Synopsis of Original Research Paper

Single cell analysis of adipocyte diversity for therapeutic application of adipocyte transplantation and development of methods to regulate adipocyte function through epigenetic regulation

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With the advent of single cell analysis, it has become possible to understand the characteristics of diverse cell populations and differences in molecular biological properties of individual cells in an integrated manner, which could not be captured by conventional bulk analysis. On the other hand, we have studied the mechanisms of adipocyte aging, functional and epigenetic regulation through bulk NGS analysis of human adipose tissue, with the aim of applying our findings to the development of innovative therapeutic methods for adipose transplantation. Indeed, we have also shown that fractionation of undifferentiated mesenchymal cells, including adipocyte progenitors, in adipose tissue by a ceiling culture method may lead to improved transplantation rates, and that differences in epigenetics including CpG DNA methylation and H3K4me3 in *PPARG* promoter have been shown to produce differences in cellular function and adipogenic differentiation potential. However, tissue-derived cell populations showed greater diversity than expected, limiting previous bulk analysis alone. Here, we performed FACS and single-cell RNA sequencing analysis (scRNA-seq) of human subcutaneous adipose tissue to investigate the cellular lineage diversity of and their molecular basis. FACS analysis identified $DPP4^{+}/CD34^{+}$ fractions, a progenitor marker of adipocytes, and that of the total number of adipocytes , 74.9% were detected. UMAP by scRNAseq classified adipose tissue into nine clusters. The $CD34^{+}/PDGFRA/B^{+}$ mesenchymal cell population, including adipocytes, was classified into four subclusters, one of which was the $DPP4^{+}$ adipocyte progenitor. Gene Ontology analysis with differentially expressed genes, specifically expressed in each cluster, demonstrated that the differences in each cluster characteristics were enriched while having commonality as mesenchymal cells. Thus, these results indicate that scRNA-seq using human adipose tissue can reveal the cellular diversity and molecular biological characteristics of subcutaneous adipose tissue-derived cells, and that detailed analysis of the diverse subclusters may detect the underlying molecular mechanisms that regulate differentiation induction efficiency.