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Deletion of Notch1 Signaling in Pancreatic Neuroendocrine Tumors Reduces Metastatic Properties

Weisheng Chen, Rachael Guenter, Brendon Herring, Renata Jaskula-Sztul, J. Bart Rose, Herbert Chen.

Department of Surgery, University of Alabama at Birmingham, Birmingham, AL, USA.

BACKGROUND

The 5-year survival rate for patients with unresectable, metastatic pancreatic neuroendocrine tumors (pNETs) remains less than 30%, emphasizing the need for new and effective treatment options for patients with advanced pNETs. Notch1 signaling is a critical cell-cell communication pathway responsible for regulating differentiation, cell fate determination, and epithelial-mesenchymal transition (EMT). Notch1 plays a critical role in the differentiation state of NE cells and is aberrantly expressed in metastatic pNETs. We hypothesized that Notch1 signaling plays a role in pNET malignancy.

METHODS

To characterize the role of Notch1, we developed a Notch1-knockout pNET cell line. The resulting cell line was established by deleting Notch1 at exon 3 in BON cells using CRISPR/Cas9. Successful knockout of Notch1 was confirmed by Sanger sequencing and western blot analyses. To confirm the loss of functional Notch1, we measured changes of specific Notch1 downstream genes at both the transcriptional and translational level. Moreover, to have a global view of signaling pathways affected by Notch1, we performed RNAseq analyses comparing wildtype (N1-WT) and Notch1-knockout cells. Reads were aligned to the transcriptome using STAR and differentially expressed genes (DEG's) determined using DESeq2. Gene set enrichment analysis was then performed using the Hallmark gene sets. Cell migration was measured by a transwell migration assay.

RESULTS

Notch1 knockout was confirmed by a decrease in downstream Hes family genes, and an increase in Ascl1, a gene repressed by Notch. RNAseq results showed an increase in expression of NE differentiation markers in Notch1-knock cells, NeuroD1, which was also confirmed by RT-qPCR. Further, RNAseq analyses showed that when Notch1 is deleted, there was a significant decrease in EMT-related genes ($p = 0.015$). This finding was confirmed with RT-qPCR, whereby Notch1-knockout cells demonstrated a reduction in expression of *Snail* [two-sample $t(4)= 3.957$, $p = 0.017$] and *Slug* [two-sample $t(4)= 1.062$, $p = 0.348$] compared to N1-WT, two genes linked to cell migration and EMT. Finally, migration assays revealed that significantly fewer Notch1-knockout cells were able to migrate when compared to wildtype [two-sample $t(6)= 5.889$, $p = 0.001$].

CONCLUSIONS

Knockout of Notch1 signaling in pNET cells inhibits migration and reduces expression of EMT-related genes. Our data suggest Notch1 may confer a more aggressive phenotype in pNET cells by facilitating metastatic spread. Inhibiting Notch1 signaling may be an effective therapeutic strategy in advanced pNETs.

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