Thiocoraline activates the Notch pathway in carcinoids and reduces tumor progression in vivo

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Carcinoids are slow-growing neuroendocrine tumors (NETs) that are characterized by hormone overproduction; surgery is currently the only option for treatment. Activation of the Notch pathway has previously been shown to have a role in tumor suppression in NETs. The marine-derived thiodepsipeptide thiocoraline was investigated in vitro in two carcinoid cell lines (BON and H727). Carcinoid cells treated with nanomolar concentrations of thiocoraline resulted in a decrease in cell proliferation and an alteration of malignant phenotype evidenced by decrease of NET markers, achaete-scute complex like-1, chromogranin A and neurospecific enolase. Western blotting analysis demonstrated the activation of Notch1 on the protein level in BON cells. Additionally, thiocoraline activated downstream Notch targets HES1, HESS and HEY2. Thiocoraline effectively suppressed carcinoid cell growth by promoting cell cycle arrest in BON and H727 cells. An in vivo study demonstrated that thiocoraline, formulated with polymeric micelles, slowed carcinoid tumor progression. Thus the therapeutic potential of thiocoraline, which induced activation of the Notch pathway, in carcinoid tumors was demonstrated.

INTRODUCTION
Carcinoids are slow-growing neuroendocrine tumors (NETs) that are characterized by hormone overproduction.1–4 Carcinoids, which make up about 0.5% of all malignant tumors,5,6 are most commonly found in the small intestine but can also be present in the lungs, rectum, appendix and stomach, among other locations. Symptoms from carcinoids are often absent, but metastases can lead to carcinoid syndrome, which includes effects such as diarrhea, bronchospasm and right-sided valvular heart lesions.1–4 Treatment of carcinoids remains an ever-present issue as they are resistant to current therapeutics: the single agent chemotherapy response rate is only 20%.4 Responses to chemotherapy are typically short-lived and not correlated with prolonged survival. Consequently, surgery remains the only curative option for treatment. Therefore, further studies are necessary to find more viable options for the treatment of carcinoids.

Activation of Notch1 signaling in carcinoids has been shown to have a role in tumor suppression.7 Notch isoforms (1–4) are transmembrane receptors that are activated by proteolytic cleavage following ligand binding.8–10 The Notch intracellular domain translocates into the nucleus where it forms a complex with centromere binding factor 1 (CBF1) and other proteins to activate gene transcription (HES and HEY genes). The Notch1 signaling pathway is not active in carcinoid tumors.7 As a result of studies implicating Notch as a tumor suppressor in carcinoid tumors, therapeutic leads that activate this signaling pathway represent promising strategies for the treatment of carcinoids.

Thiocoraline was originally isolated from a marine Micromonospora sp.11,12 and has demonstrated potent cytotoxicity against lung, breast, colon, renal and melanoma cancer cells and in vivo efficacy against human carcinoma xenografts.13–14 Thiocoraline is a bisintercalator and does not damage DNA or inhibit topoisomerase II; however, it does inhibit DNA elongation by DNA polymerase α.13 More recently, we isolated thiocoraline and new analogs from a marine ascidian-derived Verrucosispora sp.15 We also demonstrated that thiocoraline altered the neuroendocrine phenotype and activated the Notch pathway in medullary thyroid cancer.16 Because of the need for additional therapeutic options for neuroendocrine cancers, we aimed to investigate the effect of thiocoraline on carcinoids.

In this study, we investigated thiocoraline’s effect on cell proliferation in human pancreatic carcinoid tumor cells (BON) and human bronchopulmonary carcinoid cells (H727). Additionally, we determined thiocoraline’s ability to alter the neuroendocrine phenotype of carcinoids. Moreover, we have shown that thiocoraline transcriptionally activates the Notch pathway. To better understand the mechanism of action of thiocoraline’s antiproliferative effects, we performed cell cycle analysis by flow cytometry and validated the protein expression of cell cycle markers by western blotting. Finally, a formulation for thiocoraline was developed to overcome solubility problems using nanoparticle polymeric micelles in order to improve the solubility for in vivo studies. Thiocoraline slowed the progression of carcinoid tumor growth in mice. Altogether, the results from this study provided evidence for the therapeutic potential of thiocoraline against carcinoid tumors.

MATERIALS AND METHODS
Cell culture
BON human pancreatic carcinoid tumor cells,17 and H727 human bronchopulmonary carcinoid cells (ATCC no. CRL-S815) (ATCC, Manassas, VA, USA) were maintained as previously described.18,19 The BON cell line was authenticated in May 2012 at the Genetica DNA Laboratories (Cincinnati, OH, USA).20 For the purpose of in vivo study, BON cells were
Thiocoraline

*Chondrilla caribensis f. caribensis* sponge specimens were collected in the Florida Keys on 10 February 2010 as previously described. Thiocoraline was isolated and purified from the marine bacterium *Verrucosispora sp.*, as previously described. Thiocoraline was dissolved in dimethyl sulfoxide (DMSO) and diluted in standard media to achieve the desired concentrations.

**Cell proliferation assay and IC50 determination**

Cell proliferation was measured via 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay as previously described. Cells were plated in quadruplicate in 24-well plates under standard conditions and allowed to attach overnight. The following day, cells were treated with thiocoraline (0–40 μM) and incubated for up to 8 days. Control cells (0 μM) received DMSO at 0.5% final concentration. Cell proliferation was assessed after 2, 4, 6 and 8 days. Following 2 days of thiocoraline treatment, the dose–effect curve was plotted to determine the IC50 value. The MTT assay was performed by replacing the standard media with 250 μl of serum-free RPMI 1640 containing 0.5 mg ml⁻¹ MTT and incubated for 3.5 h at 37 °C. After incubation, 750 μl of DMSO was added per well. Plates were shaken for 5 min to enhance dissolution. Absorbance at 540 nm was measured via a spectrophotometer (μQuant; Bio-Tek Instruments, Winooski, VT, USA).

![Figure 1. Thiocoraline inhibits BON and H727 cell proliferation in vitro. The IC50 was determined for BON (a) and H727 (b) cells treated with thiocoraline for 48 h using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Using the MTT assay, 2-day treatment of thiocoraline for BON (c) and H727 (d) cells over the course of 8 days demonstrated a decrease in cell proliferation at concentrations as low as 20 nM. Experiments were done in quadruplicate, and data are plotted as mean ± s.e.m. ***P < 0.001.](image-url)

**Flow cytometry**

To analyze the cell cycle progression of BON and H727 cells, the DNA content was quantified via flow cytometry. BON and H727 cells were treated for 2 days with thiocoraline (0–40 nM). After treatment, cells were washed with cold 1× phosphate-buffered saline (PBS) pH 7.2 (Life Technologies, Carlsbad, CA, USA) and harvested with trypsin (Life Technologies) to enhance dissociation. Cells were then centrifuged at 1200 g.m. at 4 °C and washed twice with cold 1× PBS before fixation with cold 70% ethanol and kept at –20 °C before staining. Prior to staining, cells were again washed twice with cold 1× PBS with centrifugation after each wash. The pellet was suspended in a propidium iodide (PI) staining solution containing 20 mg ml⁻¹ RNase-A (Sigma-Aldrich, St Louis, MO, USA) and 330 μg ml⁻¹ propidium iodide was dissolved in 1× PBS. Cells were stained in the dark overnight at 4 °C. Samples were filtered prior to analysis. Fluorescence-activated cell sorting analysis was performed on a flow cytometer at 488 nm (FACSCalibur flow cytometer; BD Biosciences, San Jose, CA, USA), and results were analyzed with the ModFit LT 3.2 software (Verity, Topsham, ME, USA).

**Western blotting analysis**

**Cell lyses.** BON and H727 cells were treated for 2 days with thiocoraline (0–40 nM), and protein extracts were harvested and quantified as previously described. Denatured cellular extracts (30–40 μg) were subjected to gel electrophoresis on 7.5% or 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Invitrogen), transferred onto nitrocellulose membranes (Bio-Rad Laboratories, Hercules, CA, USA) and blocked in milk solution. Membranes were incubated overnight at 4 °C with the appropriate primary antibody. The following primary antibodies were used at the following concentrations: anti-NOTCH1 (1:2000); anti-MASH1 (mammalian ASH1) to detect ASCL-1 (1:2000; Pharmingen, San Diego, CA, USA); anti-chromogranin A (anti-Cga; 1:1000; Zymed Laboratories Inc., San Francisco, CA, USA); anti-p21 (1:2000); anti-p27 (1:2000); anti-cyclin B1 (1:1000), anti-cyclin D1 (1:1000), anti-neurospecific enolase (NSE; 1:2000), anti-glyceraldehyde-3 phosphate dehydrogenase (GAPDH; 1:10,000; Cell lysates. BON and H727 cells were treated for 2 days with thiocoraline (0–40 nM), and protein extracts were harvested and quantified as previously described. Denatured cellular extracts (30–40 μg) were subjected to gel electrophoresis on 7.5% or 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Invitro...
Trevigen Inc., Gaithersburg, MD, USA), and vinculin (1:2000; Cell Signaling, Danvers, MA, USA). Following primary antibody incubation, membranes were washed as previously described and incubated with the secondary antibody. The following secondary antibodies at the indicated dilutions were used: goat anti-rabbit (Notch1 1:4000, Cyclin D1 1:2000, p27 1:6000, CgA 1:4000, GAPDH 1:3000); and goat anti-mouse (Cyclin B1 1:3000, p21 1:6000, ASCL-1 1:5000, NSE 1:6000 and vinculin 1:2000). Following secondary antibody incubation, the membranes were washed as previously described. Proteins were visualized using SuperSignal West Femto, West Dura, West Pico (Pierce, Rockford, IL, USA) or Immunstar (Bio-Rad Laboratories) chemiluminescent substrate according to the manufacturers’ directions. The detection of GAPDH was used as a loading control.

Tumor extracts. Tumor tissue (2 mm³) was pulverized in the Cryoprep tissue homogenizer (Covaris, Woburn, MA, USA), and the tissue powder was used for protein lysate preparation as described previously. Brieﬂy, the tissue powder was dissolved in 500 µl of lysis buffer containing 50 mmol l⁻¹ Tris, pH 7.5, 150 mmol l⁻¹ NaCl, 1% Igepal CA-630, 0.1% sodium dodecyl sulfate, 0.1 µmol l⁻¹ phenylmethylsulfonyl fluoride, 5 mmol l⁻¹ ethylene diaminetetraacetic acid and 12 µl ml⁻¹ protease inhibitor cocktail (Sigma-Aldrich); incubated on ice for 45 min; and centrifuged at 13 000 r.p.m. for 30 min at 4 °C. The supernatants were collected, and the protein concentration was determined by the bicinchoninic acid protein assay kit (Pierce). Western blotting analysis for Notch1 expression was performed as described above.

Quantitative real-time PCR (qRT-PCR)

Following 2-day thiocoraline treatment, RNA was isolated using the RNeasy Mini-kit (Qiagen, Valencia, CA, USA) and reverse transcribed with the iScript cDNA synthesis kit (Bio-Rad). qRT-PCR was performed by the iCycler IQ detection system (Bio-Rad). A 25-µl volume reaction mixture containing 2 µl cDNA sample (200 ng µl⁻¹), 200 nM forward and reverse primers and 12.5 µl SYBR Green Supermix (Bio-Rad) was used. The following PCR forward and reverse primer pairs were used: Notch1 (5′-GTCAACGCCG TAGATGACCT-3′ and 5′-TTGTTAGCCCCGTTCTTCAG-3′), HES1 (5′-TTGGAGCTTCCAGGTGGTA-3′ and 5′-GGCCCCGTGGGAATG-3′), HES2 (5′-CTGACCTCCGATT-3′ and 5′-TTGGACGAGTGCTTCT-3′), HES5 (5′-ACCCATCAACAGCATT-3′ and 5′-AGGCTTTGCTGTGCTTCAG-3′), and s27 (5′-TCTTTAGCCATGCACAAACG-3′ and 5′-TTTCAAGTGCTTCCCTCCT-3′), as a loading control. The RT-PCR reactions were performed in duplicate under previously described conditions. Results were normalized to s27 mRNA levels, and the expression was plotted as average ± s.e.m.

Figure 2. Thiocoraline suppresses cell proliferation through cell cycle arrest. Western blotting analysis of cell cycle markers of BON (a) and H727 (b) cells treated with thiocoraline demonstrated cell cycle arrest. Equal loading was confirmed with glyceraldehyde-3 phosphate dehydrogenase (GAPDH). Flow cytometry analysis of BON (c, e) and H727 (d, f) cells treated with thiocoraline confirmed cell cycle arrest in G2/M.
Luciferase reporter assay
Notch1 functional activity was measured, by the degree of CBF1-binding, utilizing a luciferase construct containing four CBF1-binding sites (4 × CBF1-Luc). BON cells were transiently transfected with CBF1-luciferase reporter construct and then treated with 0 or 30 nM of thiocoraline for 48 h. To normalize for transfection efficiency, 0.5 μg of cytomegalovirus β-galactosidase (CMV-β-gal) was cotransfected as previously described.22

Preparation of thiocoraline-incorporated polymeric micelle
Poly(ethylene glycol)-block-polylol, l-lactide) (PEG-b-PLA; 7.4k-b-2.3k) was purchased from Advanced Polymer Materials Inc. (Montreal, Quebec, Canada). The solvent evaporation method was used for the preparation of thiocoraline-loaded PEG-b-PLA micelles.25 Briefly, 1, 3 or 5 mg of thiocoraline and 10, 30 or 50 mg of the polymer were completely dissolved in acetone and transferred into round bottom flasks. Acetone was evaporated under low pressure by rotary evaporation in a 60 °C water bath until a clear thin-layered film was formed. This film was rehydrated by adding 1 ml of 0.9% sodium chloride solution in 60 °C water bath. The rehydrated thiocoraline-incorporated PEG-b-PLA solution was centrifuged for 5 min at 10,000 g and filtered with 0.22-μm regenerated cellulose filter to remove unloaded thiocoraline to obtain a sterilized polymeric micelle solution. Thiocoraline acquired aqueous solubility of 4.23 ± 1.15 mg ml⁻¹ in water by forming polymeric micelles with PEG-b-PLA. Thiocoraline encapsulated in polymeric micelle was detected and quantified by UV Cary 100 Bio UV-visible spectrophotometer (Varian, Palo Alto, CA, USA) at 360 nm. Particle size distributions of PEG-b-PLA micelles containing thiocoraline were determined by dynamic light scattering measurement, using a Zetasizer Nano-ZS (Malvern Instruments, Worcestershire, UK).

Maximum tolerable dose (MTD) study
Female 6–8-week-old babo/c mice were purchased from the National Cancer Institute (Rockville, MD, USA) and randomized into six groups. Empty vehicle, 5, 10, 20, 30 and 40 mg kg⁻¹ thiocoraline-incorporated PEG-b-PLA micelles were intravenously injected to mice, and animal body weight changes were monitored for 8 consecutive days. Institutional guidelines were followed for maintenance of animals and end point of animal studies.

Xenograft study
Four-week-old male athymic nude mice were obtained from Charles River Laboratories (Wilmington, MD, USA). Before beginning the experiment, the mice were allowed to acclimate 1 week in the animal facility to reduce stress after arrival. Mice were maintained under specific pathogen-free conditions. BON cells stably transfected with the vector encoding the luciferase reporter gene luc2 were subcutaneously inoculated into the left flank of mouse (5 × 10⁸ cells/animal) in 100 μl of Hanks Balanced Salt Solution (Mediatech, Inc., Manassas, VA, USA). Fifteen mice after inoculation, mice with palpable tumors were randomized into two groups (n = 6) and intravenously (i.v.) injected with 5 mg kg⁻¹ body weight thiocoraline-incorporated micelles and vehicle (empty PEG-b-PLA micelles), respectively. The treatment was repeated at days 22 and 29. Tumor volumes were measured from day 15 by external caliper every 4 days and then were calculated by the modified ellipsoidal formula: Tumor volume = ½ (length × width²). Additionally, mice were imaged weekly using a cooled CCD camera (Xenogen IVIS, Caliper Life Science, Hopkinton, MA, USA) to validate the dynamics of tumor growth. In brief, 250 μl (3.75 mg) of substrate d-Luciferin (Caliper Life Science) in PBS was injected i.p. in each mouse 12 min prior to the whole-body imaging. The image acquisition and tumor size analysis based on the total photon counts of bioluminescence were done by using the Live Imaging software (Caliper Life Science). At the end of the experiment, mice were killed, and the tumors were dissected from the surrounding tissues and flash frozen in liquid nitrogen for storage in −80 °C. Postmortem examination of the lungs, liver, kidneys and spleen were performed to confirm that there was no evidence of metastases or tumor growth outside of the inoculation site. All experimental procedures were performed in compliance with our animal care protocol approved by the University of Wisconsin-Madison Research Animal Resources Committee in accordance with the NIH Guideline for the Care and Use of Laboratory Mice.

Statistical analysis
Statistical analyses were performed using a one-way analysis of variance and the Kruskal–Wallis rank sum test or by repeated measures analysis of variance. A value of P ≤ 0.05 was considered statistically significant.

RESULTS
Thiocoraline inhibits BON and H727 cell proliferation in vitro
Thiocoraline has previously been shown to decrease cell proliferation in medullary thyroid cancer-TT cells at nanomolar concentrations,16 and consequently, the survival of BON and H727 cells treated with thiocoraline was investigated. Thiocoraline exhibited low nanomolar potency against BON and H727 cells (Figure 1a and b). Additionally, cell survival over the course of 8 days was monitored after treatment with thiocoraline (0–40 nM; Figures 1c and d). For both BON and H727 cells, treatment of thiocoraline at concentrations between 20–40 nM resulted in a steady decrease in cell proliferation over the course of 8 days. Treatment of thiocoraline at 5 and 10 nM resulted in a decrease in cell proliferation through 6 days of treatment in H727 cells.

Thiocoraline induces cell cycle arrest in BON and H727 cells
After determining the ability of thiocoraline to reduce cell proliferation in BON and H727 cells, the mechanism of action of thiocoraline was investigated by western blotting and flow cytometry (Figure 2). Cell cycle marker proteins (p21, p27, cyclin B1 and cyclin D1) were monitored by western blotting and suggested arrest in the G2/M phase induced by thiocoraline in BON and H727 cells. An increase in the expression of p21, a cyclin-dependent kinase inhibitor that promotes cell cycle suppression, was demonstrated by western blotting after treatment of BON and H727 cells with thiocoraline. Additionally, flow cytometry analyses

Figure 3. Thiocoraline reduces neuroendocrine tumor markers in vitro. Western blotting analysis of BON (a) and H727 (b) cells treated with thiocoraline demonstrated a dose-dependent decrease of neuroendocrine tumor markers expression: achaete-scute complex like-1 (ASCL-1), chromogranin A (CgA), and neurospecific enolase (NSE). Equal loading was confirmed with glyceraldehyde-3 phosphate dehydrogenase (GAPDH).
of BON and H727 cells treated with thiocoraline (0–40 nM) suggested that thiocoraline causes cell cycle arrest in the G2/M phase. An increase in cell population in the G2/M phase with concomitant decrease in cell population in the G1 phase was displayed with increasing concentrations of thiocoraline in the BON and H727 cell lines. The percentage of cells in the S phase showed variable change in both cell lines.

Thiocoraline decreases NET markers in vitro

ASCL-1, CgA and NSE have been characterized as markers of NETs.26,27 Treatment of thiocoraline (0–40 nM) in BON and H727 cells effectively changed their NE phenotype and resulted in a dose-dependent decrease in the expression of ASCL-1, CgA and NSE as demonstrated by western blotting analysis (Figure 3).

Thiocoraline induces the expression of Notch isoforms in BON cells

After demonstrating the ability of thiocoraline to cause cell cycle arrest in BON and H727 cells, western blotting analysis and qRT-PCR were used to further investigate thiocoraline’s mechanism of action. Clinical studies have shown that clinical efficacy was correlated with activation of the Notch pathway in NETs;28,29 therefore, Notch activation was investigated. The expression of Notch1 was monitored by western blotting and qRT-PCR in BON cells treated with thiocoraline (0–40 nM). A dose-dependent increase of Notch1 expression in BON cells was apparent by western blotting analysis and qRT-PCR (Figure 4). qRT-PCR showed a fourfold increase in the expression of Notch1 between the control (0 nM) and treatment with 40 nM thiocoraline. H727 cells treated with thiocoraline did not demonstrate a statistically significant increase in the expression of Notch1 at the protein or mRNA level. To further determine whether thiocoraline
functionally activated the Notch pathway, a luciferase reporter assay incorporating four CBF1-binding sites was used. Thiocoraline treatment of BON cells resulted in a nearly threefold induction of luciferase activity (Figure 4c), indicating that this increase was caused by Notch activation followed by Notch–CBF1 binding. Additionally, activation of downstream targets of Notch was apparent by qRT-PCR. A dose-dependent increase in the mRNA levels of HES1, HES5 and HEY2 was detected in BON cells treated with thiocoraline. These results suggest that thiocoraline functionally activates Notch signaling in carcinoids.

Thiocoraline reduced tumor progression in vivo
After investigating thiocoraline’s in vitro effect against carcinoid cell lines, an in vivo study was pursued. Thiocoraline has poor solubility in DMSO and aqueous solutions, which initially hindered in vivo experiments, but a method for formulation of thiocoraline with polymeric micelles (PEG-b-PLA) increased the aqueous solubility to 4.23 ± 1.15 mg ml⁻¹. This formulation enabled an MTD study in mice; the MTD was determined to be 7 mg kg⁻¹. There was 100% survival of mice, and no loss in body weight was observed at this dose.

After determining the MTD, an in vivo study investigated the therapeutic potential of thiocoraline. Mice were inoculated with BON cells stably transfected with luciferase-expressing plasmid luc2, and tumors were allowed to grow for 14 days. Thiocoraline formulated in polymeric micelles was dosed to six mice on days 15, 22 and 29 at 5 mg kg⁻¹. Likewise, empty micelles were dosed to six additional mice at the same time points. Thiocoraline slowed the progression of tumor growth compared with the vehicle control (Figure 5). There was a 62.6% reduction in tumor volume between mice treated with thiocoraline and vehicle. Moreover, validation of tumor growth by bioluminescence imaging (73.4% reduction in tumor growth) confirmed that thiocoraline formulated in polymeric micelles has significant antitumor activity. Western blotting analysis of proteins from the tumor tissue and qRT-PCR demonstrated increased expression of Notch1 in only mice treated with thiocoraline.

**DISCUSSION**
Activation of the Notch signaling pathway has been demonstrated to have a role in NET suppression. The Notch signaling pathway is not present in carcinoid tumors; therefore, small molecules that
can activate this signaling pathway represent promising strategies for the treatment of carcinoids. Several of these Notch activators, such as valproic acid, have made it to clinical trials.28 Despite the potential for these therapeutic leads, the only effective treatment for carcinoid cancer is surgery, and therefore, there is a need for additional therapeutic options. This study demonstrated that thiocoraline causes a decrease in cell proliferation in BON and H727 cells, acting by cell cycle arrest. Additionally, a decrease in NET markers and an increase in Notch1 protein and mRNA levels suggested that thiocoraline functionally activates the Notch pathway in BON cells. In general, these results parallel recent work investigating the potential for thiocoraline as a treatment for medullary thyroid cancer.16

Although this study demonstrated that thiocoraline activates the Notch pathway in carcinoids, these results do not examine thiocoraline’s effects on other pathways that may also contribute to the antiproliferative effects. The phosphatidylinositol 3’ kinase/Ark pathway and mitogen-activated protein kinase pathway have demonstrated a role in the suppression of NETs.2,18 Future work could entail the use of siRNA to block Notch to determine whether thiocoraline’s antiproliferative effects are a result of activation of the Notch pathway.

The ability of thiocoraline to activate Notch signaling and cause a decrease in cell proliferation in carcinoids is a promising step toward finding alternative forms of treatment for NETs. Importantly, the in vivo study presented here demonstrated that thiocoraline can slow progression of carcinoid tumors. Although Faircloth et al.29 demonstrated that thiocoraline had in vivo antitumor activity, limited in vivo studies have been reported for thiocoraline. Therefore, the in vivo study presented here is significant, but additional work is necessary in order to develop thiocoraline into a viable drug lead.

This study complements other recent work on thiocoraline as a potential therapeutic. Considerable progress has been made for the total synthesis of thiocoraline, which could make it more amenable to pharmaceutical development. Thiocoraline was originally synthesized by Boger et al.,30,31 and work has been done more recently by Albericio and co-workers to complete an efficient solid-phase synthesis of thiocoraline using enzyme-labile protecting groups.32 Additionally, the biosynthesis of thiocoraline has been studied,33–37 which could help provide more potent analogs. Another issue for thiocoraline is its poor aqueous solubility, which hinders its ability to be delivered in vivo. Our formulation using polymeric micelles helps alleviate this problem. Additionally, recent work has been done with liposomes to improve the ability to deliver thiocoraline.38,39 Overall, considerable work is still necessary, but many of the pieces are in place for thiocoraline as a potential therapeutic.

Thus thiocoraline activates the Notch signaling pathway and reduces cell proliferation in carcinoids. Treatment of BON and H727 cells with thiocoraline at nanomolar concentrations resulted in a decrease in NET markers (ASCL-1, CgA and NSE). Cell cycle analyses by flow cytometry and western blotting demonstrated arrest in the G2/M phase induced by thiocoraline in BON and H727 cells. A dose-dependent increase of Notch1 on the protein and mRNA levels in BON cells treated with thiocoraline, as well as an increase of downstream targets of Notch, points towards the tumor-suppression role of thiocoraline in carcinoids. Importantly, thiocoraline slowed carcinoid tumor progression in vivo. Although further work is necessary to better understand thiocoraline’s mechanism of action, these results suggest that thiocoraline could be a potential treatment for NETs.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

R Jaska-Sztul designed the experiments. H Cho designed the thiocoraline formulation. T Wyche, A Dammalapati, H Cho, Aarrison and R Jaska-Sztul performed the experiments. T Wyche wrote the manuscript with contributions from R Jaska-Sztul and reviewed by T Bugni, G Kwon and H Chen.

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