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Epicardial epithelial-to-mesenchymal transition in injured heart

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Abstract

Cre-LoxP-mediated genetic lineage trace has been used to illuminate the cell fate of progenitor cells *in vivo*. Application of this strategy to the epicardium, a sheet of cells covering the surface of heart, revealed that it dynamically participates in both heart development and postnatal heart repair and regeneration. After myocardial infarction, epicardial cells undergo epithelial-to-mesenchymal transition (EMT) and mainly adopt myofibroblast, fibroblast and smooth muscle cell fates. Here we present the wholemount images that map epicardial EMT following myocardial infarction, taking advantage of an inducible epicardial Cre line and a double fluorescence reporter. While remote epicardium retained its epithelial cell shape, reactivated epicardium in the infarcted region showed significant EMT. This image supports active involvement of the epicardium in repair and regeneration of infarcted myocardium.

Keywords: epicardium • myocardial infarction • EMT • regeneration

Epicardium is the outmost cell layer covering the heart. While the importance of this cell layer during heart development has been studied in detail, its significance in the pathobiology of the adult heart is just beginning to be studied. Two major roles of epicardium have been reported: contribution of intramyocardial cells and production of paracrine factors [1, 2]. During development, epicardial cells proliferate and undergo epithelial-to-mesenchymal transition (EMT). The resulting mesenchymal cells migrate into the myocardium, forming fibroblast, smooth muscle and endothelial cells. A small fraction of cardiomyocytes have also been reported to arise from epicardium [3, 4], although this conclusion remains controversial [5]. The epicardium thus produces multiple cell lineages integral to the developing myocardium. In addition, epicardium is a signalling hub, as multiple bidirectional signals exchanged between epicardium and myocardium have been shown to be pivotal for normal development of the heart and the coronary vasculature [6–9].

In the normal adult heart, the epicardium does not actively undergo EMT. However, adult heart injury in the form of myocardial infarction (MI) partially reactivates foetal epicardial properties [10]. After MI, a subset of foetal epicardial genes are reactivated, and epicardial cells proliferate and undergo a partial EMT to form a thickened layer of mesenchymal cells that are active in myocardial protection [11]. These mesenchymal cells express markers of myofibroblast, fibroblast and smooth muscle lineages. Interestingly, recent work showed that priming with thymosin β 4 prior to MI expanded the fate of epicardial derivatives to include cardiomyocytes [12].

To directly visualize EMT during repair and regeneration of infarcted myocardium, we used labelled epicardium using the Cre-LoxP genetic lineage tracing strategy. We used a tamoxifen-induced Cre allele, $Wt1^{CreERT2/+}$, with epicardium-restricted cardiac activity, and the $Rosa26^{mTmG/+}$ reporter line [11], which switches from mRFP to mGFP expression (m denotes RFP and GFP variants that localize to the cell membrane) following Cre catalysed recombination. Treatment of $Wt1^{CreERT2/+}$;$Rosa26^{mTmG/+}$ selectively labelled epicardial cells with mGFP. These mice then underwent experimental MI by left descending artery ligation, and hearts were

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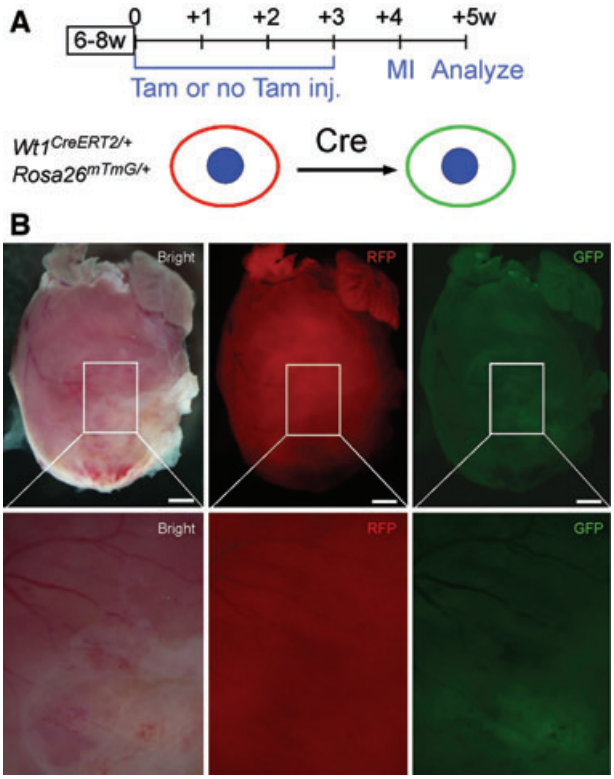

Fig. 1 (**A**) Schematic figure showing the switch of membrane RFP into GFP in cells after the induction of Cre and MI. (**B**) Wholemount view of $Wt1^{CreERT2/+}$;$Rosa^{26mTmG/+}$ heart seven days after myocardial infarction. As control, the mouse was not pre-treated with tamoxifen administration. The bottom panels of figures are magnification of the above wholemount figures. White bar = 1 mm.

examined seven days later using a fluorescent stereomicroscope (Fig. 1A). Importantly, MI stress did not significantly activate *Wt1*^{CreERT2} in the absence of tamoxifen, as demonstrated by the paucity of GFP fluorescence in control experiments in which MI hearts were not pre-labelled by tamoxifen treatment (Fig. 1B). In contrast, tamoxifen pre-treated MI hearts exhibited robust green fluorescence (Fig. 2). This image uses endogenous mGFP fluorescence to map the epicardial response to MI across the whole heart (Fig. 2A–D). A large area of green fluorescence was visible under the ligation site, corresponding to areas of epicardial thickening. Green fluorescence over myocardium remote from the infarct was more heterogeneous and generally less intense, indicating reduced and variable epicardial thickening in these regions. Magnification of remote and infarct myocardium clearly demonstrated distinct morphology of cells in these regions (Fig. 2E and F). $mGFP^{+}$ epicardial cells of the remote myocardium exhibited epithelial morphology (white arrowheads; Fig. 2E). In contrast, mGFP+ epicardium-derived cells of the infarct myocardium were spindle shaped (Fig. 2F), consistent with their expression of markers of myofibrolasts, fibroblasts, and smooth

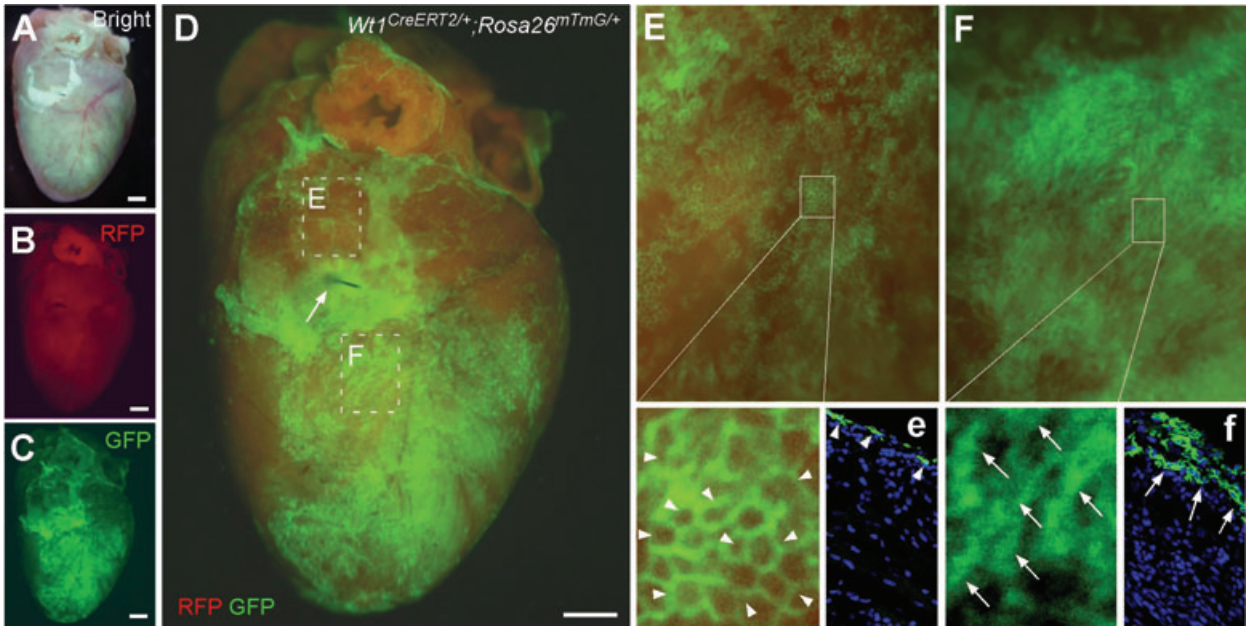

Fig. 2 Image of whole heart with reactivated epicardium after MI. (**A–D**) Wholemount image of $Wt1^{CreERT2/+}$;$Rosa^{26mTmG/+}$ heart 1 week post-MI. (**A**) bright field illumination. (**B, C**) RFP and GFP fluorescence. (**D**) Merge of green and red channels. Arrow indicates LAD ligature. Boxed areas are magnified in (**E**) and (**F**). (**E**) Magnification of remote myocardium above the LAD ligature. Arrowheads indicate epicardial cells with epithelial morphology. (**F**) Magnification of infarcted myocardium below the LAD ligature. Arrows indicate epicardium-derived cells with mesenchymal cell shape. (**E**, e) and (**F**, f), sections of heart above or below the LAD ligature. Bar = 1 mm.

muscle cells, and possibly recently discovered telocytes [11, 13–15]. The presented image illustrates the epicardial EMT process in the infarcted adult myocardium, providing insights into the role of epicardium in repair and regeneration of infarcted myocardium.

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Conflict of interest

The authors confirm that there are no conflicts of interest.

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