Alkbh5, a RNA Demethylase, Is Involved in Fine-tuning of Cell Differentiation (FS11-07-19)

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Objectives: N^6-Methyladenosine (m6A) is the most prevalent mRNA modification, which modulates mRNA export, splicing, and stability. Recent studies showed that m6A is involved in cell differentiation such as spermatogenesis, but the underlying mechanism is still unclear. This study was aimed to investigate whether alkylation repair homolog 5 (ALKBH5)-dependent m6A demethylation plays a role in metabolic-related cell differentiation.

Methods: Expression analysis upon differentiation was done using R studio on publicly available data from two independent cell differentiation models, adipogenic 3T3-L1 and myogenic C2C12. M6A levels in mRNA were measured using dot blot. Knockdown of ALKBH5 was conducted by small interference RNA at two days prior to differentiation induction. For phenotypic measures upon differentiation, staining analysis by Giemsa and Oil Red-O was conducted for 3T3-L1 and C2C12 cells, respectively, followed by histogram analysis using ImageJ. Total RNA was isolated from cells and was reverse-transcribed to cDNA, followed by qRT-PCR for gene expression analysis.

Results: Comparative analysis of adipogenesis and myogenesis models revealed common pathways, including oxidative phosphorylation. Among a-ketoglutarate-dependent demethylases, *Alkbh5* expression was substantial and its substrate, m6A level was changed upon induction of differentiation. Staining analysis showed that Alkbh5 knockdown using siRNA significantly increased production of triglycerides and myotubes in 3T3-L1 and C2C12, respectively, indicating promotion of cell differentiation. To identify putative targets, expression data using microarray and qRT-PCR were analyzed. Expression of CEBPb and myogenin among adipogenic and myogenic markers was significantly altered upon Alkbh5 knockdown in 3T3-L1 and C2C12, respectively.

Conclusions: The results together showed that ALKBH repression promoted cell differentiation in both adipogenic and myogenic models, possibly regulating early differentiating markers such as CEBPb and myogenin, respectively.

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