The Roles of E-Cadherin and MET in Papillary Renal Cell Carcinoma

Camille Barnes, Emily Roloffs, and Dr. Brendan Looyenga, Calvin College, Grand Rapids, Michigan

Background

Papillary Renal Cell Carcinoma (PRCC) arises from epithelial cells in the proximal tubule of the kidney. PRCC has distinctive genetic expression compared to healthy tissue, such as a lack of E-cadherin and an overexpression of MET. E-cadherin is a tumor suppressor that is commonly lost in carcinomas through hypermethylation. This epithelial cadherin forms adherens junctions between adjacent cells and creates contact inhibition. MET is a receptor tyrosine kinase that binds growth factors causing the protein to dimerize, auto-phosphorylate, and activate cell proliferation pathways. In normal HK2 cells, active MET decreases and E-cadherin expression increases as the cells become confluent. In PRCC Caki2 cells, E-cadherin is never expressed and MET stays active as the cells become confluent.

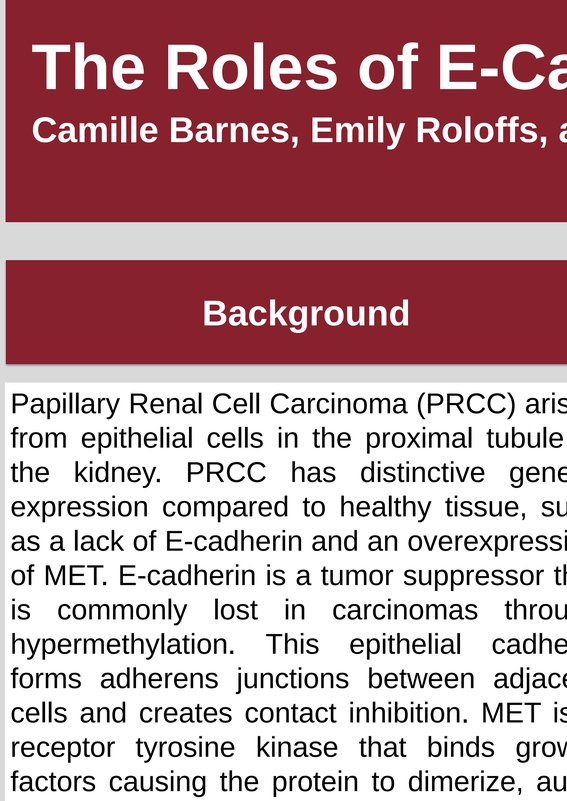

Figure 1: Immunoblot and quantitative data of HK2 and Caki2 cell lysates harvested in 24 hour increments.

Hypothesis

- Expressing E-cadherin in Caki2 cells will reduce the quantity of activated MET and decrease the rate cell proliferation.
- Knocking out E-cadherin expression in HK2 cells will increase the quantity of activated MET and the rate of cell proliferation.

Methods

Expression of E-Cadherin in Caki2 Cells in a TET-Inducible System

The E-cadherin gene or E-cadherin-GFP gene was inserted into pT2-TRE3G vectors along with the hygromycin b resistance gene. E-cadherin and E-cadherin-GFP had a TRE3G promotor that is only activated when doxycycline is present. The hygromycin b resistance gene was constitutively active. The vectors were inserted into the cells at various concentrations by transient transfection. Cells were transfected overnight before being treated with hygromycin b. Monoclonal colonies were selected and expanded. Lysates were harvested when cells became confluent and analyzed by immunoblot for E-cadherin expression.

Stable Deletion of E-Cadherin in HK2 Cells Using CRISPR/Cas9 Technology

Cells were transfected with CRISPR/Cas9 E-cadherin knock out plasmid and homologous directed repair plasmid that contained the puromycin resistance gene. Cells were transfected overnight before being treated with puromycin. Monoclonal cell lines were selected and expanded. Cell lysates were harvested when cells became confluent and analyzed by immunoblot for E-cadherin expression.

Results

Expression of E-Cadherin in 293FT Cells

- Caki2 cells were transformed with the E-cadherin gene but remained sensitive to hygromycin b, despite having the resistance gene. No Caki2 cells survived the selection process with hygromycin b, so an alternative cell line was chosen to confirm the success of transfection.
- Concentration-dependent expression of E-cadherin-GFP was successfully inserted into 239FT cells.

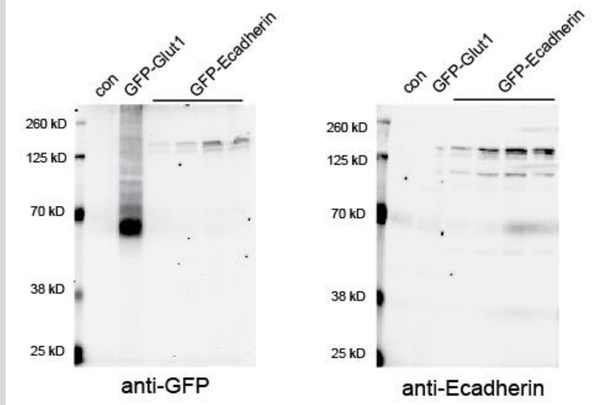

Figure 2: Immunoblot of 239FT cells transfected with E-cadherin-GFP.

Deletion of E-Cadherin in HK2 Cells

- Initial results indicate that the transfection with CRISPR/Cas9 E-cadherin knock out plasmid and homologous directed repair plasmid was successful.
- A majority of monoclonal lines had deletion of E-cadherin on only one of the chromosomes, and few lines had deletion on both chromosomes.

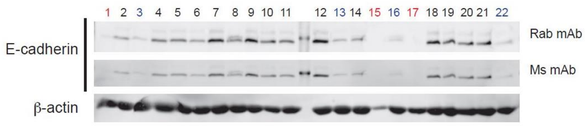

Figure 3: Immunoblot of monoclonal HK2 cell lines after transfection with CRISPR/Cas9 E-cadherin.

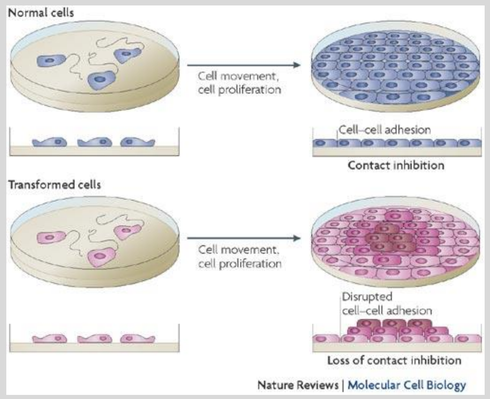

Figure 4: A depiction of contact inhibition and the loss of it (Adapted from Takai et al.)

Conclusions

- Caki2 cells are sensitive to hygromycin b, even with the resistance gene constantly expressed.
- Stable lines of HK2 cells with E-cadherin deletion can be created.

Future Research

- Determine the level of hygromycin b that Caki2 cells can tolerate or an alternative way to develop a stable line of Caki2 cells with E-cadherin expression.
- Validate the deletion of E-cadherin in HK2 cells through genomic testing and analyze the effects on MET expression.

References

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