JACC: BASIC TO TRANSLATIONAL SCIENCE VOL. 1, NO. 7, 2016

ISSN 2452-302X
http://dx.doi.org/10.1016/j.jacbts.2016.10.002

EDITORIAL COMMENT

Applying Modern Transcriptomics to Interrogate the Human Cardiac Fibroblast*

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Cardiovascular disease and heart failure remain a major cause of mortality worldwide with tremendous socioeconomic consequences (1). In the Western world, coronary artery disease and subsequent myocardial infarction injury are the leading disease sequelae contributing to heart failure, which is characterized by progressive ventricular stiffening, remodeling and fibrosis, myocyte dropout, reduced contractility, and rhythm disturbances (2). An underlying progressive fibrotic response appears to worsen cardiac function, predispose to arrhythmia, and limit any chances for minor regenerative efforts within the heart (3). Cardiac fibroblasts (CF) are presumed to be the major mediators of physiological scar formation after acute myocardial infarction that prevents wall rupture, as well as the mediators of long-standing and progressive fibrotic remodeling during heart failure. CF become activated by injury or long-standing disease during which they can synthesize and deposit collagen and other extracellular matrix proteins, as well as affect ventricular wall and septal remodeling through their newly acquired contractile activity (4). The concept of identifying novel antifibrotic therapies that target select functions of the activated fibroblast has been an ongoing research goal (5), although effective agents are still lacking.

Until recently, a major limitation in understanding the pathologic fibrotic response has been our inability to identify the origin and molecular characteristics of CF. However, in the past few years a number of genetic approaches in mouse models have begun to reveal the origins of the CF and the central molecular effector pathways that mediate their activation (6-9). These studies collectively determined that CF residing within the adult heart are a developmentally traceable cell-type differing from many other tissue resident fibroblasts (9,10). More specifically, disease-relevant CF in the adult mouse heart are derived from a developmental epicardial origin of cells best defined by the molecular markers TCF21, TBX18, and WT1 (10,11), and when activated during disease they uniformly express the marker periostin (6) and other contractile genes such as smooth muscle α-actin (12).

Far less is understood of fibroblast origins and molecular characteristics in the human heart, during both development and disease processes in adults. Indeed past studies have suggested that fibroblast characteristics can vary between large and small mammals (and humans) when compared even within the same tissues (13,14). Therefore, more studies of human-derived cardiac fibroblasts are needed, especially during developmental and adult time points during acute and chronic disease. In this issue of the *JACC: Basic to Translational Science,* Jonsson et al. (15) address this issue by performing an elaborate series of transcriptome analyses of cultured fetal versus adult human cardiac fibroblasts.

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Jonsson et al. (15) analyzed 2 primary lots of isolated human fibroblasts, 1 from the embryonic heart and 1 from the adult heart, each from different commercial vendors. Their research demonstrated vastly different cell sizes and proliferation rates of the adult and fetal CF in culture, suggesting that adult and fetal CF have intrinsically different phenotypes. Transcriptional analysis using ribonucleic acid sequencing (RNA-seq) revealed that ~13,400 genes were expressed in the

*Editorials published in *JACC: Basic to Translational Science* reflect the views of the authors and do not necessarily represent the views of *JACC: Basic to Translational Science* or the American College of Cardiology.

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adult and fetal CF, with 1,639 genes exclusively expressed in the fetal CF and 1,354 genes exclusively expressed in the adult CF. Using Ingenuity Pathway Analysis (Qiagen, Hilden, Germany) they identified gene groups that were specific to each lot of fibroblasts from the 2 different vendors. The investigators also performed transposase accessible chromatin sequencing to assess chromatin accessibility and chromatin immunoprecipitation sequencing to reveal active chromatin marks, which were further compared with the RNA-seq analysis, collectively generating a profile that showed that there was ~80% similarity in gene expression profiles in the fetal and adult fibroblast cell isolates, whereas 20% of the gene expression profiles were unique.

From their analyses, Jonsson et al. (15) were able to identify 2 unique genes in adult human CF that encoded transcription factors: *PLAGL1* and *STAT1*. Importantly, and for the first time, the investigators confirmed that these 2 genes were highly expressed in 2 additional lots of commercially obtained adult CF from the same vendor, but were not expressed in 1 additional lot of commercially obtained fetal CF. The investigators then employed a knockdown approach with GapmeR oligonucleotides in all 3 lots of adult human CF against either *PLAGL1* or *STAT1*. *STAT1* knockdown increased the roundness of the cultured CF and reduced cell number without affecting proliferation. Knockdown of both *PLAGL1* and *STAT1* also reduced expression of a number of adult CF genes such as *ELN, TNFRSF11B, COL1A1,* and *COL1A2*, suggesting that *STAT1* and *PLAGL1* are important for maintaining the adult CF phenotype.

The results of Jonsson et al. (15) represent an important first step to providing a much needed higher dimensional interrogation of the human CF and to better understand the possible developmental origins of these cells and their associated molecular signatures. The 2 target genes that were identified, *PLAGL1* and *STAT1* are interesting and should be the basis of future mechanistic analysis, which could have important therapeutic ramifications. However, the very strength of this study directed at interrogating the human CF also has several difficulties that are inherent when one studies cells obtained from human tissues. For example, the entire transcriptomic discovery analysis was conducted on 2 lots of CF that were obtained from different vendors. The limited number of samples of adult and fetal fibroblasts raises questions about the broader reproducibility of the investigators'' findings in different patient populations. Another limitation relates to the difficulties in interpreting the transcriptional program of fibroblasts that have been passaged in culture, which is likely to be different from the transcriptional program of fibroblasts that reside within the heart. A final issue to consider is that the human fetal CF is likely a cell type that is continually evolving over rather short periods of time during cardiac development. For example, studies in mice have shown that during embryonic development, epicardial precursors destined to become CF are only generated and distributed throughout the heart just prior to birth (11). Therefore, the developmental beginnings and maturation of CF precursors in the human heart need to be carefully annotated before one can infer when the CF fibroblast becomes phenotypically specified, which would better inform the molecular interrogation of these or earlier precursor cells. Despite the modest inherent limitations of the current study, Jonsson et al. (15) clearly demonstrated the tremendous potential of applying modern transcriptomics to better understanding the biology of the human CF, as well as identifying novel therapeutic targets that could be instrumental in some day preventing the development of pathological myocardial fibrosis.

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REFERENCES

1. Laslett LJ, Alagona P Jr., Clark BA 3rd, et al. The worldwide environment of cardiovascular disease: prevalence, diagnosis, therapy, and policy issues: a report from the American College of Cardiology. J Am Coll Cardiol 2012;60 Suppl 25:S1–49.

2. Thygesen K, Alpert JS, Jaffe AS, et al. Third universal definition of myocardial infarction. Circulation 2012;126:2020–35.

3. Talman V, Ruskoaho H. Cardiac fibrosis in myocardial infarction—from repair and remodeling to regeneration. Cell Tissue Res 2016;365:563–81.

4. Chistiakov DA, Orekhov AN, Bobryshev YV. The role of cardiac fibroblasts in post-myocardial heart tissue repair. Exp Mol Pathol 2016;101:231–40.

5. Brown RD, Ambler SK, Mitchell MD, Long CS. The cardiac fibroblast: therapeutic target in myocardial remodeling and failure. Annu Rev Pharmacol Toxicol 2005;45:657–87.

6. Kanisicak O, Khalil H, Ivey MJ, et al. Genetic lineage tracing defines myofibroblast origin and function in the injured heart. Nat Commun 2016;7:12260.

7. Swonger JM, Liu JS, Ivey MJ, Tallquist MD. Genetic tools for identifying and manipulating fibroblasts in the mouse. Differentiation 2016;92:66–83.

8. Moore-Morris T, Tallquist MD, Evans SM. Sorting out where fibroblasts come from. Circ Res 2014;115:602–4.

9. Furtado MB, Costa MW, Pranoto EA, et al. Cardiogenic genes expressed in cardiac fibroblasts contribute to heart development and repair. Circ Res 2014;114:1422–34.

10. Moore-Morris T, Guimaraes-Camboa N, Banerjee I, et al. Resident fibroblast lineages mediate pressure overload-induced cardiac fibrosis. J Clin Invest 2014;124:2921–34.

11. Acharya A, Baek ST, Huang G, et al. The bHLH transcription factor Tcf21 is required for lineage-specific EMT of cardiac fibroblast progenitors. Development 2012;139:2139–49.

12. Darby I, Skalli O, Gabbiani G. Alpha-smooth muscle actin is transiently expressed by myofibroblasts during experimental wound healing. Lab Invest 1990;63:21–9.

13. Agocha A, Sigel AV, Eghbali-Webb M. Characterization of adult human heart fibroblasts in culture: a comparative study of growth, proliferation and collagen production in human and rabbit cardiac fibroblasts and their response to transforming growth factor-beta1. Cell Tissue Res 1997;288:87–93.

14. Jaźwińska A, Sallin P. Regeneration versus scarring in vertebrate appendages and heart. J Pathol 2016;238:233–46.

15. Jonsson MKB, Hartman RJG, Ackers-Johnson M, et al. A transcriptomic and epigenomic comparison of fetal and adult human cardiac fibroblasts reveals novel key transcription factors in adult cardiac fibroblasts. J Am Coll Cardiol Basic Trans Science 2016;1:590–602.

KEY WORDS fibroblast, gene expression, heart, transcription