Bringing Primary Cell Relevance to Screening: Clonetics Conditionally Immortalized Cells and QBT™ Fatty Acid Uptake Assay for High Throughput Screening

Alex Batchelor, Claire Scholfield, D.G Ferneyhough, Carole Crittenden*, Zoe Damian, and Anatoliy Koval.
Cambrex Bio Science Walkersville, Inc. and *Molecular Devices Corporation

Introduction

Transfection of primary human cells with a temperature sensitive Large T-Antigen (L-Tag) or with a combination of L-Tag and catalytic subunit of human telomerase (hTERT) offers a successful approach to engineer conditionally immortalized human cell lines. These cells are driven to proliferate at the permissive temperature (33°C) but are induced to differentiate for use in assays when the temperature is raised to 37°C. The incorporation of telomerase prevents cell senescence. Conditionally immortalized cells maintain most of the valuable properties of primary cells but possess practically unlimited growth potential, thus representing an ideal model for cell based HTS applications.

Both conditionally immortalized human skeletal muscle cells and human preadipocytes have been produced. Characterization of preadipocytes, including comparative data for proliferation, differentiation and insulin response of the extended passages will be presented. The QBT™ Fatty Acid Uptake Assay kit provides a single step homogeneous *in vitro* assay for use in HTS and metabolic disease research. Defects in fatty acid metabolism have been linked to several pathological states, including Type 2 diabetes, obesity and cardiovascular disease. The experimental data will show that the combination of immortalized preadipocytes and the QBT assay represents a unique and convenient HTS model closely resembling the properties of primary human cells.

Materials and Methods

Clonetics® Conditionally Immortalized Human Preadipocytes (clone XA15A1)

Cambrex Bio Science Walkersville, Inc. has produced a mixed population of conditionally immortalized cells derived from subcutaneous adipose tissue. These cells have been immortalized by expression of temperature sensitive SV 40 Large T-antigen (u19tsA58) using an MMLV-based retroviral vector system and possess resistance to Neomycin. XA15A1 preadipocytes are able to differentiate into functional adipocytes after more than 40 Population Doublings (PD) in culture.

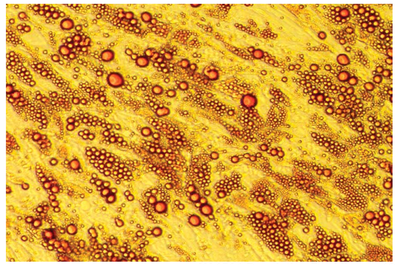
Cell Maintenance and Differentiation

In the proliferation phase cells were routinely cultured in supplemented SKGM-2 (Cambrex) at 33°C, 5%(v/v) CO_2; 95% (v/v) air. The average proliferation rate under these conditions was 42 hours/PD. The cells were processed through a transition stage, which has the potential to increase the percentage of differentiated cells, consisting of a 24-72 hours incubation at 37°C , 5%(v/v) CO_2; 95% (v/v) air in supplemented PGM (Cambrex) media. To obtain differentiated XA15A1 cells, the cells were grown in supplemented PDM (Cambrex) media at 37°C, 5%(v/v) CO_2; 95% (v/v) air.

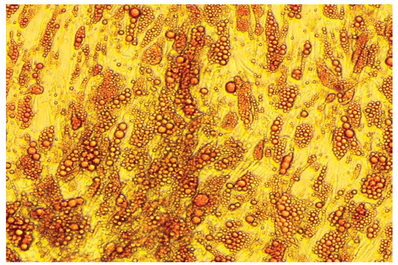
Assay Method

The QBT™ Fatty Acid Uptake Assay (Molecular Devices) is a single step homogeneous fluorescent assay that provides real-time uptake kinetics and is ideally suited for high throughput screening applications in both 96-well and 384-well formats. The kit employs a BODIPY®-dodecanoic acid fluorescent fatty acid analog coupled with Molecular Devices proprietary quench technology. The BODIPY label provides an ideal long chain fatty acid analog that behaves much like natural fatty acids: it becomes activated by acyl-CoA attachment; is incorporated into di-and triglycerides; and accumulates in intracellular lipid droplets. In addition, the BODIPY analog is a known substrate for fatty acid transporters since its uptake by adipocytes can be competed by non-labeled fatty acids. The elimination of radioactive compounds results in easier reagent handling, reduced disposal costs and eliminates safety risks associated with radiolabel assays.

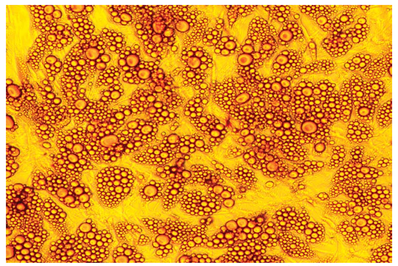
Results



XA15A1 Population Doublings = 12



XA15A1 Population Doublings = 18



Primary human adipocytes Population Doublings = 5

Figure 1: Comparison of phenotypes of differentiated XA15A1 (different Population Doublings) and primary human adipocytes during the AdipoRed™ assay. The AdipoRed assay (Cambrex) quantifies the accumulation of intracellular triglycerides.

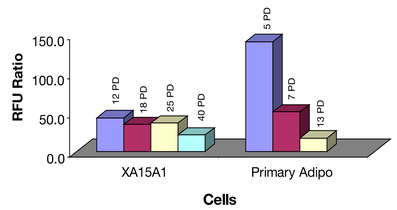


Figure 2: Comparison of the life span of functional XA15A1 and primary human adipocytes. XA15A1 cells were maintained continuously at 33°C and differentiation was initiated at the indicated passages. Primary human adipocytes were maintained and differentiated. Differentiated cells were analyzed using AdipoRed assay (Cambrex).

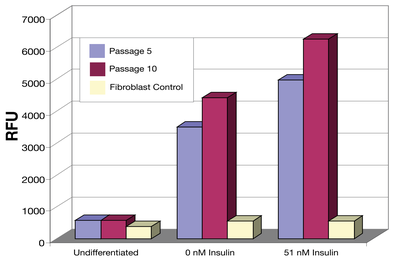


Figure 3: QBT (Molecular Devices) assay: Effect of differentiation and insulin stimulation on fatty acid uptake by different passages of XA15A1 (Passages 5-blue and 10-red). XA15A1 cells were plated in 96 well plates and, either differentiated (9 days in PDM medium and 3 days in PGM) or, left undifferentiated during the experiment. The differentiated cells were incubated in serum-free medium for 7 hours followed by stimulation with 51 nM of insulin for 40 min. The QBT assay was performed for 1 hour as recommended by the manufacturer (Molecular Devices). "Telomerized" human skin fibroblasts (yellow) were used as a negative control. Kinetic readings were performed using CytoFluor Multi-Well Plate Reader, Series 4000 (bottom read, gain 70, excitation 488/emission 536, 20 readings with 3 min intervals at 37°C). The example data shown is from the read taken at 30 min.

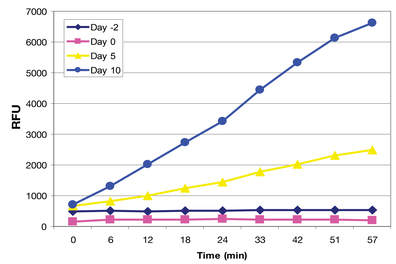


Figure 4: QBT assay time course: XA15A1 cells acquire the ability to uptake fatty acids during differentiation. Cells were plated in seven identical 96 well plates and grown to confluency at 33°C in PGM (QBT assay at Day –2). The cells were incubated at 37°C in PGM for 2 days (QBT assay at Day 0) and differentiated in PDM for 10 days (QBT assay at Days 5 and 10).

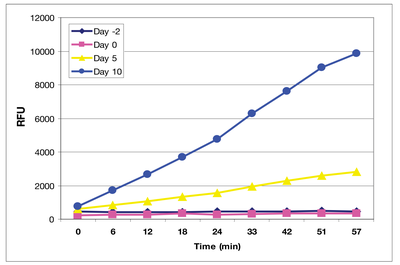


Figure 5: QBT assay time course: XA15A1 cells acquire insulin sensitivity during differentiation. Cells were plated, grown, differentiated, and analyzed as described in the figure above. Before assaying with QBT the cells were incubated in PGM for 12-24 hours, in serum-free medium for additional 6 hours, and stimulated with 20 nM of insulin for 45 min.

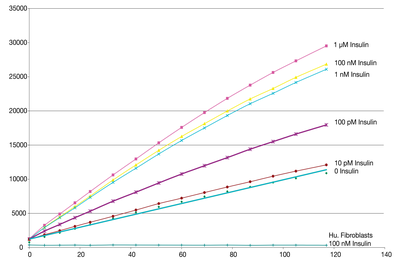


Figure 6: QBT assay: Insulin stimulation of fatty acid uptake by XA15A1. XA15A1 cells were plated in 96 well plates, grown and differentiated for 13 days (12 days in PDM and 1 day in PGM). Before the assay, cells were incubated in serum-free medium for 6 hours and treated with different concentrations of insulin for 40 min. The data for "telomerized" fibroblasts (negative control) incubated with 100 nM of insulin is presented as well.

Conclusions

- Immortalized cell lines offer the possibility of an inexhaustible supply of cells that can be used as models of animal or human tissues
- Cambrex overcomes the limitations of traditional immortalization procedures by using a temperature dependent, conditional immortalization approach. This utilizes large T-Antigen that allows the immortalization to be reversed and the cells to revert to their original characteristics.
- The homogeneous QBT Fatty Acid Uptake Assay Kit from Molecular Devices Corporation provides a fast, simple and reliable fluorescence-based assay for the detection of fatty acid uptake in cells containing fatty acid transporters.

The combination of immortalized preadipocytes and the QBT assay represents a unique and convenient HTS model closely resembling the properties of primary human cells.

CAMBREX