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PRECLINICAL RESEARCH

A Transcriptomic and Epigenomic Comparison of Fetal and Adult Human Cardiac Fibroblasts Reveals Novel Key Transcription Factors in Adult Cardiac Fibroblasts

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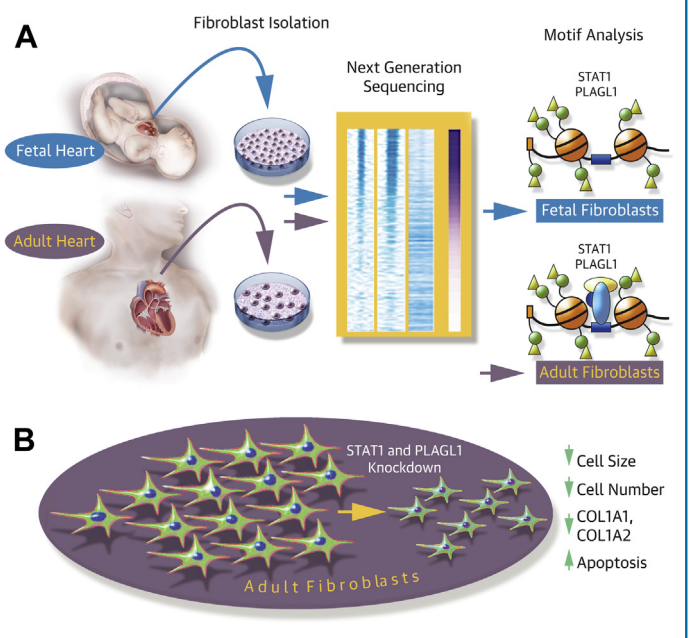
VISUAL ABSTRACT



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HIGHLIGHTS

- **The interplay between cardiomyocytes and cardiac fibroblasts is increasingly being recognized as important in cardiac disease.**
- **Fetal and adult cardiac fibroblasts influence their neighboring cardiomyocytes in different ways. A genome-wide comparison of the 2 reveals that they share >80% of gene transcripts.**
- **Motif analysis of empirical regulatory elements located next to differentially expressed genes led to identification of key differential regulators of fibroblast identity.**
- ***STAT1* and *PLAGL1* were identified and validated as key transcription factors to maintain the adult cardiac fibroblast phenotype. Loss of either factor led to a significant change in phenotype, including smaller cell size, apoptosis, reduced turnover, and down-regulated collagen gene expression.**

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SUMMARY

Cardiovascular disease remains the number one global cause of death and presents as multiple phenotypes in which the interplay between cardiomyocytes and cardiac fibroblasts (CFs) has become increasingly highlighted. Fetal and adult CFs influence neighboring cardiomyocytes in different ways. Thus far, a detailed comparison between the two is lacking. Using a genome-wide approach, we identified and validated 2 crucial players for maintaining the adult primary human CF phenotype. Knockdown of these factors induced significant phenotypical changes, including senescence and reduced collagen gene expression. These may now represent novel therapeutic targets against deleterious functions of CFs in adult cardiovascular disease. (J Am Coll Cardiol Basic Trans Science 2016;1:590–602)

ABBREVIATIONS AND ACRONYMS

aHCF = adult human cardiac fibroblast

ATAC = assay for transposase accessible chromatin

ATAC-seq = assay for transposase accessible chromatin-sequencing

CF = cardiac fibroblast

ChIP-seq = chromatin immunoprecipitation-sequencing

CM = cardiomyocyte

ECM = extracellular matrix

EMT = epithelial-to-mesenchymal transformation

FGF = fibroblast growth factor

fHCF = fetal human cardiac fibroblast

HCF = human cardiac fibroblast

IL = interleukin

IPA = Ingenuity Pathway Analysis

RNA-seq = ribonucleic acid-sequencing

RT-qPCR = reverse transcription-quantitative polymerase chain reaction

TF = transcription factor

Although cardiomyocytes (CMs) occupy most of the tissue volume and provide the mechanical force delivered by the heart, they are largely outnumbered by nonmyocyte cells (30% vs. 70%), part of which are cardiac fibroblasts (CFs) (1,2). Cross-sectional confocal microscopy of ventricular tissue reveals that each CM is in the direct vicinity of at least 1 CF (3), reflecting a significant role for CFs in the heart; that is, to create and hold a supportive environment for CMs, such as by regulation of the extracellular matrix (ECM). More than simply a "scaffold cell," CFs are understood to communicate with CMs in 3 different ways. The first method is through direct cell-to-cell contact, in which the formation of adherens junctions (cadherins) and gap junctions (connexins) play a crucial role (4). The second method is by paracrine or autocrine secretion of growth factors such as fibroblast growth factor (FGF)-2/basic FGF and transforming growth factor-β or important cytokines such as interleukin (IL)-1β and the IL-6 family, including leukemia inhibitory factor and cardiotrophin-1 (5). In the third method, cells indirectly relay signals via the ECM by modulating its composition and quantity by secretion or degradation of the ECM building blocks (4,6).

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Common features of fibroblasts are the lack of a basement membrane, profound granular material in the cytoplasm scattered along a large Golgi apparatus, and a substantial rough endoplasmic reticulum (7). A unique marker for fibroblasts or CFs is still lacking, although both fetal and adult CF express periostin, discoidin domain receptor 2, and vimentin (8). Another feature of CFs is their ability to transform into an active state; the myofibroblast. Myofibroblasts express smooth muscle cell markers (e.g., smooth muscle actin [SMA]) and may contract. They have also been implicated in wound contraction, fibrosis, and scar healing and are a source of cytokines and growth factors, such as IL-6 and transforming growth factor-β (9).

Fibroblasts are abundant throughout all tissues in the body, and the population is heterogeneous, with diverse appearances and functions depending on where the cells reside (10). Apart from the expression of common core fibroblast genes, a specific gene expression profile involving the cardiogenic transcriptional network has been described uniquely for CFs (11). Furthermore, regional differences exist in which CFs from the atrium and the ventricle express different cardiogenic transcription factors (TFs) (11,12). Important differences have also been found between rat CFs from the embryonic heart compared with the adult heart, with a differential response in insulin-like growth factor-induced collagen production (13). The influence of CFs on co-cultured CMs also varies depending on age. Embryonic CFs increase proliferation of CMs, whereas adult CFs induce hypertrophy (6). Notably, this finding is consistent with the growth of a fetal or neonatal heart, which depends on the proliferation of CMs, whereas the adult heart responds to stress by hypertrophy. The diversity of the CF population is also underlined by the finding that CF may be derived from at least 3 sources: from the proepicardium, from endocardial- or epicardial-to-mesenchymal transformation (EMT), and from bone marrow (14–16).

Despite increasing attention to the potential role that CF may play in novel disease therapeutics (17), a detailed genome-wide characterization of the genetic and epigenetic profiles of CF has yet to be performed. In the present study, we conducted next-generation sequencing experiments with primary ventricular fetal human cardiac fibroblasts (fHCFs) and adult human cardiac fibroblasts (aHCFs) to carefully map their respective transcriptomic and epigenomic