DEVELOPMENTAL LINEAGE OF HUMAN PLURIPOTENT STEM CELL-DERIVED CARDIAC FIBROBLAST AFFECTS THEIR FUNCTIONAL PHENOTYPE

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Cardiac fibroblasts (CFBs) support heart function by secreting extracellular matrix (ECM) and paracrine factors, respond to stress associated with injury and disease, and therefore are an increasingly important therapeutic target. CFBs are thought to be specialized by region and lineage as identified by recent single cell sequencing studies[1,2]. Here, we describe how developmental lineage of human pluripotent stem cell-derived CFBs, epicardial (EpiC-FB) and second heart field (SHF-FB) impacts transcriptional and functional properties. Both EpiC-FBs and SHF-FBs exhibited CFB transcriptional programs expressing canonical cardiac fibroblast markers *POSTN*, *COL1A1*, *VIM*, *THY1*, *HAND2*, and *GATA4*. When added to human pluripotent stem cell-derived cardiomyocytes at a ratio of 1:3, both hPSC-CFB subtypes improved aggregate formation and calcium handling of the cardiac tissues. EpiC-FB represent the majority of all CFBs in the adult human heart and are identifiable by expression of epicardial-lineage markers *TBX3*, *TBX18,* and *TBX20*[3]. As expected, hPSC-derived EpiC-FBs similarly expressed these markers. Phenotypically, EpiC-FBs have higher stress-induced activation potential to transition to a myofibroblast-like state than SHF-FBs and they secrete Gremlin-1, epidermal growth factor-like protein 7, and connective tissue growth factor, which have been implicated in fibrosis[4,5]. Thus, one should consider using EpiC-FBs in modeling cardiac fibrosis and anti-fibrotic drug testing where CFB activation and fibrosis are important. SHF-FBs represent a portion of CFBs which arise from the second heart field and can be identified by expression of lineage markers *TBX1* and *SALL1*. hPSC-derived SHF-FBs also expressed second heart field markers as well as noncanonical WNT signaling pathway factors including WNT5A, which developmentally is secreted from second heart field progenitors and is required for proper outflow tract development[6–9]. Additionally, they lack expression of factors identified in the EpiC-FBs as associated with fibrosis, and therefore may be ideal for cell therapy into an injured or diseased heart, or for other applications where activation is undesired. These phenotypic differences highlight the diversity and complexity of cardiac cell subtypes and inform CFB *in vitro* model selection. We expect that hPSC-CFBs will be used in a range of applications spanning drug testing, disease modeling, tissue engineering, and regenerative medicine cell therapies.

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