B-9

5-Azacitidine Inhibits Neuroendocrine Tumors via the Induction of Notch3 by theTransactivator BORIS

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BACKGROUND: Induction of Notch3 signaling has been shown to inhibit neuroendocrine tumor (NET) growth. We have previously shown that DNA methyltransferase inhibitor 5-azacytidine (AZA) can effectively induce Notch3 in BON cells (NET cell line). Brother of the Regulator of Imprinted sites (BORIS) is a transactivator found in multiple cancers that is capable of binding unmethylated DNA and previously implicated in Notch3 transcriptional modulation. We hypothesize that the mechanism for AZA associated induction of Notch3 is via BORIS mediated epigenetic modulation.fore, we aimed to investigate the role of MAML3 in NET tumorigenesis.

METHODS: Luciferase reporter plasmids containing variable lengths of the Notch3 promoter region were created and co-transfected with beta-galactosidase into BON cells to map promoter region. Then, the role of BORIS induction and deletion were tested. BORIS was overexpressed in PLX304 vector and transfected with lipofectamine. BORIS gene was knocked down via siRNA transfection with scrambled siRNA as control. Transcript and protein expression were quantified by qRT-PCR and immunoblot respectively.

RESULTS: Luciferase signal was present with Notch3 promoter constructs ranging from full length to one containing 120bp upstream from the transcription start site, but lost after truncated below 109bp. In silico analysis and comparison to previously published data showed this region of the Notch3 promoter corresponded with a binding site for BORIS.

Transfection of BON cells with a BORIS overexpression system showed an increase in Notch3 protein expression and a corresponding 20% decrease in relative viability compared to an empty vector alone. BON cells treated with AZA showed Notch3 and BORIS expression levels increase in both message and protein. AZA induction of Notch3 could be reversed on the transcript level by transfection with siRNA against BORIS.

CONCLUSION: Modulation of the tumor suppressor Notch3 is possible through pharmacologic induction of BORIS with 5-azacytidine. Thus BORIS may serve as a novel target for NET pharmotherapy.

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