

# ACTIVATION OF MEK1, A RAF-1 PATHWAY EFFECTOR, ALTERS MORPHOLOGY AND NEUROENDOCRINE PHENOTYPE IN MEDULLARY THYROID CANCER

Amal Alhefdhi, MD; Herbert Chen, MD, FACS; and Muthusamy Kunnimalaiyaan, PhD  
Department of Surgery, University of Wisconsin, Madison, WI

## Introduction

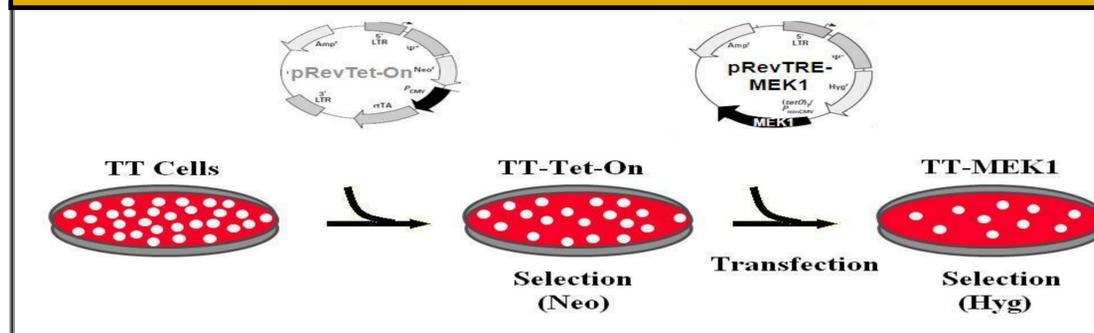
- Medullary thyroid cancer (MTC) is a prototypic neuroendocrine (NE) tumor of the thyroid C cells. Other than surgery, there are no curative therapies for MTC.
- Activation of raf-1 in MTC cells resulted in growth suppression and NE marker reduction. However, the exact mediator of this effect is not clearly understood. We hypothesize that MEK1, a key downstream target of raf-1 pathway, may be involved in the effect seen with raf-1 activation in MTC.
- To determine effect of MEK1, we established a doxycycline inducible MEK1 in TT cells and assess the effects on morphology and NE phenotype.

## Method

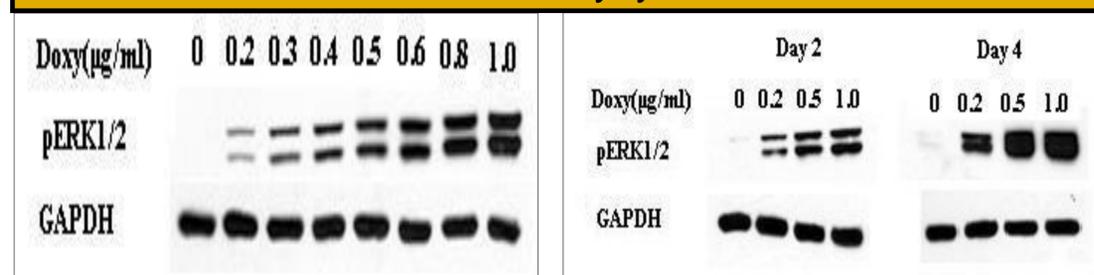
- Doxycycline inducible TT-MEK1 cells were created by stable transfection of pRevTRE-MEK1 plasmid in TT cells expressing Tet-on responsive protein. TT-MEK1 cells were treated with control, 0.2, 0.5, or 1.0  $\mu\text{g/ml}$  of doxycycline for up to 4 days.
- The level of MEK1 expression and its function was determined by western analysis using MEK1 and phosphorylated ERK1/2 antibodies. In addition, the lysates were analyzed for levels of NE markers achaete-scute complex-like 1 (ASCL1) and chromogranin A (CgA).
- Morphology of the treated and control cells were observed under a light microscope.

## Results

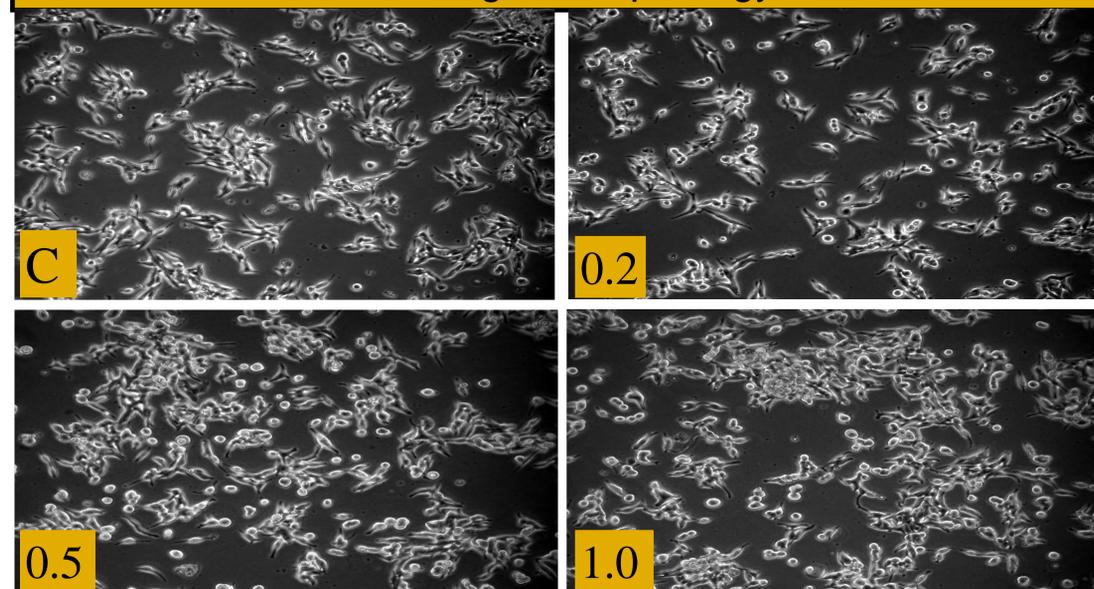
### Creation of doxycycline inducible TT-MEK1 Cells



### Dose-dependent activation of ERK1/2 is dependent on the amount of Doxycycline treatment

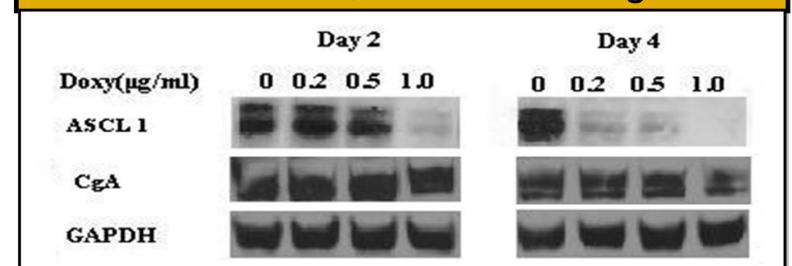


### MEK1 activation changes morphology of the TT cells



## Results Continued

### MEK1 activation reduces the levels of NE markers, ASCL1 and CgA



## Summary

- Treatment of TT-MEK1 cells with doxycycline led to an induction of MEK1 protein which led to an activation of ERK1/2 in a dose-dependent manner.
- Similar to raf-1 activation, there was a striking morphology change of rounding up cells with activation of MEK1.
- Importantly, the levels of NE markers were reduced with increasing amount of MEK1.

## Conclusion

- We demonstrate, for the first time, that the over expression of MEK1 in TT cells resulted in similar effects of raf-1 pathway activation. Thus, MEK1 may serve as a molecular target and activation of MEK1 could be a therapeutic strategy to treat patients with MTC.