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KICKING THE EPICARDIUM UP A NOTCH

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The epicardium is a layer of fibrous mesothelium that covers the external surface of the heart. Until recently, the main function of this tissue was thought to be protective and to contribute to production of pericardial fluid. Recently, however, renewed interest in the function of the epicardium has identified important contributions to cardiac development, disease and regeneration.

In the developing embryo, the epicardium derives from the proepicardial organ, a cluster of multipotent progenitor cells located dorsal to the looped heart tube during early stages of embryogenesis[1, 2]. Some proepicardial cells undergo an epithelial-to-mesenchymal transition (EMT) to generate migratory cells that encase the heart, invade the myocardium and ultimately give rise to fibroblasts, coronary smooth muscle, and possibly endothelial cells and cardiomyocytes[3–9]. The embryonic epicardium provides factors necessary for the normal development and expansion of the myocardium; disruption of critical epicardial signaling pathways leads to thin and poorly functioning myocardium[10–12]. These discoveries have raised the question of whether the epicardium might play a role in adult cardiac homeostasis or response to injury by providing cells or growth factors that impact cardiac function.

Several recent studies, including one by Russell et al. in *Circulation Research*[13], have begun to establish a novel paradigm for the adult epicardium as a tissue able to undergo dynamic activation in response to stress reminiscent of embryonic epicardial EMT. Prior studies in zebrafish have demonstrated generalized activation of the epicardium following amputation of the apex of the heart, which subsequently provides a conducive environment for cardiac regeneration[14, 15]. Although mammalian hearts do not regenerate as well as those of zebrafish, studies in mice have also demonstrated generalized activation of the epicardium of adult animals after injury and in response to specific factors induced by injury. For example, Bock-Marquette et al. showed reactivation of fetal genes and up-regulation of

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nuclear beta-catenin with evidence for EMT after injury or after administration of the growth factor thymosin beta 4 [16]. Interestingly, thymosin beta 4 is necessary for coronary artery development and may function to enhance neovascularization, perhaps by activating epicardium, after myocardial infarction[17].

Russell et al. identify a novel population of epicardial-associated cells in the adult mouse heart that are activated and expanded by injury[13]. This heterogenous population of cells is defined by evidence of Notch activation (and are therefore named “Notch-activated epicardial-derived cells” or NECs). These cells resemble multipotent stromal cells (mesenchymal stem cells) by gene expression profiling, yet express some cardiac markers in addition to those of the fibroblast lineage. They are c-kit negative, do not efflux Hoechst dye, and only a sub-population express sca1, suggesting that they are distinct from previously studied cardiac progenitor populations.

NECs become more abundant after myocardial infarction (MI) or thoracic aortic banding, though the kinetics of activation and the pattern of cellular localization within the heart are distinct. Injury provokes up-regulation of genes promoting fibroblast differentiation and down-regulation of muscle-specific genes when compared with NECs from uninjured hearts, consistent with the propensity of the mammalian heart to form a fibrotic scar in response to myocardial damage. Scar formation is both beneficial and detrimental—in the short-term it stabilizes the damaged myocardial wall and prevents rupture. However, in the long-term, scar formation may inhibit endogenous myocardial regeneration, fibrosis may impede diastolic and systolic function, and this process may augment the development of heart failure and lethal ventricular arrhythmias. To what extent the regenerative capacity of subepicardial progenitors is conserved from zebrafish to mammals remains to be seen. However, broad activation of the epicardium and conditioning of the subepicardial environment in response to injury is at least partially conserved. It is intriguing to speculate that blocking epicardial EMT may affect the fibrotic response to pressure overload, and perhaps a better understanding of the role of epicardial-derived cells in the repair process will provide new therapeutic targets for the modulation of post-infarct remodeling and regeneration.

The signaling cascades that result in generalized activation of the entire epicardium, even at sites remote from injury, remain unclear. However, if the same phenomenon occurs in humans, this phenomenon might help to explain a troublesome complication of myocardial infarction and cardiac surgery. Dressler’s syndrome, which presents days to weeks after a myocardial infarction or surgical intervention, is associated with inflammation of the pleura and pericardium, chest pain and fever. This phenomenon has been attributed to an immune-mediated response to myocardial injury, but perhaps it reflects an evolutionarily conserved mesothelial activation response contributing to repair.

Whether or not epicardially-derived cells can give rise to newly regenerated cardiomyocytes is controversial[3, 8, 9]. To determine whether NECs have cardiac differentiation potential, Russell et al. transplanted these cells in vivo into uninjured myocardium. After one week they observed colonies derived from these cells that expressed the cardiomyocyte marker α-actinin, although the cells did not adopt histological characteristics of mature

myocytes. Whether NECs have the potential to become fully functional cardiomyoctes that integrate with host myocardium remains to be determined. If they are able to express some cardiomyocyte markers, perhaps further differentiation can be enhanced with small molecules or other interventions. NECs might also be important modulators of a regenerative or healing response via the secretion of cardiotrophic paracrine factors[18]. Indeed, Russell et al. show that neonatal rat cardiomyocytes co-cultured for several months with NECs are able to maintain a fully differentiated cardiomyocyte phenotype and continue vigorous beating, whereas neonatal cardiomyocytes that are not co-cultured with NECs maintain less vigorous function. It will be interesting in future studies to elucidate the paracrine factor(s) that mediate this effect.

Activation of the Notch signaling pathway was used to identify these novel cells with inherent fibrosis-repair potential. Notch is a cell-to-cell signaling pathway implicated in the regulation of key cellular functions including multipotency, cell fate decisions, proliferation, and apoptosis within virtually every organ system, including the heart[19]. It is notable that Notch activity is evident only within a subset of epicardial cells at baseline. It will be important to identify the endogenous Notch ligand responsible for this selectivity, and to determine on which cell type the ligand is expressed. It remains unclear if Notch activation is required in NECs for proper response to stress, or whether Notch activation is simply a convenient marker. It is intriguing to speculate that Notch may enable a subset of epicardially-derived cells to maintain an undifferentiated, mutipotent state poised for a response to injury. Perhaps migration away from the epicardium and the activating ligand may result in a fate restriction (for instance, to the fibroblast lineage in mammals). Along these lines, it would be interesting to determine whether activation, expansion and differentiation of NECs in response to injury coincides with dynamic changes in Notch activity within these cells. Understanding the role of Notch activation in these cells may also be important for predicting and assessing cardiac side effects of drugs that can modulate Notch signaling, such as gamma secretase inhibitors, which are being evaluated for use in cancer, neurodegeneration, and other disorders.

Although mammals have less cardiac regenerative capacity than zebrafish, it is now becoming clear that at least some components of the injury response are conserved. Activation of the epicardium and contribution of epicardial-derived cells to the repair process is a shared phenomenon. Whether epicardial-derived cells also function to promote the survival, growth and differentiation of resident cardiomyocyte progenitors after injury in mammals remains to be seen. In any case, a better understanding of NECs and other epicardial-derived cell populations that are activated in response to injury may lead to the discovery of novel therapies to improve outcomes after myocardial infarction.

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