Respiratory Capacities and Differentiation of Novel Gluconeogenic Renal Cell Lines

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Background:

The renal proximal tubule relies on oxidative phosphorylation to carry out metabolically intensive transport. Cultured primary cells rapidly switch to a less differentiated glycolytic phenotype characteristic of most immortalized renal cell lines. Glucose starvation of non-gluconeogenic cell lines can induce and select for cells that have switched on gluconeogenesis as a survival mechanism, as glucose is essential for the production of ribose-5-phosphate, a precursor for nucleic acid biosynthesis. The selective pressure of glucose starvation not only induces gluconeogenesis, but the cells also exhibit other unique features that are characteristic of renal proximal tubular epithelial cells.

Methods:

LLC-PK1 cells were adapted to serum-free media and starved of glucose after the method of Gstraunthaler and Handler. Gluconeogenic colonies were isolated and expanded in glucose-free medium. Initial characterization of the mitochondrial oxidative capacity and glycolysis was carried out using a 96-well high throughput Seahorse Bioscience assay.

Results:

Numerous colonies of cells growing in glucose-free medium appeared after 3-4 weeks, and were sub-cloned and expanded. The phenotypic appearance of the colonies was quite variable, with many showing a pronounced differentiation compared to the parental cells. Cells typically formed compact, very dense monolayers, with extensive dome formation, even in very small colonies. Cell proliferation was observed primarily in peripheral cells, while contact inhibited cells rapidly differentiated. Initial characterization of cell lines found significant differences in oxygen consumption rates and glycolysis,
compared to parental control cells, with and without glucose.

Conclusions:

Gluconeogenic cell lines were induced from LLC-PK1 cells by culturing in glucose-free media. Several cell lines had a more differentiated appearance and enhanced oxygen consumption with reduced glycolysis, compared to parental cells. Preliminary results suggest that cell lines with enhanced renal function in vitro can be obtained via forced gluconeogenesis.