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A Bouquet for a Broken Heart: Can flowers repair a damaged heart?

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Keywords

Angiogenesis; Coronary Vessels; Endocardium; Endocardial Flowers; Endothelial Cell; Myocardial Infarction

Ischemic heart disease (IHD) is the major underlying cause of myocardial infarction, scarring, and hypertrophy that can progress to heart failure. Each year, almost 300,000 individuals have recurring myocardial infarctions, which is projected to about 40% of the US population in the next 15 years. More recently, angiogenic therapy for myocardial ischemia has received significant attention. These therapeutic angiogenic strategies include gene therapy, delivery of growth factor proteins, and stem cell implantation^{1–6}. While these approaches are attractive, there are limitations and concerns, including delivery modalities, uncontrolled angiogenesis, limited half-life of growth factors, and off-target effects on other organs^{7–9}. Moreover, limited success of therapeutic angiogenesis in myocardial revascularization left us with more questions than answers^{9, 10}. Is the damaged heart capable of inducing new vessel growth? If so, what is the source of coronary vessel growth? What are the molecular mechanisms involved? Fortunately, recent studies on coronary vascular development have begun to answer these questions.

Emerging evidence on the generation of coronary vasculature during development identified more than one source for the origin of coronary vascular endothelial cells that include epicardium, sinus venous, and endocardium¹¹. By employing conditional inducible Apln-Cre-ERT2-knockin mice, Tian *et al* showed that subepicardial endothelial cells are the major source of intramyocardial coronary arteries¹². Further, using single cell labelling and clonal analysis, Red-Horse *et al* demonstrated that coronary vessels developed from sinus venous endothelial cells via sprouting angiogenesis and de-differentiation¹³. In contrast, Wu *et al* found that endocardial endothelial cells significantly contribute to intramyocardial vascular endothelial cells using a Cre knockin mouse strain, Nfatc1Cre, which showed specific expression of Nfatc1 in the endocardium but not endothelial cells in the myocardium¹⁴. Perhaps most studies on the source of coronary vessels have focused on developmental

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Disclosures

None.

stages. Intriguingly, recent studies by Zhou *et al* revealed that the endocardium *de novo* forms coronary arteries in post-natal hearts, suggesting an important role for the endocardium in the origin of coronary vasculature in the post-embryonic stages¹⁴. However, the importance of the endocardium in the neovascularization of the myocardium in the injured/infarcted adult heart is not well known.

Endocardial Flowers: A bouquet of arterial endothelial cells generated from new and pre-existing endothelial cells within the endocardium of infarcted heart

In this issue of *Circulation Research*, Miquerol *et al* investigated the role of the endocardium in the generation of new vessels in the damaged myocardium of mouse hearts¹⁵. They found novel, previously undescribed, endothelial foci within the infarct zone of endocardium 7 days post-MI in Connexin-40-GFP mice. These mice express GFP only in endothelial cells of coronary arteries, but not in the endocardium, veins or capillaries¹⁶. These endothelial foci, termed by authors as endocardial flowers due to the appearance, exhibited a distinct arterial phenotype displaying positive expression of Cx40 and VEGFR2 and negative expression for endoglin (Cx40⁺ and VEGFR2⁺, Endoglin⁻) with accumulation of smooth muscle cells. These findings were in contrast to the surrounding endocardium, which was negative for Cx40 and VEGFR2 and positive for endoglin (Cx40⁻ and VEGFR2⁻, Endoglin⁺). Furthermore, time course analysis of arterial marker expression, revealed that VEGFR2 expression continued into the endocardium 3 days post-MI, followed by the formation of endocardial flowers, which progressively acquired the arterial phenotype with increased expression of Cx40-GFP. To unequivocally confirm the genetic tracing of the endothelial lineage of endocardial flowers (i.e. generated from new Cx40 expression or from pre-existing vessels), the authors used a tamoxifen-inducible Cx40-Cre-RFP mouse line¹⁷ crossed with R26-LacZ or R26-YFP. Induction of MI in tamoxifen injected mice generated Cx40-RFP endocardial flowers that were negative for either LacZ or YFP, suggesting that these structures resulted from arteriogenesis of Cx40 negative, endocardial cells. However, a subset of Cx40-RFP positive endocardial flowers showed YFP positive endothelial cells, indicating arteriogenesis may have occurred from pre-existing vessels. These findings demonstrated unprecedented endothelial plasticity between the endocardial and coronary arterial compartment in the infarct zone. Finally, endocardial flower formation appeared to be mediated via sprouting angiogenesis from the endocardium, which contained VEGFR2 expressing tip cells, surrounded by proliferating smooth muscle and endocardial cells.

Back in the saddle: Re-deployment of developmental mechanisms for coronary vessel growth

Although this study elegantly demonstrated the generation of new coronary vessels from a combination of endothelial cells from pre-existing arteries and endocardium *de novo*, two important questions remain to be answered. First, the molecular mechanism by which sprouting angiogenesis and arteriogenesis occurs from the endocardium is not known. Second, the functional significance of endocardial flowers has not been investigated. The

answer to the first question can be gleaned from the findings of Wu *et al*, which may provide mechanistic insights into sprouting angiogenesis and arteriogenesis in the heart. In their study using Nfatc1-Cre mice, Wu *et al* demonstrated that in between embryonic stage 11.5 and 13.5, cells from the endocardium significantly contributed to the intramyocardial arteries. Importantly, coronary vessel formation was abolished when they knocked down VEGF-A expression in the myocardium or VEGFR2 expression in the endocardium, suggesting that VEGF signaling plays a critical role in the induction of angiogenesis and coronary artery formation¹⁴.

What are the signals that trigger expression of VEGF-A in the myocardium or VEGFR2 in the endocardium? Based on the oxygen environment in the developing embryo, Wu *et al* proposed that myocardial proliferation may trigger a VEGF-A gradient across the ventricular wall (possibly regulated by a reverse gradient of oxygen) that promotes vessel growth from VEGFR2 expressing endocardial cells. Interestingly, Zhao *et al* demonstrated that VEGF-A expression increases within an hour after MI and continued to increase for 24 hours into the border zone and endocardium, which supports a role for VEGF-A in triggering sprouting angiogenesis in the endocardium¹⁸. Further, Zhao's study also demonstrated the appearance of new vessels at day 3, with a peak vascular density at day 7, correlating with the formation of endocardial flowers¹⁸. Although oxygen levels were not measured, it is conceivable that an oxygen gradient would be present, with the highest level in the endocardium to the lowest level in the infarct zone. Based on these findings, a proposed molecular mechanism (Fig.1) could involve the generation of a VEGF-A gradient in response to the hypoxic environment at the infarct/border zone of the myocardium, which may initiate sprouting angiogenesis by VEGFR2 expressing endocardial cells. These cells then form endocardial flowers by progressively acquiring the arterial phenotype through the proliferation of endothelial cells and recruitment of smooth muscle cells (SMC). This mechanism is reminiscent of the one proposed by Wu *et al* in coronary vascular development in the embryo and strongly supports the idea that the myocardium may re-deploy embryonic mechanisms during pathological conditions in the adult to induce new vascular growth¹⁴. Although hypoxia can be an underlying trigger for VEGF-A expression, the mechanisms by which VEGFR2 expression is modulated is not yet known. One of the critical regulators of VEGF-A and VEGFR2 expression/activation in endothelial cells are mechanical forces, generated by shear stress and mechanical strain^{19–22}. Since the heart tissue is continuously exposed to mechanical forces, it is plausible that mechanosignaling can play a significant role in angiogenesis, as previously demonstrated^{23, 24}.

Clinical Significance and Future Perspective

The answer to the second question is more pertinent and complicated. What is the functional significance of endocardial flowers? Do they supply blood to restrict the damage caused by an infarction and preserve cardiac function? Time course analysis revealed that endocardial flowers appeared at day 7 post-MI and peaked at day 14, implying that they may participate in the initial prevention of damage to the infarcted myocardium as well as in the preservation of cardiac function. However, the current study is somewhat limited as cardiac function measurements were not performed, which may reveal if endocardial flowers are indeed beneficial. One of the reasons for the limited success of therapeutic angiogenesis could be

our limited understanding of the mechanism behind coronary revascularization. The findings from this study identify the endocardium as a source for *de novo* endothelial cells in the adult heart, as well as pre-existing vessels from coronary arteries. The molecular mechanism underlying this process appears to be the VEGF-A/VEGFR2 signaling pathway that induces angiogenesis and arteriogenesis from the endocardium (Fig.1), as demonstrated in embryonic cardiovascular growth. The process of re-deployment of ontogenic mechanisms in pathological conditions appear to be common step^{25–27}, and understanding this molecular machinery in the adult heart could provide new avenues for future therapeutics in revascularization of the injured myocardium. In conclusion, the big question is: can flowers repair a broken heart? They may or may not, but they definitely bring a smile to one's face. Likewise, endocardial flowers may not repair or revascularize the damaged myocardium but they provide a potential avenue for therapies aimed at mending broken hearts.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Schematic of the proposed mechanism of endocardial flower formation and endothelial plasticity in the post-MI endocardium (adapted from¹⁴). Ligation of the LAD (left anterior descending artery) induces patchy expression of VEGFR2 in endocardial cells (beige) 3 days post-MI. At the same time, hypoxia in the infarction/border zone may induce VEGF-A expression creating a VEGF-A gradient (red arrow) in the direction opposite to oxygen gradient (black arrow). The VEGF-A gradient, together with mechanical forces, may induce sprouting angiogenesis in these VEGFR2 expressing endocardial cells (light green) with the appearance of tip cells (cell with projections). These cells then recruit stalk cells (dark green) to form endocardial flowers, with distinct connecting vessels 7 days post-MI. Endocardial flowers exhibit Cx40-GFP expression and clustering of smooth muscle cells (SMC) at this stage and progressively acquire the arterial phenotype. Genetic tracing experiments with Cx40-Cre; RYFP mice revealed the presence of YFP expressing endothelial cells (pre-existing) in a subset of RFP positive endocardial flowers (new), suggesting that arteriogenesis also occurs by outgrowth of pre-existing coronary arteries.