Inhibition of serotonin biosynthesis suppresses tumor growth *in vivo*

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Introduction

Small bowel neuroendocrine tumors (SBNETs) are a subgroup of NETs originating from enterochromaffin cells in the intestine. SBNETs express high level of Tryptophan Hydroxylase 1 (TPH1), a key enzyme involved in serotonin biosynthesis. High levels of serotonin cause carcinoid syndrome and potentially promote tumor progression. Current symptomatic treatment of carcinoid syndrome consists of using somatostatin analogs and the TPH1 inhibitor telotristat ethyl (TE) in severe cases. Although somatostatin analogs have been demonstrated to have anti-tumor effects in clinical trials, the effects of TE on tumor growth remains inconclusive. Several research groups have found that TE has no growth inhibitory effect on NET cells *in vitro* but one recent study showed inhibition of tumor growth in patients. To investigate this discrepancy, we studied the effect of TPH1 inhibition both *in vitro* and *in vivo* using genetic and pharmacologic approaches, and whether serotonin biosynthesis can be further suppressed using inhibitors targeting the nicotinamide dinucleotide (NAD) pathway.

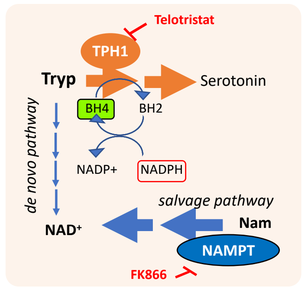

Figure 1. Serotonin and NAD biosynthetic pathways. Tryptophan is an essential amino acid that serves as a precursor for serotonin and NAD biosynthesis. The rate limiting enzyme of serotonin biosynthesis is Tryptophan Hydroxylase 1 (TPH1). TPH1 requires tetrahydrobiopterin (BH4) as a cofactor. NADPH is the electron donor that regenerates BH4. In addition to being synthesized from the de novo pathway, NAD can be generated from the salvage pathway and nicotinamide mononucleotide phosphotransferase (NAMPT) is the key enzyme involved. TPH1 can be inhibited by telotristat and NAMPT by FK688.

Methods

We generated stable TPH1 knockdown BON-1 cells using specific shRNAs, assessed their growth rate and angiogenesis potential in vitro and in vivo by measuring cell division, serotonin level, endothelial cell tube formation, tumor weight, and tumor vascularity staining. In addition, we performed similar experiments where we treated mice harboring BON-1 tumors with a vehicle control, TE, or TE with an NAD inhibitor (FK866).

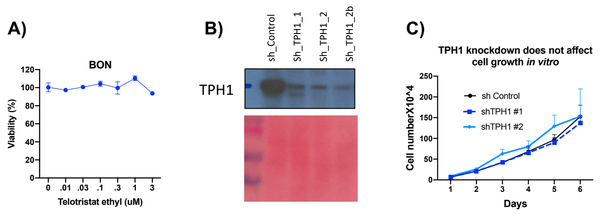

Figure 2. Characterization of BON cells with TPH1 inhibition. A) The effect of telotristat on BON cells in culture. B) Clones of BON cells expressing shRNAs against TPH1 showed decreasd expression of TPH1. C) TPH1 knockdown cells divides at similar rates as control BON cells.

Results

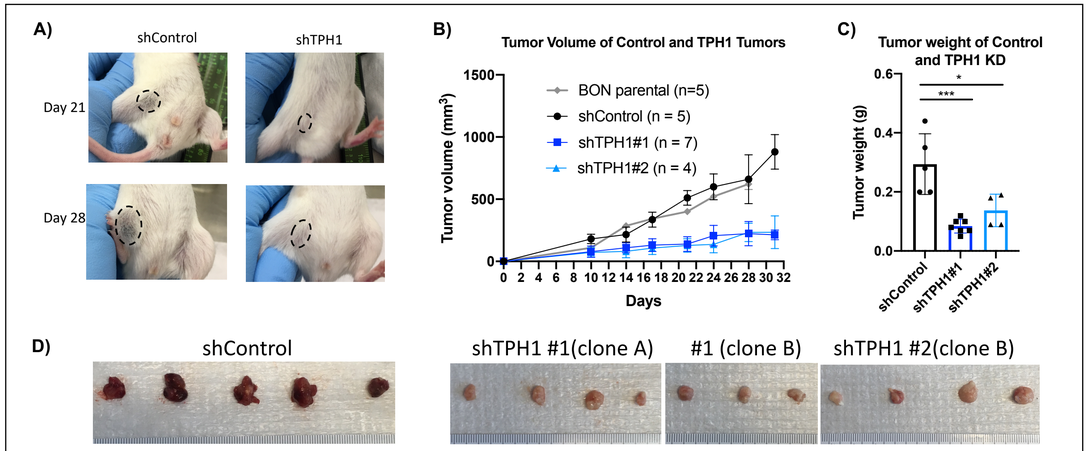

Figure 3. BON cells with TPH1 knockdown form smaller tumors *in vivo*. NSG mice were injected with 1 million BON cells in the flank. A) Xenograft model of BON cells expressing control shRNA (shControl) and shRNA targeting TPH1 (shTPH1). B) Tumor volume measurement with respect to time. C) Weight of xenograft tumors from BON cells expressing shControl and 2 different shRNA targeting TPH1 (shTPH1 #1 and shTPH1 #2). D) Picture of BON xenograft tumors.

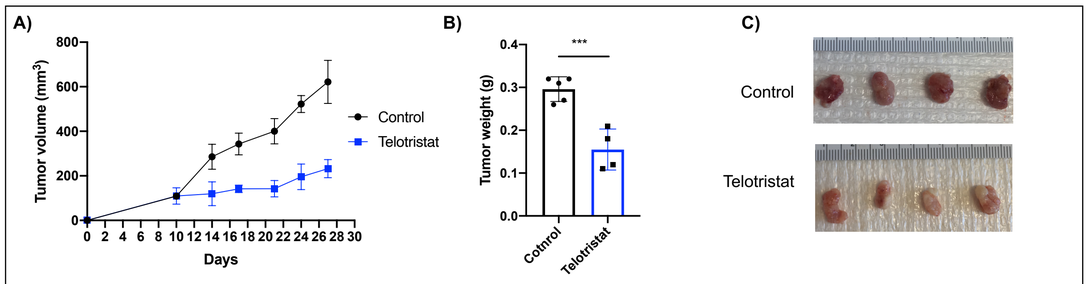

Figure 4. Inhibition of TPH1 with telotristat reduces tumor formation in a subcutaneous BON xenograft model. NSG mice were injected with 1 million BON cells in the flank. A) Tumor volume measurement with respect to time. Ten day post BON cell tumor implantation, 5 mice were given a vehicle control and 4 mice were given telotristat ethyl (TE) 3 times per week at 30mg/Kg by intraperitoneal injection. B) Weight of xenograft tumors from BON cells with and without TE treatment . C) Picture of BON xenograft tumors with and without TE treatment.

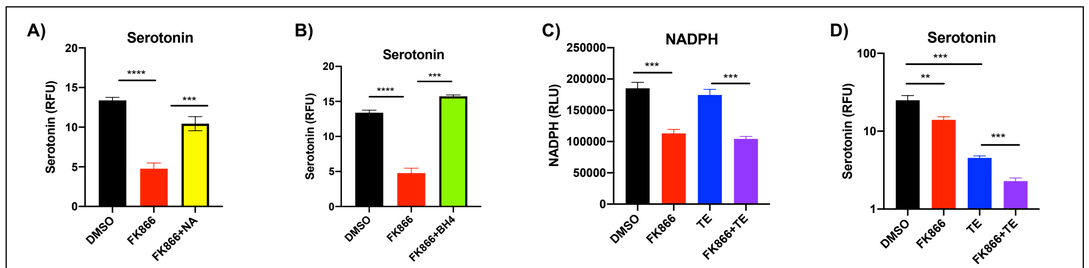

Figure 5. Modulation of serotonin and NAD metabolite levels by FK866 and TE. A) Serotonin level in BON cells treat with FK866 or FK866 in combination with nicotinic acid (NA). B) Serotonin level in BON cells treated with FK866 or FK866 with BH4. C) NADPH levels in BON cells treated with FK866, TE, or FK866+TE. D) Serotonin levels in BON cells treated with FK866, TE, or FK866+TE.

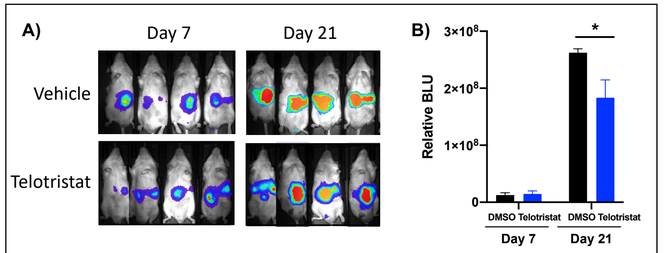

Figure 6. Telotristat reduces metastatic tumor burden. Intrasplenic injections of 1 million Luciferase-labelled BON cells per mouse. Mice with metastatic tumors were imaged each week. Data are represented in bioluminescent units (BLU).

Discussion / Conclusion

TPH1 knockdown cells and TE treated cells showed similar growth rate as control cells *in vitro*. However, TPH1 knockdown cells formed smaller tumors *in vivo* and tumors were less vascularized.

The combination of TE and FK866 additionally reduces serotonin biosynthesis and could result in an improved anti-tumor effect.

Pairing TE with NAD inhibitor represents an efficient strategy to metabolically target NETs.

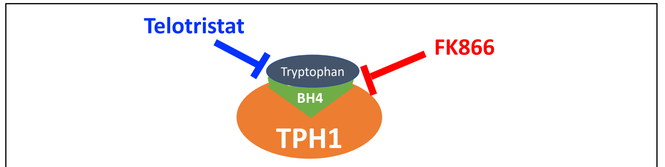

Figure 7. Targeting both serotonin and NAD pathway to maximize the inhibition of TPH1.

Ongoing / Future Studies

Ongoing *in vivo* studies to do determine the additive or synergistic effect of tumor inhibition using both TE and FK866.

Understand the mechanism of these drugs on angiogenesis *in vivo*.

References

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