

## B1

### **Activation of MEK1, A RAF-1 Pathway Effector, Alters Morphology and Neuroendocrine Phenotype in Medullary Thyroid Cancer**

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**Background:** 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 MTC-TT cells and assess the effects on morphology and NE phenotype.

**Methods:** Doxycycline inducible TT-MEK cells were created by stable transfection of pRevTRE-MEK plasmid in TT cells expressing Tet responsive protein. TT-MEK cells were treated with control or 0.2, 0.5, or 1.0 µg/ml of doxycycline for four 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), chromogranin A (CgA) and calcitonin. Morphology of the treated and control cells were observed under a light microscope.

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

**Conclusion:** We demonstrate, for the first time, that the over expression of MEK1 in MTC 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.