Inducible Gene Expression to Drive Cell Differentiation in In Vitro Renal Tubule Cells

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INTRODUCTION

- Scarcity of organ donations decreases treatment options for patients with chronic kidney disease
- Novel bioartificial kidneys present new solutions for organ failure and require using in vitro cell cultures
- Renal epithelial cells are specialized for reabsorption and secretion in the kidney
- In vitro proximal tubule epithelial cells have a de-differentiated transcriptional profile compared to in vivo counterparts
- Overexpression of LKB1 and STRADs improve differentiation in cultured intestinal epithelial cells
- Liver Kinase B1, LKB1, is a tumour suppressor gene that aids in cell metabolism and polarity
- STE-20 Related Adaptor Protein, STRAD, regulates the localization of LKB1

We aim to study how changing the expression of LKB1 and STRADs influences in vitro renal cell differentiation and their transcriptional profile.

METHODS

- Human renal proximal tubule epithelial cells (RPTECs) were cultured on standard 6-well polystyrene plates
- Cells were transfected with piggyBac (PB) transposase + Cadherin or Cad in vivo vector
- RPTECs were cultured in a 1:1 ratio of DMEM/F12 media with 5% glucose and supplemented with KGF, Insulin, Hydrocortisone, T3, 0.5% FBS, and ascorbic acid

RESULTS

Inducible Gene Expression and LKB1 Localization

Figure 4. Confirmation of gene expression induction of STRAD and LKB1 in cells transfected with piggyBac - Cad vector. A) STRAD expression significantly increased for three days post-transfection. LKB1 expression significantly induced in the presence of cumate amongst the first clone, LKB1-1. Expression of STRADs significantly increases in transfected Cad-LKB1 cells.

Genetic Variation and In Vivo–like Transcriptional Profiles Amongst Clones

Figure 6. Expression of proximal tubule biomarkers in transfected clones. A) Expression of N-Cadherin (abundant protein in proximal tubule cells in vivo) along the cell membrane in cultures B) Significant N-Cadherin presence despite cumate addition.

DISCUSSION

Conclusion

- RPTECs contain significant levels of transcriptional variability amongst clones
- The high genetic variability is NOT due to inducible STRADs or LKB1 gene expression
- The STRADs and LKB1 genes were significantly inducible with the addition of cumate

Future Directions

- Begin trying to isolate different clones from cell cultures and start new independent cultures for each clone and assess the genetic variability
- Test each culture and perform assays for water and ion transport
- Investigate other mechanistic pathways by which cell differentiation can occur
- Create new inducible vectors with other genes to test their impact on cell differentiation in vitro
- Explore more efficient transfection methods such as electroporation

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