Progress in Biophysics and Molecular Biology xxx (2015) 1–6

Contents lists available at ScienceDirect

Progress in Biophysics and Molecular Biology

journal homepage: www.elsevier.com/locate/pbiomolbio

A review of the literature on cardiac electrical activity between fibroblasts and myocytes

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ARTICLE INFO

Article history:
Received 22 September 2015
Received in revised form
12 November 2015
Accepted 16 December 2015
Available online xxx

Keywords:
Fibroblasts
Heart
Arrhythmias
Gap junctions
Ion channels
Myocardial infarction

ABSTRACT

Myocardial injuries often lead to fibrotic deposition. This review presents evidence supporting the concept that fibroblasts in the heart electrically couple to myocytes.

1. Introduction

Myocardial injuries often lead to scarring and fibrotic deposition. Fibrous tissue contains a large amount of "non-excitable" cells such as fibroblasts. Fibroblast function has been defined by their active deposition of extracellular matrix but accumulating evidence suggests that an electrophysiological function is also likely. The following review presents evidence to support the concept that fibroblasts and/or other non-myocyte cells can electrically couple to excitable cardiac myocytes and relay electrical current. This includes a review of the molecular players that may be involved such as ion channels and connexin proteins to electrophysiological studies performed *in vitro* and *in vivo*.

1.1. Fibroblast ion channels

While ion channels and their functions have been extensively studied in myocytes, their expression and physiological roles in fibroblasts are less clear, however, there is substantial interest in the ion channels that fibroblasts express. Some of these have been described using real-time polymerase chain reaction (RT-PCR) paired with whole cell patch-clamp recording of single currents from isolated cells (Hamill et al., 1981; Mery et al., 1991). Recently, it was reported that rat ventricular fibroblasts possess an inward rectifier K+ current (I_{Kir}) and a delayed rectifier K+ current (I_{Kr}) (Chilton et al., 2005; Rose et al., 2007; Shibukawa et al., 2005). In addition, human cardiac fibroblasts were shown to express calcium-activated big conductance potassium channels (BK_{Ca}) (Wang et al., 2006), as well as additional nonselective channels (Du et al., 2010; El Chemaly et al., 2006; Kamkin et al., 2003a, 2005, 2003b; Ma et al., 2008; Rose et al., 2007). In addition, recent studies have indicated fibroblasts isolated from normal hearts and maintained in culture express ATP-sensitive K+ channel (K_{ATP}) subunits (Benamer et al., 2013; Chilton et al., 2005). Patch clamp studies also demonstrate that K_{ATP} currents appear in fibroblasts in culture within days, similar to the nascent expression of αSMA, suggesting the expression of K_{ATP} may be a response mechanism in fibroblasts due to pathological signaling. An alternative way of studying fibroblast electrophysiology has been through transfection of ion channels into the cells. With this technique it has been shown that fibroblasts transfected with potassium channels are able to modulate membrane potentials and are involved in electrical signaling (Chilton et al., 2005; Shibukawa et al., 2005; Walsh and Zhang, 2008).

Finally, several studies have indicated that cultured fibroblasts express sodium channels. Chatelier et al. (2012) reported the

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http://dx.doi.org/10.1016/j.pbiomolbio.2015.12.006
0079-6107/

presence of a fast inward voltage gated sodium current in atrial fibroblasts beginning after 7 days of culture. The appearance of this current was associated with the expression αSMA expression. Quantitative RT-PCR indicated transcripts of $Na_v1.5$ were four times higher in cultured fibroblasts compared to freshly isolated fibroblasts (Chatelier et al., 2012). Patch clamp indicated that sodium currents in atrial fibroblasts exhibited similar biophysical characteristics compared to currents recorded from myocytes (Chatelier et al., 2012). These studies suggest that fibroblast sodium currents may generate a persistent sodium entry into aSMA positive fibroblasts or myofibroblasts, which may in turn influence proliferation, migration, and secretion (Chatelier et al., 2012). While voltage gated sodium channels are well known to be important in electrophysiological properties of myocytes, there is also evidence that these channels are involved in other cellular functions in unexcitable cells. For example, voltage gated channels may modulate cell invasion process in cancer (Gillet et al., 2009; Roger et al., 2003), participate in human angiogenesis (Andrikopoulos et al., 2011), and assist in microglia and keratinocyte secretion (Black et al., 2009; Zhao et al., 2008). While these studies have provided some insight about fibroblast channel expression, additional work is still needed to determine the functional consequences of these currents and to obtain channel expression profiles from intact tissue. Furthermore, a persistent sodium entry into aSMA positive fibroblasts might influence myocyte electrophysiology particularly if both populations are electrically coupled. The magnitude of this effect might require mathematical simulation studies as has been performed for the effect of fibroblast stretch activated currents (Zhan and Xia, 2013).

It should be noted that most of the results described in this section were obtained from cultured fibroblasts, and numerous studies have demonstrated that fibroblasts have a propensity to alter their expression profiles when cultured. For example it is well known that fibroblasts plated under standard tissue culture plates differentiate into myofibroblasts that express αSMA in 24–48 h after isolation (Miragoli et al., 2006, 2007; Wang et al., 2003). This is particularly important given that the electrophysiological characteristics of cultured fibroblasts that express aSMA differ from fibroblasts (discussed below). Therefore, care should be taken before extending *in vitro* findings to cardiac fibroblasts *in situ*.

1.2. Fibrosis and indirect roles in arrhythmogenicity

Fibrotic remodeling involves a disproportional accumulation of collagen and is an integral feature of pathological remodeling. Collagen thickens the ventricular myocardium, which can impair contractility and may also mechanically separate myocytes (Gardner et al., 1985). Fibroblast aggregation and increased extracellular matrix production can mechanically separate myocardial bundles this may cause conduction slowing, conduction block or lead to a lengthier conduction path between myocytes. If the latter occurs propagation of activation across myocytes follows a zigzag course, which inherently causes conduction delay (de Bakker and van Rijen, 2006). Rohr also proposed that conduction delay may arise from tissue discontinuities (de Bakker and van Rijen, 2006). In some cases, conduction delay or block is caused by the mismatch between current supply as the wave encounters varying sizes of myocardial bundles caused by excessive deposition of connective tissue. Fibroblasts have also been implicated in wave front curvature alteration. As fibrosis is mostly aligned with myocyte fiber directions, it would follow that separation of myocytes by connective tissue would not significantly affect longitudinal propagation, unless fibers run in the transverse direction. However, conduction slowing may arise at the pivoting point, where fiber direction changes. Thus, the wave front curvature alternation is a potentially major determinant of reduced conduction velocity at such barriers (de Bakker and van Rijen, 2006).

Fibroblasts may also contribute to electrical remodeling by the secretion of paracrine factors which can alter myocyte membrane electrophysiology (Brown et al., 2005; LaFramboise et al., 2007). For example, TNF-α can inhibit the inward rectifier channel, I_{K1}, the transient outward current, I_{to}, and the ATP sensitive potassium channel, I_{KATP} (Fernandez-Velasco et al., 2007; Isidoro Tavares et al., 2009) thus prolonging ventricular action potential duration and inducing electrical abnormalities (Abramochkin et al., 2013). Fibroblasts are highly sensitive to circulating hormones, and they react by modifying their proliferative and synthetic response (Brilla et al., 1995; Griffin et al., 2000). Fibrotic tissue remodeling is associated with increased expression of matrix metalloproteases (MMPs) and growth factors such as transforming growth factor beta (TGF-β), angiotensin II (AT2), endothlein-1 (ET1), and tumor necrosis factor alpha (TNF-α) (Baudino et al., 2006). AT2, ET1, and TNF-α have been shown to regulate myocyte hypertrophy (Baudino et al., 2006). A study by LaFramboise et al. found that chemical mediators secreted by neonatal fibroblasts affected the size, contractile capacity, and phenotype plasticity of cardiac myocytes in culture (LaFramboise et al., 2007). In another study, rat cardiac monolayers were exposed to cardiac fibroblast-conditioned media for 24 h. The cardiac fibroblasts released paracrine factors into the media that caused dose dependent and partially reversible effects (with the removal of media) on cardiac monolayers. Cardiac myocytes cultured with fibroblast-conditioned media showed significant reduction in conduction velocity and upstroke velocity, as well as a significant prolongation of action potential duration, electrophysiological changes that are mirrored in many states of cardiac pathology (Pedrotty et al., 2009). While it is more difficult to identify the influences of fibroblast secretion of paracrine factors *in vivo*, these experiments demonstrate that fibroblasts may modulate myocyte electrophysiology through indirect methods.

While secreting paracrine factors and providing physical obstruction to electrical conduction are indirect mechanisms by which fibroblasts likely contribute arrhythmogenicity, relevant data that suggests fibroblasts play a more active role in arrhythmogenesis. A growing body of *in vitro* and clinical evidence suggests fibroblasts can electrically couple to myocytes and directly modify myocyte electrophysiology. The next section will review the evidence for electrical coupling between fibroblasts and between fibroblasts and myocytes.

1.3. Evidence for fibroblast electrical coupling

1.3.1. In vitro evidence

There is growing evidence that fibroblasts can directly communicate to myocytes by electrically coupling through connexins. The significance of these interactions is not yet fully understood. Numerous studies have demonstrated connexin proteins are expressed at sites of fibroblast-myocyte contact in culture (Chilton et al., 2007; Doble and Kardami, 1995; Gaudesius et al., 2003; Miragoli et al., 2006; Rook et al., 1989, 1992b). The gap junctional plaques connecting fibroblast–fibroblast or fibroblast-myocyte are structurally more discrete and likely much smaller than myocyte–myocyte plaques (Zhang et al., 2008). While the fibroblast gap junctions are likely much smaller than myocyte–myocyte counterparts, it is also possible that immunohistological handling of the tissue may damage the gap junctional plaques found in fibroblasts. In the past, it has been difficult to convincingly demonstrate gap junctional plaques in fibroblasts. Although connexin staining is not always visible in appositional membranes, fibroblast cultures exhibit a consistent level of intercellular coupling, which suggests that the gap junctions are not as

Please cite this article in press as: Mahoney, V.M., et al., A review of the literature on cardiac electrical activity between fibroblasts and myocytes, Progress in Biophysics and Molecular Biology (2015), http://dx.doi.org/10.1016/j.pbiomolbio.2015.12.006