A TRP to cardiac fibroblast differentiation

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Keywords: cardiac fibroblast, differentiation, mechanical signaling, myofibroblast, integrin, ECM stiffness, TRPV4, Rho/ROCK

The differentiation of cardiac fibroblasts to myofibroblasts is one of the key events during cardiac remodeling, however, the molecular mechanism underlying this process is not well known. Calcium signaling plays an important role in the regulation of cardiac fibroblast function, but its role in the differentiation of fibroblasts is undefined. Recently four Transient Receptor Potential (TRP) channels TRPM7, TRPC3, TRPC6 and TRPV4 were shown to be crucial for the differentiation of cardiac fibroblasts to myofibroblasts. This addendum sums up the roles described for these four TRP channels in cardiac fibroblast differentiation, and discusses the possible molecular mechanisms underlying this process and its relevance for cardiac remodeling in disease.

Cardiac remodeling is critical for the function of the heart in response to injury or insult.1-3 Cardiac fibroblasts (CF) are the predominant cell types that secrete extracellular matrix (ECM) and help maintain the structural integrity of the heart.4 CFs proliferate, migrate and differentiate to myofibroblasts during cardiac remodeling in disease states such as myocardial infarction (MI), atrial fibrillation (AF), and pressure overload-induced hypertrophy.4-7 Myofibroblasts are hyper-secretory, highly contractile and deposit excessive ECM proteins resulting in cardiac fibrosis that eventually form the scar. However, uncontrolled production of ECM proteins by prolonged survival of myofibroblasts can lead to pathological fibrosis. Therefore, most of the studies on cardiac fibrosis focus on understanding the molecular mechanism(s) controlling differentiation of cardiac fibroblasts to myofibroblasts. Fibroblast differentiation involves two major events including de novo expression of α-SMA and its incorporation into the stress fibers which are mediated by soluble and mechanical signaling.8-13 Most of the studies in the field have been focused on the profibrotic soluble factors such as transforming growth factor (TGFβ), angiotensin II (Ang II) and endothelin (ET-1) working through canonical SMAD and ERK1/2 pathways. Interestingly, intracellular calcium regulates a number of functions in fibroblasts such as contractile activity,14 however, it is not known whether calcium signaling plays a role in the differentiation of fibroblasts to myofibroblasts. Recently four different Transient Receptor Potential (TRP) channels, TRPM7, TRPC3, TRPC6 and TRPV4 have been shown to be critical for fibroblast differentiation to myofibroblasts.15-18

TRP channels are non-selective calcium entry channels19 and are widely expressed in a number of cells including fibroblasts. Du et al. (2010) demonstrated that atrial fibroblasts isolated from human cardiac tissue exhibit endogenous currents of TRPM7 and calcium entry is exclusively mediated through TRPM7 but not TRPC1, TRPC6, TRPV2 or TRPV4 which are also expressed in these cells.17 Importantly, they found that TRPM7 expression and activity is upregulated in fibroblasts from AF patients and that knockdown of TRPM7 significantly reduced basal differentiation of fibroblasts (from AF patients) to myofibroblasts. Further, TGFβ-mediated increased expression of TRPM7 was shown to be correlated with increased differentiation...
of human atrial fibroblasts to myofibroblasts in vitro. These results suggest an exclusive role for TRPM7 in fibroblast myofibroblast transition in the human atrium and that TRPM7 expression and activity is regulated by TGFβ. However, this study did not provide any evidence for the mechanisms by which TGFβ regulates TRPM7 expression/activity or differentiation. Two additional studies provided mechanistic insights. First, using animal models of atrial fibrillation, isolated fibroblasts and pharmacological inhibitors, Harada et al. (2012) demonstrated that TRPC3 channels are critical for atrial fibroblast differentiation.\textsuperscript{19} Further they described that NFAT-mediated downregulation of microRNA-26 increased TRPC3-dependent fibroblast proliferation via ERK1/2 pathway leading to increased fibroblast differentiation. Although this study provided a possible mechanism(s) for TGFβ-induced increased expression of TRPC3 and cardiac fibroblast proliferation, no mechanism(s) were given for the differentiation process. Davis et al. (2012) in the second study addressed this point albeit the candidate TRP channel here was TRPC6 not TRPC3. Using a genome-wide screen they identified TRPC6 as a calcium channel necessary and sufficient for fibroblast differentiation to myofibroblasts.\textsuperscript{20} Importantly, they demonstrated that the profibrotic mediators TGFβ and Ang II, independent of other, induced TRPC6 expression through a p38 MAPK/inosensitive ion channel, TRPV4 mediates fibroblast differentiation via integration of soluble and mechanical signals.\textsuperscript{22} TRPC3 and TRPC6 were both demonstrated to play role in mechanotransduction during pressure overload-induced cardiac hypertrophy.\textsuperscript{24} However, TRPC6 mechanosensitivity requires the expression of Angiotsin II receptor (AT1R), importantly, even without the ligand binding to the receptor.\textsuperscript{25} Although these three TRP channels have mechanosensitive functions, there are no reports on their involvement in mechanotransduction in cardiac fibroblasts. In a recent study, we addressed this question and demonstrated that a mechanosensitive ion channel, TRPV4 mediates fibroblast differentiation via integration of soluble and mechanical signals.\textsuperscript{25} By using a TRPV4-specific antagonist AB159908 and siRNA knockdown of TRPV4, we conclusively demonstrated that TRPV4 is required for TGFβ1-induced differentiation as measured by incorporation of α-SMA into stress fibers. Furthermore, using bioengineered gels of varying stiffness we have shown that TGFβ1-induced fibroblast differentiation was dependent on ECM stiffness and was attenuated by TRPV4 blockade. Similar to TRPM7, TRPC3 and TRPC6, the expression of TRPV4 was also increased by TGFβ treatment. Our study proposed two roles for TRPV4. First, TRPV4 acts as a mechanosensor of ECM stiffness and induce fibroblast differentiation by increasing the expression and incorporation of α-SMA in to stress fibers\textsuperscript{13,25} may be via a Rho/ROCK dependent pathway.\textsuperscript{26} Second, increased cell contraction and tensional forces may activate additional integrins\textsuperscript{27,28} leading to the activation of latent TGFβ\textsuperscript{29} forming a positive mechanical feedback loop that drives the differentiation of fibroblasts. Importantly, in contrast to the exclusive role of TRPM7 in atrial fibroblast differentiation shown by Du et al.,\textsuperscript{17} our study demonstrated that TRPM7 channels do not play a role in ventricular fibroblast differentiation.

The findings from the above studies suggest that all four different TRP channels are critical for fibroblast differentiation to myofibroblasts in a cell-specific manner. This raises an important question, how and why would nature employ different TRP channels for the same function? The answer to this question comes partially from the studies discussed. TRPM7 and TRPC3 are exclusively involved in atrial fibroblast differentiation whereas TRPC6 and TRPV4 appear to participate in ventricular fibroblast differentiation. The fact that TRPM7 is exclusively required for atrial fibroblast differentiation and TRPV4 is critically utilized for ventricular differentiation, despite both being sufficiently expressed in atrial and ventricular fibroblasts, confirms the cell-specific roles for these TRP channels. This also could be due to the local mechanical environment, since ventricles are mechanically more active than atria, which may preferentially activate TRPV4. The local mechanical environment cannot explain the roles for TRPC3 and TRPC6 in fibroblast differentiation, however, the molecular signaling may shed light on this issue. Mechanistically, the unifying point of all these TRP channels is that their expression is regulated by soluble factors such as TGFβ irrespective of the origin of fibroblast. Furthermore, NFAT signaling is implicated in both TRPC3- and TRPC6-dependent fibroblast proliferation and α-SMA expression, respectively. Although these studies demonstrate that each of these TRP channels is critical for fibroblast differentiation, this is based on a...
implicated in fibroblast proliferation, α-SMA expression and stress fiber formation leading to the differentiation of fibroblasts to myofibroblasts. Unraveling specific roles for each TRP channel and their downstream signaling will further our understanding of myofibroblast differentiation and may provide the basis for the development of novel therapies to limit myocardial fibrosis and its complications.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Acknowledgments
This work is supported by start-up funds from NEOMED (C.K.T.) and, in part, by NIH-1R15HL106442–01 (J.G.M. and C.K.T.).

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