Abstract

Obesity-driven adipose tissue dysfunction is a key contributor to insulin resistance and the progression of type 2 diabetes mellitus (T2DM). The 3T3-L1 murine preadipocyte cell line is widely used to model adipocyte biology in vitro; however, successful differentiation is not always explicitly confirmed prior to downstream experimentation. This study aimed to characterise 3T3-L1 adipocyte differentiation through morphological and molecular approaches, and to establish a validated model for investigating adipocyte dysfunction and insulin resistance. Cells were differentiated using the standard MDI protocol over 15 days, with brightfield microscopy performed at six timepoints (Day 0–15) to assess morphological changes. Gene expression of five adipogenic markers (*PPARG*, *FABP4*, *LPL*, *ADIPOQ*, and *GLUT4)* was quantified by qRT-PCR and analysed. Brightfield microscopy confirmed progressive lipid droplet accumulation from Day 6 onwards, consistent with successful adipogenesis. All five genes showed significant and progressive upregulation throughout the time course. *PPARG* and *FABP4* were significantly upregulated from Day 3, confirming early induction. *LPL* showed a steady linear increase, while *ADIPOQ* and *GLUT4* displayed a delayed but dramatic upregulation from Day 6, confirming terminal adipocyte identity and acquisition of insulin-responsive molecular machinery. These findings demonstrate that 3T3-L1 adipocytes acquire functional adipocyte characteristics in a temporal hierarchy, providing a foundation for future studies of obesity-associated metabolic dysfunction.

Keywords: 3T3-L1, adipogenesis, adipocyte differentiation, insulin resistance, gene expression