Vesicle and Protein Trafficking in Renal Cancer

Andrew Folkerts, Patrick Jonker, Emily Rolof and Brendan Looyenga

Calvin College Department of Chemistry and Biochemistry

Introduction

This research focused on Type 1 papillary Renal Cell Carcinoma and a protein that is overexpressed in this type of cancer, called MET. MET is a receptor Tyrosine kinase that is phosphorylated and activated in the presence of its ligand Human Growth Factor (HGF). In its activated state MET phosphorylates and activates other proteins that mediate a growth response including STAT3, AKT, ERK1/2, and S6. Depending on where the activated MET is trafficked to in the cell however, different proteins will be activated and therefore a different growth response.

This research sought to elucidate the details of this activation and internal trafficking with special interest in the protein LRRK2 and if it had a role in MET trafficking.

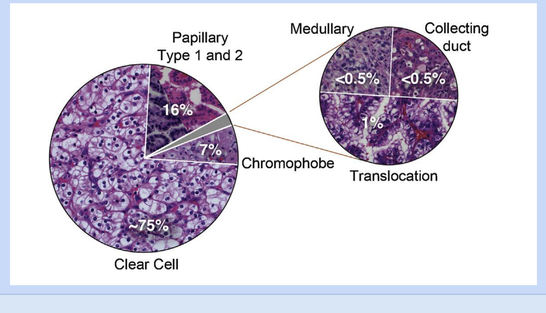

Figure 1. Shows the histology of the different types of renal cancer and their relative prevalence. Image taken from: Shuch B, et al. Understanding Pathologic Variants of Renal Cell Carcinoma: Distilling Therapeutic Opportunities from Biologic Complexity *European Association of Urology.* Jan 2015. 67: 85-97.

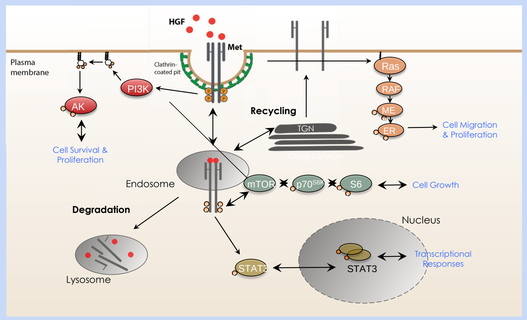

Figure 2. A cartoon of MET activation, which proteins it activates, where those proteins are activated, and the different trafficking options. *Adapted from* www.intechopen.com/source/html/19955/media/image3.png

Methods

Western Blots: This method makes use of protein that were collected after the cells are lysed. It was used for gross protein activation through phosphorylation and for quantification of overall protein. This also was able to give information about many proteins simultaneously. This data is shown in images of the gels.

Flow Cytometry: Maintains the structure of the cell and so is able to give information about if the protein is on the surface or if it is internalized. It is also able to give quantification of relative amount of proteins. This was mostly used for comparing surface and internal amounts of MET. This data is shown in the various other types of graphs.

Results

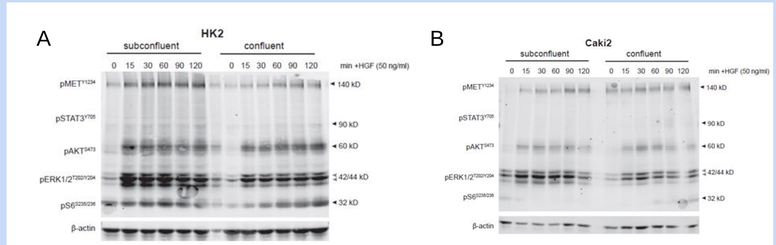

Figure 3. Western Blot data following time-couse stimulation with 50ng/mL HGF. Both cell types were grown until desired cell density and then exposed to serum free media for roughly 24 hours before the experiment. Serum free media was again added before the start of the experiment so all cells were exposed to fresh media for same amount of time. 3A: Normal kidney epithelial cells (HK2) show that cell to cell contact downregulates the sensitivity of MET to its ligand HGF. 3B: MET driven kidney cancer epithelial cells (Caki2) show little to no decrease in activation of MET with cell density. No matter how dense the cells are, it is still just as easy to activate the growth pathways that should be inhibited with confluency.

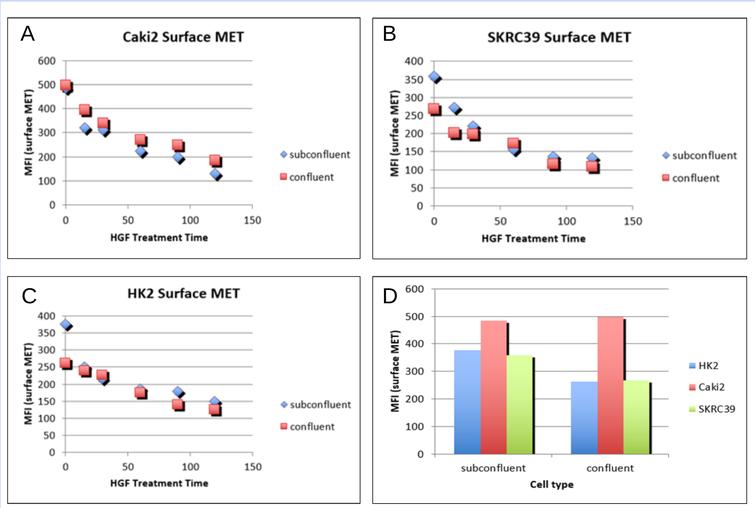

Figure 4: Serum starved confluent and subconfluent cells were given treatment of 50ng/mL HGF containing media for the designated time before being exposed to NaN3 containing trypsin. The internalization kinetics of surface MET is shown. Figures 4A and 4B show similar desensitization while 4C shows a faster internalization from its higher starting surface MET level.

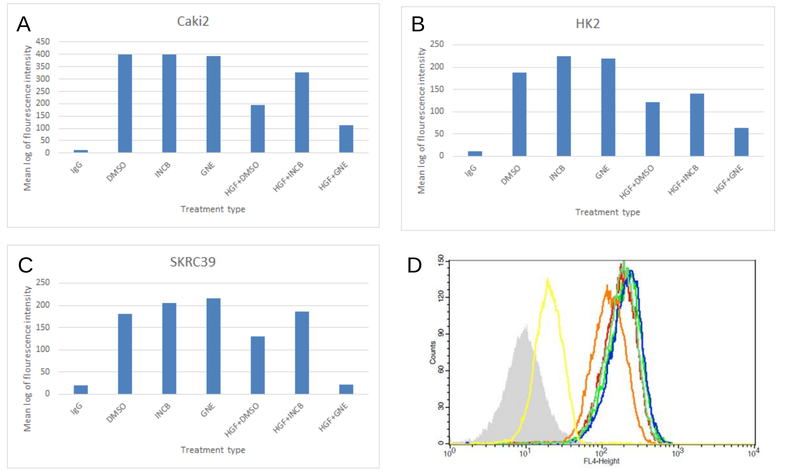

Figure 5. Cells were treated with either a MET inhibitor, INCB, a LRRK2 inhibitor, GNE, or a vehicle control, DMSO, for 1 hour and then with the same solution but containing 50 ng/mL HGF. Flow cytometry data showing a decrease of surface MET when treated with the LRRK2 inhibitor as opposed to a vehicle control or a MET inhibitor for all cell lines. This seems to suggest that LRRK2 does in fact recycle MET to the cell surface. Figure 5D shows the flow cytometry overlay of the SKRC39 data which is also presented in bar graph form to the right of it.

Conclusions & Future Directions

In the future we hope to generate validated LRRK2 KO cells to further test the data supporting that there is LRRK2 interacting with MET. We also plan to determine which organelles the MET is being trafficked using fluorescent microscopy.

References

This project was funded by the National Institutes of Health-National Cancer Institute (NCI) grant 1-R15-CA192094. We also acknowledge Calvin College Chemistry Department for lab space.

1. Albiges L, et al. MET is a potential target across all papillary renal cell carcinomas.*Clinical Cancer Research*. 1 July 2014. 20(13):3411-21. PMID: 24658158
2. Looyenga B, et al. Chromosomal amplification of leucine-rich repeat kinase-2 (LRRK2) is required for oncogenic MET signaling in papillary renal and thyroid carcinomas *Proceedings of the National Academy of Science.* 25 Jan 2011. 108(4): 1439-44. PMID: 21220347.
3. Shuch B, et al. Understanding Pathologic Variants of Renal Cell Carcinoma: Distilling Therapeutic Opportunities from Biologic Complexity *European Association of Urology.* Jan 2015. 67: 85-97.
4. Trusolino L, et at. MET signalling: principles and functions in development, organ regeneration and cancer *Nature.* Dec 2010. 11: 834-48.