B-10

Notch1 and Notch3 signaling interplay regulates pancreatic neuroendocrine cell proliferation

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BACKGROUND

The 5-year survival rate for patients with advanced pancreatic neuroendocrine tumors (pNETs) is less than 30%. Notch signaling is a transmembrane receptor pathway with four distinct isoforms, activation of which is linked to cellular differentiation, cell fate, and viability. Both Notch1 and Notch3 signaling have been shown to be dysregulated in pNETs. Notch3 activation has been shown to repress Notch1 activity in other cancers. We hypothesize that activation of Notch3 can reduce Notch1-mediated proliferation in pNETs.

METHODS

Genetic deletion of Notch1 (N1-KO) in a pNET cell line (BON) was achieved using CRISPR/Cas9 to create a compound heterozygous knockout at exon 3. An inducible Notch1 overexpression cell line was generated by stably transfecting BON with a plasmid construct containing the Notch1 active form (N1ICD) under a Tet-On regulator. A constitutively on Notch3 cell line was generated by lentiviral transduction of BON with a plasmid overexpressing the Notch3 active form (N3ICD). Real-time quantitative PCR was used to measure mRNA transcript levels, and western blot was used to measure protein expression. Two-dimensional (2D) viability was measured by MTT assay, and three-dimensional (3D) proliferation was measured by spheroid size.

RESULTS

We found that deletion of Notch1 reduced the expression of Notch3 at both the mRNA and protein levels. Increased cell density (ligand availability) or treatment with valproic acid (known Notch1 inducer) lead to increased N3ICD in wild-type (WT) BON but not in N1-KO. Overexpression of N1ICD was associated with increased expression of known downstream target Hes1, as well as Notch3. In contrast, forced overexpression of N3ICD lead to increased Notch1 expression but not Hes1 when compared to a BON cell line transfected with an empty vector. Functionally, N1-KO and N3ICD overexpression cells both had reduced proliferation in both 2D and 3D cell culture.

CONCLUSIONS

The Notch1 and Notch3 receptors produce signaling cascades that regulate growth in pNET cells through distinct mechanisms. Reduction of Notch1 signaling suppresses growth, while the same effect can be achieved with Notch3 overexpression. Thus, specifically upregulating Notch3 may be a strategy to block Notch1-mediated proliferation in pNET cells.

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