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Investigating serotonin metabolism in neuroendocrine cancers

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BACKGROUND

Small bowel neuroendocrine tumors (SBNETs) originate from enterochromaffin cells in the intestine which synthesize and secrete serotonin. Other NETs and other cancers may also produce serotonin but do not store them in vesicles. The rate limiting enzyme of serotonin biosynthesis is tryptophan hydroxylase 1 (Tph1). Patients with high serotonin level could develop carcinoid syndrome, which can be treated with somatostatin analogues and the Tph1 inhibitor telotristat ethyl (TE) in severe cases. Little is known about the effect of serotonin on tumor cells during the dynamic process of neuroendocrine cancer growth. Here, we determined the effect of serotonin inhibition on tumor growth *in vitro* and *in vivo* using genetic and pharmacologic approaches. We identified improved tumor inhibition by combining TE with sunitinib, a tyrosine kinase inhibitor (TKI). In addition, we engineered a serotonin biosensor to track changes in serotonin levels in real-time.

METHODS

The levels of Tph1 in various cancer cell lines were determined. The biological effects of Tph1 inhibition using shRNAs targeting TPH1 stable knockdown and TE +/- sunitinib treatment were tested. Control and knockdown lines were assessed for their growth rates, angiogenesis potential, serotonin levels, endothelial cell tube formation, tumor weight, and tumor vascularity. To create a biosensor to detect endogenous serotonin levels in live cells, we fused the serotonin binding domain and *Renilla* luciferase reporter. Mass spectroscopy, immunofluorescence, and western blotting were used to study serotonin metabolism under different conditions.

RESULTS

TPH1 is highly expressed in SBNETs and several other cancer types. TPH1 knockdown cells and TE treated cells showed similar growth rates as control cells *in vitro*. However, TPH1 knockdown cells formed smaller tumors *in vivo* and tumors were less vascularized. The combination of TE and sunitinib led to a further decrease in tumor growth and lower serotonin levels in both tumor and blood samples. Moreover, we detected the dynamic changes in serotonin levels in tumor cells undergoing anchorage-independent growth and during serum starvation.

CONCLUSIONS

Although Tph1 inhibition with TE showed no effect on tumor cell growth *in vitro*, Tph1 inhibition reduced tumor formation *in vivo* and is potentiated in the presence of a TKI. Our serotonin biosensor enables real-time detection of alterations in serotonin synthesis in living cells under various growth conditions and has the potential to provide greater insight into serotonin metabolism in different stages of tumor progression and to identify therapeutic strategies to target cancer metastases and carcinoid crisis.

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