly belonging to the activated pro fibrotic pool) into new cardiomyocytes, i.e., iCMs (Fig. 1). In this scenario, CSCs signify convenient targets already *in situ* and integrated with the tissue to be repaired and regenerated. Importantly, cardiac fibroblasts have been shown to be much more prone to induced reprogramming towards mature iCMs compared to heterologous cell sources (Ieda *et al.*, 2010), thus making CSCs essential protagonists of this approach. Ideally, a strategy targeting specifically pro-fibrotic stromal cells, directly reprogramming them towards iCMs, would fulfill both aims of potentiating a constructive healing response while refilling the lost cardiomyocyte pool.

Conclusions

In conclusion, the functional definition of CSCs has been profoundly changed through the years, with exciting novel approaches for exploiting them in cardiac repair strategies. Future developments in targeting or potentiating their tasks may further change their role as specialized actors in cardiac physiopathology.

Acknowledgement: Funders are thanked for supporting the work of this writing contributors.

Availability of Data and Materials: No original data is included in this Viewpoint.

Author Contribution: All authors contributed to conception and drafting of the manuscript, and approved its final version.

Ethics Approval: No ethical approvals are required for this manuscript.

Funding Statement: IC is supported by Grant # RG11916B85CDBF76 from Sapienza University. VP is supported by Grant # AR120172B8B543B3 from Sapienza University. FP is supported by Grant # A0375-2020-36621 from Regione Lazio (POR-FESR 2014-2021).

Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

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