Single-cardiomyocyte RNA-seq analysis of human heart tissue reveals dynamic transcriptomic features in advanced heart failure

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Background: Heart failure is caused by a variety of molecular pathways. Using molecular pathology analysis, we have demonstrated that quantification of DNA damage in cardiomyocytes can predict clinical prognosis and drug response before treatment in patients with heart failure (Fujita et al. *JACC Basic Trasl Sci*. 2019). However, there is no method to analyze molecular pathogenesis of each patient with heart failure at the single-cell level in an unbiased manner.

Methods: Left ventricular tissue was obtained from patients with advanced heart failure who had received implantation of left ventricular assist device or heart transplantation, and control tissue from autopsy cases whose antemortem heart function was normal. Cardiomyocytes were freshy digested with collagenase for single cell RNA-sequencing. Left ventricular tissue was also used for single-molecule RNA *in situ* hybridization to analyze spatial expression profiles at the single-cell level.

Results: Single-cell RNA-sequencing divided cardiomyocytes from patients with heart failure into several clusters and revealed their molecular signatures such as DNA damage response, extracellular matrix, and type 1 interferon signaling. Single-molecular RNA *in situ* hybridization uncovered their spatial expression profiles, including extracellular matrix genes expressed by cardiomyocytes adjacent to fibrotic regions. Pseudotime analysis reconstructed trajectories into several types of failing cardiomyocytes, and epigenomic analysis identified specific transcription factors regulating their trajectories. By integrating with pathological findings, we found that genes involved in extracellular matrix and DNA damage response are linked with cardiomyocyte hypertrophy and cardiac fibrosis.

Conclusions: Our study provides a foundation for understanding pathogenesis of heart failure in human and developing precision medicine for heart failure.