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Metastatic Pancreatic Neuroendocrine Tumors Have Decreased Somatostatin Expression and Increased Akt Signaling

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BACKGROUND: Patients with pancreatic neuroendocrine tumors (PNETs) often present with metastases, which reduce survival. Molecular features associated with PNET tumorigenesis have been reported, but mechanisms of metastasis remain incompletely understood.

METHODS: Gene expression was determined using RNA Sequencing (RNA-Seq) of primary and metastatic samples from 43 patients with grade 1 or 2 well-differentiated PNETs. Differentially expressed genes were identified by nonparametric Wilcoxon rank sum test and log2 fold changes estimated using the voom package in R. Quantitative PCR (qPCR) confirmed expression differences. BON cells were transfected with siRNA and shRNA to create knockdown cell lines. Knockdown was confirmed by qPCR and Immunofluorescence, and Western blots quantified protein expression.

RESULTS: Nodal and hepatic metastases had 4.2 and 35.0-fold lower expression of somatostatin (SST), respectively, compared to primary tumors ($p = 0.003$) by RNA-Seq. Validation by qPCR in an additional 12 patients confirmed 5.3-fold lower SST expression nodal and hepatic metastases compared to primary ($p = 0.043$). There was no difference in SST receptor, synaptophysin, or chromogranin A expression, suggesting metastases did not lose their neuroendocrine markers or differentiation. SST-knockdown cells had increased cell viability by alamarBlue assay and increased cell growth by manual counting. SST-knockdown cells had a 1.8-fold increase in phosphorylated Akt protein compared to controls ($p = 0.049$). SST-knockdown cells exhibited a trend towards increased phosphorylated mTOR and total mTOR compared to controls.

CONCLUSION: PNET metastases have lower expression of SST than primary tumors without loss of neuroendocrine markers of differentiation. SST knockdown increased cell viability and growth in PNET cell lines. SST knockdown was also associated with increased activation of Akt, suggesting SST as an upstream regulator of Akt. These findings identify upregulation of the Akt/mTOR pathway as a potential mechanism by which loss of SST expression promotes a metastatic phenotype.

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