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Overexpression of MAML3 Increases Tumorigenicity and Invasion in Neuroendocrine Tumor Cells

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BACKGROUND: Metastatic transformation in neuroendocrine tumors (NETs) is not well understood. MAML3 is a transcriptional co-activator which binds intracellular NOTCH to activate gene transcription. High circulating MAML3 has been associated with metastatic disease in patients with small intestine NETs. Recurrent MAML3 fusion genes were found in pheochromocytomas associated with aggressive disease. The MAML3 fusion gene lacks exon 1 containing the NOTCH binding site, suggesting non-canonical activity of the protein. Therefore, we aimed to investigate the role of MAML3 in NET tumorigenesis.

METHODS: Three NET cell lines, SK-N-SH, QGP1, and BON1, were transiently transfected with either full-length MAML3 (FL) or exon 1 deleted MAML3 (dEx1), and we examined the biologic effects of overexpression using in vitro assays. All assays were performed in triplicate with at least three biological replicates. Statistical significance was determined by ANOVA.

RESULTS: Overexpression of FL and dEx1 MAML3 increased invasion compared with vector control (SK-N-SH: 53% ($p=0.0004$) and 25% ($p=0.0324$) increase with FL and dEx1, respectively. QGP1: 81% ($p< 0.0001$) and 50% ($p=0.0020$) increase; BON1: 38% ($p<0.0001$) and 35% ($p<0.0001$) increase), and increased soft agar colony formation (QGP1: 94% ($p< 0.0001$) and 68% ($p=0.0009$) increase with FL and dEx1, respectively; BON1: 60% ($p=0.0313$) and 100% ($p=0.0017$) increase). Transient transfection did not result in protein expression lasting long enough to test colony formation in SK-N-SH cells. Since biological effects were similar for both FL and dEx1, which lacks the NOTCH binding site, we hypothesized

that overexpressed FL and dEx1 MAML3 can act on non-canonical targets. We found increased TCF promoter activation assayed by luciferase activity with both MAML3 constructs, suggesting WNT signaling pathway activation. Co-immunoprecipitation confirmed interaction between MAML3 and β-catenin.

CONCLUSION: MAML3 overexpression is associated with increased tumorigenicity and invasion in NET cells. The mechanism of action is unknown, but may involve WNT signaling pathways leading to more aggressive/metastatic disease.