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Deletion of Notch1 inhibits pancreatic neuroendocrine tumor growth in vivo

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BACKGROUND

Pancreatic neuroendocrine tumors (pNETs) are uncommon neoplasms developing from islet cells in the pancreas. Current studies show that Notch1 plays an essential role in the development of pNETs. Notch1 signaling is a cell-cell communication pathway responsible for regulating cell growth, differentiation, and cell fate determination. We hypothesized that Notch1 signaling plays a significant role in pNET progression.

METHODS

We generated a Notch1 pNET cell line, BON-N1-KO, using CRISPR-Cas9 technology. Successful Notch1 knockout was confirmed by Sanger sequencing and Western blot analysis. To assess tumor growth *in vivo*, we subcutaneously implanted wildtype (WT) BO and BON-N1-KO into different groups of nude mice. The study included 12 mice (6 males and 6 females), with three replicates per group. Tumor sizes were measured twice weekly using calipers. When tumors reached a volume of approximately 180mm^3, the mice were euthanized. The tumors were then sectioned for Ki-67 immunohistochemistry staining. To obtain a comprehensive view of signaling pathways affected by Notch1 deletion, we performed proteomic analysis using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) followed with Ingenuity Pathway Analysis (IPA).

RESULTS

Deletion of Notch1 significantly inhibited tumor growth in both sex. At day 58, the average WT BON tumor size in female was 124.88 mm^3, compared to 20.23 mm^3 in BON-N1-KO ($p = 0.001$). In contrast, at day 36, the average WT BON tumor size in male was 132.02 mm^3, compared to 23.65 mm^3 in BON-N1-KO ($p = 0.04$). Ki-67 staining showed no significant difference between the BON and BON-N1-KO groups. Principal Component Analysis (PCA) of the proteomics data reveals distinct clustering between WT BON and BON-N1-KO groups. IPA identified the EIF2 signaling pathway as the most significantly affected by Notch1 deletion ($p = 7.34e-10$). Furthermore, molecular and cellular function analysis indicated significant differences between WT BON and BON-N1-KO groups in "cell death and survival" ($p = 9.93e-3 – 4.12e-11$) and "cellular growth and proliferation" ($p = 1.03e-2 – 2.06e-8$) processes.

CONCLUSIONS

Knockout of Notch1 signaling in pNET cells inhibit tumor growth *in vivo*. Our data suggest that this inhibitory effect highlighting the potential of Notch1 as a therapeutic target.

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