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Regeneration in heart disease – Is ECM the key?

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Abstract

The heart possesses regeneration potential derived from endogenous and exogenous stem and progenitor cell populations, though baseline regeneration appears to be sub-therapeutic. This limitation was initially attributed to a lack of cells with cardiomyogenic potential following an insult to the myocardium. Rather, recent studies demonstrate increased numbers of cardiomyocyte progenitor cells in diseased hearts. Given that the limiting factor does not appear to be cell quantity but rather repletion of functional cardiomyocytes, it is crucial to understand potential mechanisms inhibiting progenitor cell differentiation. One of the extensively studied areas in heart disease is extracellular matrix (ECM) remodeling, with both the composition and mechanical properties of the ECM undergoing changes in diseased hearts. This review explores the influence of ECM properties on cardiomyogenesis and adult cardiac progenitor cells.

Keywords

Regeneration; Cardiac progenitor cell; Extracellular matrix; Heart disease

Regeneration in Heart Disease

Evidence from rodent and human studies challenges the view of the heart as a terminally differentiated organ (Bergmann et al. 2009; Hsieh et al. 2007). The heart possesses regenerative capacity attributed to endogenous and exogenous progenitor cell populations (Liao et al. 2007) recently identified in adult myocardium. Intrinsically, this regenerative capacity is insufficient to prevent the progression toward heart failure following various insults. To address this limitation, a common strategy has been to deliver stem/progenitor cells to the diseased heart through local implantation or systemic migration (Segers and Lee 2008). The utility of this approach is called into question by human (Kubo et al. 2008) and murine (Mouquet et al. 2005) studies showing that progenitor cell quantities are in fact

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normal or increased in diseased versus healthy hearts. Further, these progenitor cells retain the capacity to differentiate when isolated from the surrounding diseased tissue. Thus, the heart's limited regeneration may be attributed to processes that inhibit progenitor cell differentiation in situ.

ECM Changes in Heart Disease

Cardiomyocytes account for only one-third of the cell quantity in the adult mammalian heart (Zak 1973). Fibroblasts are the largest population of non-myocytes, producing the intricate network of extracellular matrix (ECM) proteins providing orientation and anchorage to surrounding cardiomyocytes (Weber 1989). The physiologic and pathologic importance of the ECM network is well studied. The primary components of the ventricular ECM network are Collagen I and III, among other structural proteins. Collagen I predominates in healthy myocardium, whereas Collagen III is produced when the heart is injured. The changes in ECM properties during progression toward heart failure have been extensively reviewed elsewhere (Jourdan-Lesaux et al. 2010). Briefly, all ECM components are deposited in larger amounts leading to extensive myocardial fibrosis (Weber 1989). Studies using volume or pressure overload models of heart failure suggest that gene expression of ECM components is largely reversed following treatment with beta-blockers, ACE inhibitors or Angiotensin II receptor blockers (Champetier et al. 2009; Grimm et al. 2001; Grimm et al. 1998; Masutomo et al. 2001). The orientation of ECM proteins also changes following an insult. In healthy myocardium, ECM fibers are arranged longitudinally alongside cardiomyocytes with few crosslinks. In diseased myocardium, the crosslinks become more pronounced (Pick et al. 1989) thereby changing the mechanical properties of the ECM matrix (Fomovsky et al. 2010). The myocardial elastic modulus increases from a baseline of 16.6 kPa to 31.8 kPa, 53.3 kPa, and 90.22 kPa at one, two, and three weeks post-infarction, respectively (Zhang et al. 2011). This phenomenon gave rise to the hypothesis that changes in stiffness and ECM composition influence stem/progenitor cell function in heart disease (Zhang et al. 2009). Reciprocally, recruitment of mesenchymal progenitor cells to the heart may further contribute to changes in ECM composition (Sopel et al. 2012). Considering the vast changes in myocardial ECM composition and mechanics during disease progression, it is worthwhile to examine the effects on cardiac development and on the behavior of cardiomyocyte progenitor cells.

Cells Feel Their Surroundings

Cells display methods to sense their surroundings and can react to perceived changes. This was first discovered through the observation that cell shape controls proliferation in cell cultures (Folkman and Moscona 1978). Afterwards, ECM components were recognized to either interact directly with cells or to modulate growth factor stimulation (Hynes 2009). Mechanical properties, such as stiffness, constitute a third factor by which the ECM controls cell function (Guilak et al. 2009; Tulloch et al. 2011).

A cell adheres to its substrate through complexes of proteins known as focal adhesions. The adhesions couple the substrate to the cell's actin cytoskeleton, which is capable of generating and monitoring tension. The pathways responsible for relaying mechanical information have

been explored and are extensively reviewed elsewhere (Borg and Baudino 2011; Buxboim et al. 2010; Discher et al. 2009; Kuraitis et al. 2012; Vogel and Sheetz, 2006). Here, we will discuss key players in brief.

The RhoA/ROCK signaling pathway, which was originally described in the control of cytokinesis and other cell structure changes (Loirand et al. 2006), is commonly implicated in the stiffness response. Rho GTPase is activated by cell distortion, among other soluble and ECM-based signals (Huang and Ingber 1999). Rho acts through p27, an inhibitor of the cyclin-dependent kinases (CDKs), to regulate cell cycle progression and therefore proliferation (Huang and Ingber 1999). The RhoA/ROCK pathway is involved in adaptation of neonatal cardiomyocytes to changes in stiffness (Jacot et al. 2008): In untreated cells, force production is greatest within a physiologic range of stiffness (8-10 kPa). In cells treated with chemical inhibitors of either RhoA or ROCK, the force generation continues to increase above the 8-10 kPa range—suggesting a loss of cell adaptation to the stiffness of the environment.

Other mediators of stiffness-sensing are non-muscle myosins. These were shown to be necessary for mediating the effect on lineage specification to nervous tissue, muscle, or bone (Engler et al. 2006). Specifically, Non-muscle myosin (NMM) II is upregulated in cells cultured on stiff substrates and downregulated on soft substrates. Chemical inhibition of NMM II prevents the influence of stiffness on differentiation.

Other cellular mechanosensors include structural motifs containing cryptic peptides exposed by tension and ion channels sensitive to force and receptor-ligand bonds that become stronger when strained (Vogel and Sheetz 2006). Notably, specific tyrosine kinases acting as mediators of stiffness sensing are unique to the ECM protein composition. FAK senses stiffness on surfaces coated with collagen for instance, while Src kinase does so on surfaces coated with fibronectin.

ECM Effects on Cardiac Development

Studying cardiac development can guide the understanding of influences of ECM composition and mechanical properties on stem/progenitor cells. During cardiac development, tissue stiffness changes (Young and Engler 2011). Further, cell shape and arrangement vary --particularly during looping of the embryonic heart tube (Manasek et al. 1972) -- coinciding with changes in ECM composition (Bowers and Baudino 2010; Chan et al. 2010). When cells from embryonic cardiac fields are cultured on hydrogels mimicking the specific stiffness coinciding with changes in cardiac development, the cells show greater progression toward organized cytoskeletons as well as a higher expression of cardiac-specific markers (Young and Engler 2011). It is yet unclear whether these factors are cause or consequence of ongoing cardiac development. To examine whether extracellular matrix stiffness plays a role in development, embryonic stem cells (ESC) and fetal/neonatal cardiomyocytes have been studied in culture on substrates of varying stiffness. ESCs show preferred differentiation toward cardiomyocyte precursors in relation to mechanical strain, with higher strain resulting in more beating foci and expression of cardiomyocyte markers (Schmelter et al. 2006).

Cell spreading in culture is an indicator of cytoskeletal organization, and this is shown to vary with different ECM components. The spreading area of neonatal cardiomyocytes cultured on ECM-coated dishes was found to be dependent on the ECM components specific to the dishes (Hilenski et al. 1991). Fibronectin was associated with the largest spreading area, and ECM coating of any kind was associated with two-fold higher spreading versus uncoated dishes (Hilenski et al. 1992). In addition, cells react to patterns in ECM components. If ECM substrates are layered in an organized fashion, neonatal cardiomyocytes organize themselves in the direction of the ECM fiber structure and form rod-shaped cells similar to adult cardiomyocytes (Simpson et al. 1994). Besides the organized myofibrils, ECM patterns can also influence the nuclear alignment of neonatal cardiomyocytes *in vitro* and result in anisotropic nuclei direction similar to that of cardiomyocytes *in vivo* (Bray et al. 2010). Maturation of neonatal cardiomyocytes requires structured assembly of myofibrils, as proper assembly of the cytoskeleton is required for cells to perform mechanical work. This assembly is influenced by ECM composition (Hilenski et al. 1991), myocyte (Bray et al. 2008; McCain and Parker 2011) and matrix stiffness (Engler et al. 2004). As the cells become functional, both beating frequency and synchrony of cardiomyocytes is stiffness-dependent (Engler et al. 2008; Shapira-Schweitzer and Seliktar 2007). When neonatal cardiomyocytes are cultured on relatively stiff substrates, they stop beating after several days in culture (Engler et al. 2008) and contract asynchronously (Shapira-Schweitzer and Seliktar 2007). When cultured on substrates with a stiffness approximating healthy myocardium, the cells retain their beating frequency and synchrony (Engler et al. 2008; Shapira-Schweitzer and Seliktar 2007).

The main mediator of ECM/cell interactions in development appears to be β 1-Integrin, and it is involved in cell spreading across ECM matrices (Hilenski et al. 1991). Further β 1-integrin is shown to regulate cell proliferation (Hornberger et al. 2000), cardiomyocyte differentiation (Fassler et al. 1996) and hypertrophic response (Pham et al. 2000; Ross et al. 1998).

The importance of ECM/cell interactions in cardiac development is demonstrated by work done in β 1 integrin knockout mice. Murine ESCs lacking β 1 integrin show different patterns of cardiomyogenic differentiation. Sarcomere alignment was impaired in cardiomyocytes lacking β 1 integrin. The mice were viable but showed significant defects in cardiomyocyte maturation (Fassler et al. 1996). Further evidence reveals the preferential differentiation of ESCs along the cardiovascular lineage when the cells are cultured on scaffolds resembling cardiac ECM composition (Schenke-Layland et al. 2011). Recently developed protein and ECM microarrays for cell-based experiments are facilitating high-throughput studies of the influence of cell microenvironment on cellular function (Flaim et al. 2005). In one report, ECM and cell signaling effects on cardiomyogenic differentiation were studied using ESC cells transfected with an α -MHC reporter. Collagen IV appeared to increase α -MHC expression, whereas Collagen I and III -- both overexpressed in diseased hearts -- appeared to decrease the expression (Flaim et al. 2008). In another report, culturing ESCs on Collagen I substrates appeared to promoted differentiation into endothelial cells (Li et al. 2009).

ECM Effects on Progenitor Cells

Stem and progenitor cells in various tissues reside in specific niches (Moore and Lemischka 2006). These niches are defined by cell-cell as well as cell-ECM interactions and are known to determine stem cell function and fate (Votteler et al. 2010). Similarly, progenitor cells in the heart reside in defined niches (Bearzi et al. 2007; Urbanek et al. 2006). Ckit⁺ cardiac progenitor cells were found to express β 1 integrin and are in close contact with laminin and surrounding cardiomyocytes (Urbanek et al. 2006). Flk-1⁺ cardiac progenitor cells proliferated more on 3D scaffolds coated with laminin or vitronectin than those on collagen IV-coated scaffolds (Heydarkhan-Hagvall et al. 2012). Cardiosphere-derived cells – another cardiac progenitor cell population – show similar cell-cell and cell-ECM interactions (Li et al. 2010). In MSCs cultured on ECM substrates coated with different collagen subtypes, Collagen V promoted cardiomyogenic differentiation whereas Collagen I and III demonstrated no effect (Tan et al. 2010).

As stated earlier, ECM stiffness is another changing variable in development and disease. The influence of matrix stiffness on differentiation was examined using MSCs cultured on substrates of varying stiffness. Very soft substrates favored neurogenic commitment, stiff substrates favored osteogenic commitment, and substrates with a stiffness closest to muscle promoted myogenic differentiation (Engler et al. 2006; Wang et al. 2010). The stiffening of post-infarction myocardium appears to favor differentiation of bone marrow mononuclear cells toward endothelial lineages in vitro (Zhang et al. 2011). Analogous behavior can be observed in vivo: MSCs injected into the rigid microenvironment of the scar in infarcted murine hearts show osteogenic differentiation (Breitbach et al. 2007). Incidentally, MSC migration is also influenced by stiffness. If MSCs are cultured on substrates displaying stiffness gradients, they tend to migrate toward the region of higher stiffness. Furthermore, the cells' myogenic differentiation is related to their position on the gradient (Tse and Engler 2011). Finally, stiffness is not the only material property involved in directing stem cell fate. When isolated cardiac mononuclear cells were cultured on three-dimensional substrates having different pore sizes, cardiomyogenic differentiation potential was influenced (Forte et al. 2008).

It is worth noting that progenitor cell ECM interactions are not unidirectional. Cardiac progenitor cells are themselves able to synthesize ECM components and recent evidence suggests an increase in ECM component production by such cells during differentiation (Bax et al. 2012). Additionally, studies on Angiotensin II-induced hypertrophy suggest that progenitor cells in fact contribute to cardiac fibrosis (Sopel et al. 2012; Sopel et al. 2011). Thus, progenitor cells influence -- either directly or through paracrine effects -- the changes in ECM properties observed in diseased hearts.

Conclusions

ECM composition and mechanical properties play a significant role in cardiac development, and these factors are significantly altered during the pathogenesis of heart disease (Figure 1). Little is known regarding the mechanical influence of ECM on progenitor cell fate, and the resultant effects of altered ECM composition on adult cardiac progenitor cells and cardiac

regeneration potential remain to be determined, particularly in order to realize the potential of therapeutic regeneration. One key set of experiments to elucidate these issues would be to study the effects of varying ECM composition, material properties, and geometry on cardiac progenitor cell behavior in a combinatorial fashion. This would expand our current understanding of the complex relationship between ECM changes and cell behavior in the myocardium and elsewhere (Table 1). To this end, for instance, multiple techniques have been developed to culture cells on substrates of varying stiffness and to study the effects on proliferation and differentiation. Future investigation into the interplay between cardiac cells and healthy versus diseased cellular microenvironments may deepen our understanding of heart biology and facilitate the effort to optimize regeneration potential in diseased hearts.

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Figure 1.

Recent publications have established the existence of resident cardiac stem/progenitor cells in adult myocardium. Myocardial injury not only lead to cardiomyocyte cell death but also triggers a series of event with tissue remodeling including the hypertrophy of surviving cardiomyocytes, fibrotic replacement of last tissue, activation of resident stem cells to proliferate and differentiate, and changes to the ECM milieu. Together, the new established disease microenvironment may further affect resident progenitor cell function and their ability to continue regenerate and heal injured myocardium.

Table 1

Effects of ECM properties on *in vitro* cell behavior

Cell Type		Lin-/Sca-1+	Flk+	C-kit+	MSC	ESC
ECM property						
Optimal ECM protein(s)	Fibronectin					↑Endothelial cell differentiation ¹
	Laminin, Collagen IV				↓Myogenic/osteogenic differentiation ²	
	Laminin		↑Proliferation ³		↑Smooth muscle cell differentiation ⁴	↑Cardiomyocyte differentiation ⁵ ; ↑Pneumocyte differentiation ⁶
	Vitronectin		↑Proliferation ³			
	Collagen IV		↑Cardiovascular & hematopoietic differentiation ⁷			↑Cardiovascular & hematopoietic differentiation ⁷
	Collagen V				↑Cardiomyocyte differentiation ⁸	
	Cardiac ECM			↑ Proliferation, adhesion and survival ⁹		
Surface geometry	Square pores	↑Cardiomyocyte differentiation ¹⁰				
Substrate stiffness	Physiologic stiffness				↑Myogenic differentiation ¹¹	
	Scar tissue stiffness				↑Osteogenic differentiation ¹²	

¹Battista et al. 2005²Rowlands et al. 2008³Heydarkhan-Hagvall et al. 2012⁴Suzuki et al. 2010⁵Flaim et al. 2008⁶Lin et al. 2010⁷Schenke-Layland et al. 2008⁸Tan et al. 2010

- 9 French et al. 2012
- 10 Forte et al. 2008
- 11 Engler et al. 2006
- 12 Breitbart et al. 2007