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Mechanobiology of cardiomyocyte development

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ABSTRACT

Cardiac cells are under constant, self-generated mechanical stress which can affect the differentiation of stem cells into cardiac myocytes, the development of differentiated cells and the maturation of cells in neonatal mammals. In this article, the effects of direct stretch, electrically induced beating and substrate elasticity on the behavior and development of cardiomyocytes are reviewed, with particular emphasis on the effects of substrate stiffness on cardiomyocyte maturation. In order to relate these observations to *in vivo* mechanical conditions, we isolated the left ventricle of Black Swiss mice from embryonic day 13.5 through post-natal day 14 and measured the elastic modulus of the epicardium using atomic force microscope indentation. We found that the elastic modulus of the epicardium significantly changes at birth, from an embryonic value of 12 ± 4 kPa to a neonatal value of 39 ± 7 kPa. This change is in the range shown to significantly affect the development of neonatal cardiomyocytes.

1. Introduction

Cardiac tissue is subjected to dynamic mechanical stresses from very early development, without pause, for a person's entire life. Portions of the heart experience shear stress that can be pulsatile, oscillatory or even turbulent at times. Much of the heart experiences both active stretching during filling and self-generated mechanical force during ejection. Because cardiomyocytes, or the contractile cells that comprise heart muscle, rhythmically contract, the stresses and strains that these cells experience are very sensitive to the mechanical properties of the surrounding cardiac tissue as well as the hydrostatic properties of the blood being pumped.

Cardiomyocytes respond to these mechanical forces, along with other possible signals, by proliferating, hypertrophying and altering the heart geometry to enable the development of a fully functioning four-chambered heart from a small heart tube, all while continuing to pump plasma and blood. Cardiomyocytes in the fully formed heart continue to grow and mature even after birth. In the adult human heart, cells will hypertrophy or remodel in response to changing loads or conditions, sometimes in a compensatory fashion and sometimes pathologically, and the recent discoveries of some adult cardiac stem cell populations show that the heart can often repair small areas even into adulthood.

In this article, we will review the mechanical sensitivity and mechanically induced responses in cardiac cell differentiation, development and maturation. We will also present some original data on the changing mechanical properties of cardiac tissue during development and in ischemic regions of the adult heart.

2. Differentiation and cardiomyocyte development

Differentiation of a cardiac progenitor into a cardiomyocyte can be the first step in the development of a heart. Researchers have been able to generate cardiomyocytes from human embryonic stem cells (Boheler et al., 2002) and induced pluripotent stem cells (Mauritz et al., 2008; Narazaki et al., 2008) using a variety of chemical additives and culture techniques. Similar effects have also been observed in other stem cell sources not typically thought of as cardiomyogenic, including bone marrow-derived mesenchymal stem cells (MSC) (Fukuda, 2003; Xu et al., 2004; Yamada et al., 2007; Antonitsis et al., 2008), bone-marrow stromal cells (Makino et al., 1999), umbilical cord blood-derived stem cells (Pereira et al., 2008) and adult cardiac stem cells (Bearzi et al., 2007; Smith et al., 2007). The true cardiogenic potential of MSC has been a matter of debate and several studies have found that MSC can express cardiac markers but not differentiate into functional cardiac cells (Jiang et al., 2002; Davani et al., 2003; Rose et al., 2008a). Many successful differentiation studies have involved direct co-culture of MSC and mature cardiomyocytes, and some have noted that cardiac cells and stem cells can fuse in ways that make them difficult to distinguish from differentiated

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cells (Nygren et al., 2004; Rodic et al., 2004). Additionally, it has been noted that some successful techniques use MSC that have some degree of CD45 marker expression, which might indicate contamination with hematopoietic stem cells and those cells may be the only ones differentiating into cardiomyocytes (Rose et al., 2008b).

Studies have demonstrated that mechanical conditioning by direct stretching can affect cardiomyogenesis. The expression of cardiac differentiation markers sarcomeric α-actinin, MEF2c and GATA-4 increased in mouse embryonic stem cells after static stretching at 10% strain for 2 h (Schmelter et al., 2006). It is interesting to note, however, that other studies have found that low frequency direct stretch of 10% strain for 10 cycles/min could reduce differentiation of human embryonic stem cells, maintaining an undifferentiated state (Saha et al., 2006). Clearly, complex mechanotransduction signaling can impact multiple systems and specifics of the cell culture and cell state may strongly affect the stem cell mechanotransductive response.

One study found that laminar shear flow of 10 dynes/cm^2 s over cultures of mouse embryonic stem cells resulted in an upregulation of expression of some cardiac markers, including MEF2c and sarcomeric α-actinin (Illi et al., 2005). Cultures of bone marrow-derived mesynchymal stem cells on substrates of various rigidity have found that general myocyte marker expression increases when cells are plated on a substrates with a vary narrow range in elastic modulus, centered around 10 kPa (Engler et al., 2006), though to our knowledge, no study has specifically demonstrated substrate stiffness effects on cardiomyogenesis from undifferentiated stem cells.

A larger amount of experimental data exists on the effects of mechanical stimulation on cells already partially differentiated toward the cardiac lineage and expressing cardiac markers. This could indicate that major mechanotransductive pathways are not expressed in pluripotent cells but are expressed and activated during the process of cardiogenesis. Cells hand-selected for beating colonies and expression of the cardiac markers cardiac α-myosin heavy chain (MHC), cardiac α-actin and the transcription factors GATA-4 and Nkx2.5 not only continued to increase expression of those markers when continuously stretched at 10% strain and 1.0 Hz for 2 weeks, but also formed cell–cell connections and synchronously beat both in culture and upon implantation onto infarcted rat hearts (Gwak et al., 2008). Furthermore, mouse embryonic stem cells genetically selected for expression of cardiac α-MHC showed a sensitivity to stretch frequency, increasing cardiac marker expression upon direct stretching at 3 Hz but decreasing expression at 1 Hz (Shimko and Claycomb, 2008).

3. Cardiomyocyte maturation

Cardiomyocytes will mature in the weeks following birth. This maturation can be viewed histologically through the appearance of well-defined sarcomeres (Lucchesi and Sweadner, 1991), marked cardiomyocyte hypertrophy (Li et al., 1996) and cell binucleation (Soonpaa et al., 1996). In addition, the mechanisms of calcium handling are altered in maturing cardiomyocytes, with extracellular calcium current through membrane channels accounting for most of the calcium transient in embryonic and neonatal cardiomyocytes, and calcium release from internal sarcomeric stores accounting for the calcium transient of adult cardiomyocytes (Gomez et al., 1994; Husse and Wussling, 1996). This change in calcium handling is concurrent with an increase in expression of the sarcoplasmic/endoplasmic calcium ATPase (SERCA2a) and the Ryanodine receptor (RyR), which acts as the sarcomeric calcium release channel (Lodish et al., 2000).

Direct stretch of cardiomyocytes can directly affect the activity of several ion channels and increase gap junction-mediated cell coupling, as reviewed in Jacot et al. (2009). In maturing cardiomyocytes, experiments on neonatal rat atrial cells found that 13% biaxial strain results in differences in gene expression of specific potassium channels and currents, ultimately resulting in reduced action potential duration (Saygili et al., 2007; Rana et al., 2008). However, another group found that neonatal rat ventricular myocytes respond to anisotropic static stretch of 10 min by an increase in action potential duration (Zhang et al., 2008). The effect of both static and dynamic stretch to upregulate expression of connexin-43, which forms gap junctions that electrically couple cardiomyocytes, and the effective coupling of cells in response to stretch has been very well documented (Wang et al., 2000; Zhuang et al., 2000; Shyu et al., 2001; Pimentel et al., 2002; Shanker et al., 2005; Yamada et al., 2005).

In addition to the role of mechanical factors in influencing the maturation and development of an aligned sarcomeric structure in neonatal cardiomyocytes, cell shape has also been shown to play a role. The use of geometric boundaries that force neonatal rat ventricular myocytes to spread into an elongated shape, similar to that of cardiomyocytes *in vivo*, leads to more sarcomeric alignment and clear axes of contraction (Bray et al., 2008; Parker et al., 2008).

Finally, in partially or wholly differentiated cells, electrical stimulation of the cells can induce beating, creating a dynamic loading of cell–matrix connections as well as portions of the cytoskeleton without the need for direct stretch. One group found that neonatal rat cardiomyocytes stimulated by an electric field in the direction of a micro-pattern-induced alignment increase elongation (Heidi et al., 2009) and that neonatal rat cardiomyocytes, cultured in a PEG-diacrylate gel with endothelial cells, were more excitable and expressed more connexin 43, which forms gap junctions to electrically couple the cells, when stimulated with electrical pulses during culture (Chiu et al., 2008).

The elastic modulus of the extracellular matrix surrounding cardiac cells has multiple possible methods of signaling. In other cell types, integrins binding to the extracellular matrix have been shown to develop more mature focal adhesions and mediate signaling pathways that affect complex cell behaviors when bound to a substrate with a higher elastic modulus (Beningo et al., 2001). Because neonatal cardiac cells will spread and elongate on a surface, and become more rounded in suspension, it is likely that this same integrin-mediated signaling plays a role in cardiomyocytes. In addition, a softer substrate, by definition, allows for a greater length of contraction for the same generated force allowing for signaling mediated by contractile strain. Finally, the direct relationship between the force generated by a cardiomyocyte and its sarcomere length (Bluhm et al., 1995) links these two signaling pathways. A high degree of shortening can result in shorter sarcomeres (and faster shortening velocities), and therefore lower force on the connections to the matrix. Also, a high degree of spreading and prestress force generation can result in longer sarcomeres, and still higher force on the connections to the matrix.

Given these relationships, it is not surprising that studies have observed increasing cardiomyocyte force with increasing matrix stiffness. However, when neonatal rat cardiomyocytes were cultured for 7 days on substrates of varying elasticity, they produced lower contractile force on substrates with elastic modulus of 25 kPa or above (Jacot et al., 2008). These cells on these stiffest gels appeared to lack the maturation seen in cells on the softer gels—they had fewer defined sarcomeres that spanned the width of the cell and they expressed less SERCA2a calcium pump resulting in less stored calcium, lower calcium transients and therefore lower force. However, by inhibiting one pathway,