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Notch1 receptor-mediated metabolic flexibility promotes a survival advantage in pancreatic neuroendocrine neoplasms

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BACKGROUND

Cancer cells utilize both oxidative phosphorylation (OXPHOS) and glycolysis to generate energy. Switching between OXPHOS and glycolysis can promote tumor progression. The mechanisms governing oncogenic metabolic flexibility are largely unknown, but recent data has suggested that Notch1 dysregulation in cancer cells can contribute to altered metabolic phenotypes. We hypothesized that Notch1 signaling supports metabolic flexibility in pancreatic neuroendocrine tumor (pNET) cells.

METHODS

We established a Notch1-knockout (N1-KO) pNET cell line by deleting Notch1 at exon 3 in BON cells using CRISPR/Cas9. Seahorse Glycolytic Rate Assay and Mitochondria Stress Test were used to measure the glycolytic and mitochondrial activities of cells. A glycolysis deprivation assay was employed to determine cell viability at varying glucose concentrations. Single end RNAseq was performed using the Illumina NGS platform, to a read depth of 50M.

RESULTS

Compared to wild-type (WT) BON, the N1-KO cells had reduced basal oxygen consumption rate (28.2 ± 1.3 vs. 40.7 ± 1.4; p=0.02, ATP production (21.9 ± 1.0 vs. 29.6 ± 0.98; p=0.04), and maximal respiration (39.4 ± 1.7 vs. 63.7 ± 2.1; p=0.004). N1-KO cells also had a reduction in basal glycolysis, as measured by proton efflux rate, compared to WT (48.7 ± 2.6 vs. 57.7 ± 6.2; p=0.2). To test metabolic flexibility, WT and N1-KO cells were starved of glucose (0mM), compared to normal glucose (17.5mM) and viability was measured over time. By day 5, the N1-KO group had a viability of 0%, whereas 13% of WT cells were alive. To determine if Notch1 loss was associated with altered expression of established metabolic genes, we performed RNAseq on WT and N1-KO cells. Differential gene expression analysis found multiple OXPHOS-related genes (*UQCC2, COX15, COX20*) and glycolysis-related genes (*Slc1a1, Slc2a4, Hk1, Hk2*) were significantly down-regulated in N1-KO cells.

CONCLUSIONS

Our study shows that Notch1 signaling facilitates metabolic reprogramming as a survival advantage in pNET cells. Targeting Notch1 signaling to mediate cellular metabolism may be a novel therapeutic strategy in pNETs.

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