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Oncogenic Signaling in the PNETs of Multiple Endocrine Neoplasia Type 1 is Epigenetically Regulated by the LncRNA Meg3

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BACKGROUND: Patients with multiple endocrine neoplasia type 1 (MEN1) syndrome exhibit MEN1 germline mutations and tissue-specific loss of the encoded tumor suppressor protein menin. Consequently, tumors develop in multiple endocrine organs - the pancreatic islets, the pituitary, and the parathyroids. Menin loss is known to cause promoter hypermethylation and downregulation of the long non-coding RNA (LncRNA) Maternally Expressed Gene 3 (MEG3). MEG3 loss in pancreatic neuroendocrine tumors (PNETs) corresponds with aberrant upregulation of the oncogenic HGF receptor c-MET. Meg3 overexpression in a mouse insulin-secreting PNET cell line, MIN6, downregulates c-Met expression. However, the molecular mechanism by which MEG3 regulates c-MET is unknown.

METHODS: We interrogated multiple mechanisms for the Meg3 regulated oncogenic c-Met signaling. Chromatin isolation by RNA purification and sequencing (ChIRP-Seq) was used to identify Meg3 genomic binding sites. RNA chromatin immunoprecipitation (RNA-ChIP) assays were used to validate Meg3 interaction with the epigenetic regulator Polycomb Repressive Complex 2 (PRC2). Effects on c-Met expression were assessed by MIN6 transfections with Meg3 isoforms, deletion constructs, and GA-GT rich Meg3 RNA sequences called triplex forming oligos (TFOs).

RESULTS: We provide the first direct evidence for inhibition of the highly abundant c-Met transcript in MIN6 cells by ectopic expression of alternatively spliced isoforms of Meg3. RNA ChIRP-Seq identified Meg3 binding to unique genomic regions, in and around the c-Met gene. In the absence of Meg3, these c-Met regions displayed distinctive enhancer-signature histone modifications. Meg3, acting in concert with PRC2 components, such as EZH2, epigenetically silenced c-Met expression. Additionally, Meg3 TFOs suppressed c-Met transcription in MIN6 cells, but enhanced proliferation.

CONCLUSION: Combined, these data offer mechanistic insight into MEG3 loss as an important epigenetic determinant of oncogenic c-Met expression in PNETs. These findings have clinical relevance for targeting c-MET in PNETs and the potential to ameliorate ß-cell loss in diabetes utilizing c-MET regulation for pancreatic islet ß-cell expansion.