Characterization of conditionally immortalized human adipocytes and skeletal muscle cells.


Abstract

Conditionally immortalized adipocytes and skeletal muscle cells were generated using AliGems (Lonza) and were used to characterize their ability to differentiate into muscle and adipocyte-like cells in vitro.

Materials and Methods

Conditionally immortalized cells: generation, growth, and differentiation

AliGems (Lonza) conditionally immortalized human skeletal muscle cells (HUMC1) and human adipocytes (HUMA1) were generated using the AliGems (Lonza) system. HUMA1 were immortalized using the AdipoRed assay (Lonza) and were used to compare the differentiation of adipocytes and skeletal muscle cells.

Mircroarray expression analysis

Experiments were performed in collaboration with GenoFinder Corporation (Tokyo, Japan). Total RNA was isolated using TRIzol and purified using RNeasy Mini Kits (QIAGEN). The RNA samples were hybridized to Human Myobase 18K Microarray Genechip probesets (Affymetrix) and functional enrichment analysis was performed using Ingenuity Pathway Analysis. Expression profiles were compared using BioVinci software (Genebio Inc. France). Replicate studies were performed to determine the reproducibility of the microarray data.

Lipogenesis

The AdipoRed assay (Lonza) was performed according to the manufacturer’s instructions. Briefly, 96-well plates were coated with a solid-phase insulin in adipogenesis medium (DMEM/F12+2% FBS) and incubated at 37°C for 16 h. The insulin was removed and adipogenesis medium (DMEM/F12+2% FBS) containing acetyl-CoA at 465 nm and enolase at 530 nm were measured.

Conlydentially immortalized adipocyte and skeletal muscle cell phenotypes

Conlydentially immortalized adipocytes and skeletal muscle cells can be differentiated into adipocytes and skeletal muscle cells in vitro. The AdipoRed assay (Lonza) was performed according to the manufacturer’s instructions. Briefly, 96-well plates were coated with a solid-phase insulin in adipogenesis medium (DMEM/F12+2% FBS) and incubated at 37°C for 16 h. The insulin was removed and adipogenesis medium (DMEM/F12+2% FBS) containing acetyl-CoA at 465 nm and enolase at 530 nm were measured.