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MAML3 Overexpression Increases Tumorgenicity in Several Neuroendocrine Tumor Types

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BACKGROUND: The development of metastatic disease in neuroendocrine tumors (NETs) is not well-understood. MAML3 is a transcriptional co-activator. Recurrent MAML3 fusion genes are found in pheochromocytomas/paragangliomas (PPGLs) associated with aggressive disease. Increased MAML3 protein expression is associated with metastatic disease in small bowel NETs, and high mRNA expression is seen in a subset of small cell lung cancers (SCLCs). Therefore, we aimed to investigate the role of MAML3 in NET tumorigenesis.

METHODS: Human PPGLs were used for immunohistochemistry and genetic analysis. Four NET cell lines, neuroblastoma (SK-N-SH), PNET (QGP1 and BON1) and SCLC (DMS114), were transiently transfected with MAML3 (FL) or exon 1 deleted MAML3 (dEx1; mimicking the fusion), and biologic effects of overexpression were examined in vitro. All assays performed in triplicate with >3 biological replicates. Statistical significance determined by ANOVA.

RESULTS: Through RT-PCR, 7% (4/55) of human PPGL have UBTF~MAML3 fusions, similar to TCGA, and all were sporadic cases with metastatic disease. Fusion-positive PPGL had intense MAML3 nuclear staining and increased ß-catenin by IHC and showed increased WNT4 mRNA expression.

In vitro, overexpression of FL and dEx1 MAML3 increased invasion in SK-N-SH, QGP1, and BON1 (all $p<0.05$), and increased soft agar colony formation in QGP1 and BON1 (all $p<0.05$). In DMS114, only FL overexpression increased invasion ($p<0.01$) whereas both FL and dEx1 increased colony formation (all $p<0.01$). When investigating mechanism, we found increased TCF promoter activation by luciferase activity and co-immunoprecipitation between MAML3 and ß-catenin in SK-N-SH, QGP1 and BON1 cells, suggesting WNT signaling pathway activation. Interestingly, this mechanism was not seen in DMS114 and transcriptome analysis suggested different pathway activation.

CONCLUSION: MAML3 overexpression is associated with increased tumorigenicity in several different NET cells. The mechanism of action may involve WNT signaling pathways in neuroblastoma and PNET cells, leading to metastatic disease.

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