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Hedgehog signaling drives glial cell plasticity and oncogenic reprogramming in gastroenteropancreatic neuroendocrine tumors

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BACKGROUND

Gastroenteropancreatic neuroendocrine tumors (GEP-NETs) represent heterogenous malignancies whose cellular origins remain poorly understood. *Men1*-driven reprogramming of neural crest-derived glial cells was recently implicated in GEP-NET development. In these studies, hyperactivation of the Sonic hedgehog (SHH) signaling pathway known to pattern neural crest cell fate coincided with the neuroendocrine phenotype in mice. Here, we investigated the hypothesis that loss of MENIN encoded by the *MEN1* gene promotes SHH-mediated reprogramming of enteric glial cells to acquire a neuroendocrine cell fate.

METHODS

Men1 was deleted in glial cells by expressing Cre recombinase downstream of the human glial fibrillary acidic protein promoter (*GFAP*$^{\Delta Men1}$). Hedgehog (HH) activation of *Men1*-deficient glial cells was blocked by deleting the gene encoding primary ciliary protein KIF3Arequired for transducing SHH signaling. The resulting *GFAP*$^{\Delta Men1}$ mice were evaluated for NET development and dysregulated hormone activity. Induction of HH signaling was confirmed in primary enteric glial cultures upon *Men1* silencing. Hyperactivation of SHH in human and mouse GEP-NETs was evaluated by immunofluorescent staining and western blot. Human and mouse tumoroids were treated with an agonist and inhibitors of HH signaling and evaluated for ERK/AKT activation, proliferation, and transcript fluctuations indicative of neural crest cell reprogramming.

RESULTS

GFAP$^{\Delta Men1}$ mice developed NETs in the pancreas, pituitary, and small intestine. Impaired SHH activation in GFAP^{+}/*Men1*$^{-/-}$ cells abolished the development of NETs and restored hormone levels to that of wild type mice. *Men1* silencing in enteric glial cultures stimulated HH signaling and upregulated the expression of neural progenitor and neuroendocrine transcripts coincident with downregulation of glial lineage genes. Human and mouse GEP-NETs overexpressed SHH and HH pathway components. Functionally, SHH treatment activated ERK/AKT signaling, cell proliferation, and the expression of neuroendocrine transcripts in GEP-NET tumoroids whereas pharmacological inhibition of HH signaling reversed these effects.

CONCLUSIONS

Our observations implicate neural crest-derived glial cells as potential neuroendocrine cell precursors that are susceptible to transformation through increased HH signaling upon loss of MENIN. These studies warrant future investigation into the delivery of Hedgehog inhibitors in the adjuvant setting for the treatment of GEP-NETs.

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