

Original Article

Repurposing the bitter taste receptor TAS2R14 as a pro-ferroptotic driver in thyroid cancer via the STAT3-HCG11-miR-450b-5p axis

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Abstract

Taste Receptor Type 2 (TAS2R) has an obvious function of sensing bitterness and transmitting neural signals. Our study aims to elucidate the upstream regulatory pathways of TAS2R14 in thyroid tumors, revealing new facets of TAS2R investigation. Bioinformatics analyses are conducted utilizing UALCAN, TargetScan, ENCORI, and GEPIA. Gene expression is assessed via microarray, qPCR, and western blot analysis. Molecular interactions are probed through dual-luciferase reporter assay, biotin-coupled pull-down, and chromatin immunoprecipitation (ChIP) assay. Ferroptosis assessment is performed using incorporated iron-centric techniques. Cellular phenotypes are determined by cell invasion, viability, apoptosis, and wound healing assays. *In vivo* studies employ a nude mouse xenograft model. We discover that TAS2R14 can inhibit thyroid cancer cell proliferation, invasion, and migration, concurrently inducing ferroptosis. The expression of TAS2R14 is governed by miR-450b-5p, which is sponged by the lncRNA HCG11 via a STAT3-dependent pathway. TAS2R14 promotes cell ferroptosis and arrests thyroid cancer progression through an upstream regulatory cascade that involves STAT3, the lncRNA HCG11, and miR-450b-5p. Our research reveals an unsuspected regulatory paradigm of the TAS2R family, shedding new light on its function and extending our understanding beyond its established role in bitter taste sensation.

Key words bitter taste receptor, thyroid cancer, ferroptosis, TAS2R14, STAT3, ncRNA

Introduction

Bitter taste receptors (Taste Receptor Type 2, TAS2R) constitute a distinct subfamily within the G protein-coupled receptor (GPCR) superfamily. TAS2Rs are evolutionarily conserved chemosensory detectors with more than 20 members. They are instrumental in discerning noxious substances and selecting beneficial nutrients [1–3]. Current research has focused primarily on taste perception itself [4]. A significant portion of this work is dedicated to examining downstream signaling pathways [5–8]. In recent years, a cascade of pivotal discoveries concerning the TAS2R family has emerged, encompassing the modulation of animal behaviors [9], elucidation of structural underpinnings [10], cholesterol binding

mechanisms [11,12], and degradation of lipid droplets in neutrophils [13]. These findings are anticipated to substantially advance our comprehension of the diverse biological roles of TAS2Rs, extending well beyond the scope of gustatory sensation. Their functions beyond the gustatory realm represent a burgeoning frontier in GPCR research.

Thyroid cancer is a distinct subtype of endocrine malignancy. It is particularly common among head and neck cancers, accounting for an estimated 3%–4% of all cancer diagnoses [14,15]. The median age at diagnosis is in the early 50s. However, thyroid cancer also disproportionately affects adolescents and young adults, with the highest prevalence occurring in people aged 16–33 years [16,17].

Over the past four decades, a striking 313% increase in thyroid cancer incidence has been documented [16,18]. Despite global variability in incidence rates, a consistent upwards trajectory has been noted across multiple countries [19]. This trend underscores the critical need for an in-depth investigation into the molecular pathways that drive the etiology and progression of thyroid cancer. Such research is essential for improving diagnostic precision, accelerating drug development, and refining prognostic assessments.

Our previous research extensively explored the extra-lingual functions of bitter taste receptors, transcending their established role in bitter taste perception [20–22]. In particular, we identified the clinical significance of the TAS2R14 receptor in thyroid cancer [23]. In this study, we aimed to elucidate the upstream regulatory mechanisms of TAS2R14 in thyroid tumors, thereby shedding new light on the TAS2R family. Our findings indicate that TAS2R14 may inhibit cell proliferation, invasion, and migration while simultaneously promoting ferroptosis in thyroid cancer cells. Furthermore, we determined that the expression of TAS2R14 is modulated by the STAT3-mediated lncRNA HCG11-miR-450b-5p axis, a regulatory network that has not been characterized previously. This finding highlights the novel instance in which the TAS2R family is modulated by the intricate interplay of long and short noncoding RNAs. These novel findings not only reveal a unique regulatory signal for the TAS2R family but also implicate its role in a novel cell death paradigm.

Materials and Methods

Bioinformatics approaches

mRNA expression data, comprising 337 normal samples and 512 tumor samples—as well as clinical data up to December 19, 2022, for thyroid cancer—were sourced from The Cancer Genome Atlas (TCGA) via the UALCAN dataset portal. A differential analysis was subsequently performed to identify lncRNAs and mRNAs with significant expression differences, applying stringent criteria of $|\log_2\text{FC}| > 1.5$ and $P_{\text{adj}} < 0.05$. The subsequent step involved the use of TargetScan to predict target miRNAs, specifically identifying TAS2R14 as having binding sites for miRNAs. The interaction between miR-450b-5p and the lncRNA HCG11 was further explored using the ENCORI platform. Additionally, the expression data for STAT3 across various cancers were extracted from the GEPIA dataset.

Sample collection

For this study, a cohort of 42 thyroid cancer tissues and their corresponding adjacent normal tissues were meticulously collected. Each tissue sample was procured from patients who underwent surgical procedures. The selection of the sample size was guided by literature, and the samples were meticulously matched at a 1:1 ratio. The inclusion criteria for the study were stringent, ensuring that all participants met the following conditions: (a) a definitive diagnosis of thyroid cancer confirmed by post-operative pathology; (b) receiving initial treatment at our hospital, with no history of prior locally curative surgery or radiation therapy involving the head or neck region. Upon excision, all the tissue samples were promptly snap-frozen in liquid nitrogen to preserve their integrity. The samples were subsequently transferred to a -80°C freezer for long-term storage prior to analysis. The collection and utilization of these human tissue samples were conducted with the explicit informed consent of the patients involved. Furthermore, the study was granted approval by the Institutional Ethics Committee of our

hospital, adhering to the ethical standards for the use of human samples in research.

Cell culture

A panel of cell lines, including the normal thyroid epithelial cell line NTHY-ORI 3-1 and the thyroid cancer cell lines TPC-1, KTC-1, WRO, B-CPAP, SW579, and FTC-133, was obtained from the American Type Culture Collection (ATCC; Manassas, USA). NTHY and TPC-1 cells were cultured in T-75 flasks using Dulbecco's modified Eagle's medium (DMEM; HyClone, South Logan, USA) supplemented with 10% fetal bovine serum (FBS; Beyotime, Shanghai, China). The culture conditions were maintained at 37°C in a humidified atmosphere containing 5% CO_2 , with media replenishment occurring every 48 h. To inhibit ferroptosis in cells, cells were treated with 5 μM ferrostatin-1 (Fer-1; Sigma-Aldrich, Shanghai, China) for 24 h at 37°C . To obtain separate cytoplasmic and nuclear fraction, a total of 4×10^6 cells were collected and rinsed twice with cold PBS, followed by incubation in hypotonic buffer [50 mM HEPES (pH 7.4), 10 mM KCl, 350 mM sucrose, 1 mM EDTA, 1 mM DTT, 0.1% Triton X-100, 1 $\times$ Protease Inhibition Cocktail (Sigma-Aldrich, Shanghai, China), and 100 U/ml RNase Inhibitor (Thermo Fisher Scientific, Shanghai, China)] on ice with intermittent vortexing for 10 min. After centrifugation at 2000 g for 5 min, the supernatant was collected as the cytoplasmic fraction. The remaining pellet was subsequently washed and resuspended in lysis buffer [10 mM HEPES (pH 7.0), 100 mM KCl, 5 mM MgCl_2 , 0.5% NP-40, 10 μM DTT, 1 $\times$ Protease Inhibition Cocktail, and 100 U/ml RNase Inhibitor] to obtain the nuclear fraction.

Cell transfection

Mimics of miR-450b-5p, negative control mimics, the pcDNA3.1-TAS2R14 plasmid encoding TAS2R14, pcDNA3.1-HCG11, and the empty pcDNA3.1 plasmid (OE-NC) vectors were all purchased from RiboBio (Guangzhou, China). In accordance with the manufacturer's guidelines, LipoFiter transfection reagent (Beyotime) was used to transfect the mimics or plasmids into TPC-1 and WRO cells. The cells were utilized for subsequent experiments 24 h after transfection.

Small interfering RNA (siRNA) for human TAS2R14 or STAT3 was purchased from Genechem Co. Ltd. (Shanghai, China). The oligonucleotide primers utilized in this study are detailed in [Supplementary Table S1](#). The siRNAs were dissolved in RNase-free water (final concentration of 20 μM), and the Lipofectamine RNAiMAX transfection reagent (Thermo Fisher Scientific) was stored at 4°C and equilibrated to room temperature before use. Adherent cells were seeded in 6-well plates 24 h prior to transfection (70% confluency). The siRNA-lipid complex was prepared by mixing 5 μL of siRNA (20 μM) with 250 μL of Opti-MEM serum-free medium (tube A), and 7.5 μL of Lipofectamine RNAiMAX was diluted with 250 μL of Opti-MEM (tube B). Tubes A and B were combined and incubated at room temperature for 5–15 min to allow complex formation. The complexes were added dropwise to the cell culture wells (500 μL /well), and the plate was gently swirled to mix, resulting in a final siRNA concentration of 50 nM. The cells were incubated at 37°C with 5% CO_2 for 4–6 h, after which the medium was replaced by complete medium containing serum. The culture was continued for 24–72 h before gene silencing efficacy was assessed [24]. Anti-miR-450b-5p and anti-miR-Control oligonucleotides were also delivered into cells using Lipofectamine RNAiMAX

as carriers. The anti-miR sequences used in the study are shown in [Supplementary Table S2](#).

Cell viability analysis

The assessment of cell viability was performed by CCK-8 assay. TPC-1 or WRO cells were plated in 96-well plates at an initial density of 6×10^3 cells per well and incubated for 24 h to allow for attachment. The CCK-8 reagent (Abcam, Cambridge, UK) was subsequently used. The mixture was added, and the cells were incubated for an additional 3 h. The absorbance at 450 nm was measured via a microplate reader (PerkinElmer, Waltham, USA), which provides a quantitative assessment of cell viability.

Wound healing assay

TPC-1 or WRO cells were inoculated into 6-well plates. Once the cells reached 80% confluence, a wound was created by gently scraping the monolayer with a 200- μ L pipette tip. The cells at the wound edges were washed twice with culture medium to remove any detached cells. Fresh medium was used to facilitate cell regrowth (24 h). Wound closure was monitored over a period of 24 h for both the experimental and control groups, and the migration of the cells was documented by photography at 0 and 24-h post-wounding.

Cell cycle and apoptosis analysis

The cell cycle analysis was conducted 24 h post-transfection using a cell cycle detection kit (Abcam). The cells were fixed in 95% ethanol overnight, followed by staining with 50 μ g/mL propidium iodide (PI) in the dark for 30 min. Flow cytometry was used to determine the distribution of the cells throughout various phases of the cell cycle.

Cell invasion analysis

The migratory capacity of the cells was evaluated using a Transwell chamber with an 8- μ m pore size (Corning, New York, USA). For the Transwell assay, 1×10^5 cells suspended in serum-free DMEM were evenly seeded into the upper chamber, and the lower chamber was filled with medium containing 10% FBS. After a 30-h incubation period, the cells on the upper surface of the filter were removed by gentle abrasion, and the cells on the lower surface of the filter were fixed with methanol and stained with 0.5% crystal violet, imaged and quantified under a microscope.

Western blot analysis

Protein lysates were extracted from cultured cells via radio-immunoprecipitation assay (RIPA) buffer (Beyotime). Protein quantification was performed via the bicinchoninic acid (BCA) assay kit (Beyotime). Equal amounts of protein samples were resolved on a 12% polyacrylamide gel (Bio-Rad, Hercules, USA) and transferred to a 0.45- μ m polyvinylidene fluoride (PVDF) membrane (GE Healthcare, Chicago, USA). After being blocked with non-fat dry milk (Beyotime), the membranes were probed with primary antibodies overnight at 4°C, followed by incubation with horseradish peroxidase (HRP)-conjugated secondary antibodies for 2 h at room temperature. Immunodetection was accomplished via a ChemiDoc MP Imaging System (Bio-Rad) with a luminescent HRP substrate (Millipore, Billerica, USA). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) served as an endogenous loading control. Details of the antibodies used are provided in [Supplemen-](#)

[tary Table S3](#).

RNA extraction and quantitative real-time polymerase chain reaction (qPCR) analysis

Total RNA was extracted from both the cellular and tissue samples using Trizol reagent (Life Technologies, Gaithersburg, USA). Following RNA isolation, complementary DNA (cDNA) was synthesized via a cDNA synthesis kit (Vazyme, Nanjing, China) in accordance with the manufacturer's protocol. qPCR was performed with the SsoFast EvaGreen Supermix system (Bio-Rad). All gene expression data were normalized relative to the housekeeping gene *GAPDH*. The oligonucleotide primers utilized in this study are detailed in [Supplementary Table S4](#).

Assessment of iron content and MDA, GSH and 4-HNE levels

The total cellular iron content was quantified using the Iron Assay kit (Abcam) which provides a sensitive and specific method for measuring iron levels. Concurrently, the concentration of malondialdehyde (MDA), a biomarker for lipid peroxidation, was evaluated using the MDA Assay kit (Abcam). Furthermore, the level of glutathione (GSH), a critical antioxidant in cells, was determined with the mouse GSH ELISA kit (Abcam), which is optimized for the accurate quantification of GSH. The concentration of 4-hydroxynonenal (4-HNE) was determined according to the method described by Ding *et al.* [25]. Briefly, the cells were digested with trypsin and then centrifuged at 1000 g for 5 min. The resulting cell pellet was resuspended in PBS and subjected to repeated freeze-thaw cycles for cell lysis. After further centrifugation at 1500 g for 10 min, the supernatant was collected. The biotin-labelled antibody working solution was mixed with the sample homogenate and incubated at 37°C for 45 min. Following washing, 100 μ L of HRP-conjugated streptavidin working solution was added to each sample, and the mixture was incubated at 37°C for 30 min. After another washing step, 90 μ L of chromogenic substrate was added, and the mixture was incubated at 37°C for 15 min. The chromogenic reaction was terminated, and the optical density (OD) at 450 nm was immediately measured using a microplate reader (PerkinElmer, Waltham, USA).

Microarray analysis

Total RNA was extracted from paired tissue samples obtained from six thyroid cancer patients via the Trizol reagent (Life Technologies), followed by purification with the RNeasy Mini kit (Qiagen, Beverly, USA) according to the manufacturer's protocols. RNA integrity and purity were confirmed using an Agilent 2100 Bioanalyzer. miRNA species isolated from total RNA were labeled and annealed to 100 ng Cy3-conjugated RNA following the protocol of the miRNA Complete Labeling and Hyb Kit (Agilent Technologies, Santa Clara, USA). Thereafter, the samples were subjected to scanning with an Agilent Microarray Scanner (Agilent Technologies). Microarray analysis was conducted to identify differentially expressed microRNAs (miRNAs), employing methods previously described in the literature [26].

Dual-luciferase reporter assay

A dual-luciferase reporter assay was performed in strict accordance with the protocol provided by the kit manufacturer (Beyotime). To elucidate the promoter activity of the *TAS2R14* gene, various

lengths of promoter sequences (350, 500, 1000, and 6000 base pairs) were cloned and inserted into the pGL3 vector. These recombinant plasmids were transiently transfected into 293T, TPC-1, or WRO cells, which were subsequently cultured in 12-well plates. The transfection process utilized Lipofectamine 2000 (Thermo Fisher Scientific), with a mixture comprising 1 µg of the reporter plasmid and 1 µg of *Renilla* luciferase plasmid for normalization. After a 48-h incubation period, the cells were lysed using the PLB buffer supplied with the kit, and the resulting luciferase activities were measured. The firefly luciferase activity, indicative of promoter strength, was normalized to *Renilla* luciferase activity to account for variations in transfection efficiency.

Biotin-coupled miRNA pull-down assay

The 3'-end of miR-450b-5p or the negative control mimics was conjugated with biotin, enabling the specific pull-down of miRNA-associated molecules. A total of 1×10^6 TPC-1 or WRO cells were transfected with 100 pmol of biotinylated miR-450b-5p (Bi-miR-450b-5p) or the corresponding negative control using Lipofectamine 2000 (Thermo Fisher Scientific) following the manufacturer's protocol. After a 48-h incubation period, the cells were harvested via centrifugation at 300 g. The cell pellets were washed twice with PBS and then resuspended in 0.7 mL of ice-cold lysis buffer containing 5 mM MgCl₂, 100 mM KCl, 20 mM Tris-HCl (pH 7.5), 0.3% NP-40, 50 U of RNase OUT (Invitrogen), and a complete protease inhibitor cocktail (Roche Applied Science, Indianapolis, USA). The cells were incubated on ice for 10 min to facilitate lysis. The cell lysate was clarified by centrifugation at 10,000 g for 10 min to remove insoluble materials. The miRNA biotin pull-down experiments were conducted according to established methodologies reported in the literature [27]. The enrichment of target RNAs, such as HOXD-AS1 or SOX4, in the pull-down samples was quantified by qPCR, providing insights into the miRNA interactome.

Chromatin immunoprecipitation (ChIP) assay

The ChIP assay was carried out using the Millipore EZ Magna ChIP™ kit (Millipore) following the manufacturer's instructions. In brief, the TPC-1 and WRO cell lines were treated with a 1% formaldehyde solution at 25°C for 10 min during the precipitation process. This was followed by an overnight co-incubation with either the STAT3-specific antibody (Sigma-Aldrich, St Louis, USA) or the control IgG antibody (Sigma-Aldrich) in conjunction with protein A/G agarose beads, leading to the immunoprecipitation procedure. The quantity of extracted DNA was then determined by quantitative PCR. The *HCG11* primers used in the assay were as follows: forward: 5'-ATTCGACCATGGCATCTG-3', reverse: 5'-AGAAGGCCTTGAGACAACA-3'.

Xenograft tumor model experimentation

Female BALB/c mice, aged 4–6 weeks and weighing 18–20 g, were obtained from Shanghai SLAC Laboratory Animal Co., Ltd. (Shanghai, China) and maintained under specific pathogen-free conditions. The animals were provided with food and acidified water ad libitum. A total of forty mice were randomly assigned to eight experimental groups in a blinded manner, with each group comprising five mice. TPC-1 or WRO cells, at a dosage of 2×10^6 cells per mouse, were subcutaneously implanted to establish the xenograft tumor model. The experimental groups were categorized as follows: Control vector group (Groups 1 and 2); LncRNA HCG11

overexpression group (Group 3); LncRNA HCG11 knockdown group (Group 4); LncRNA HCG11 overexpression combined with miR-450b-5p group (Group 5); LncRNA HCG11 overexpression combined with the miR-control group (Group 6); LncRNA HCG11 knockdown combined with the anti-miR-450b-5p group (Group 7); and LncRNA HCG11 knockdown combined with the anti-miR-control group (Group 8). The sample size was meticulously determined to ensure statistical power and compliance with ethical guidelines. The inclusion and exclusion criteria for the animals were independently assessed in accordance with the AAALAC standards. All xenograft tumor experiments were conducted following the Guidelines for the Care and Use of Laboratory Animals, and the study protocol was approved by the Institutional Animal Care and Use Committee of our hospital.

Statistical analysis

The statistical analysis was conducted via the SPSS software suite, version 17.0 (SPSS Inc., Chicago, USA). The quantitative data were subjected to one-way analysis of variance (ANOVA) to discern variances among the group means. Subsequent to ANOVA, Dunnett's multiple comparison post hoc tests were applied to delineate statistically significant differences between individual groups. Data are presented as the mean $\pm$ standard deviation (SD), with the threshold for statistical significance established at a *P* value of less than 0.05.

Results

TAS2R14 suppresses the viability of thyroid cells

Our prior research revealed the decreased expression of TAS2R14 in thyroid cancer tissues [23]. The findings prompted us to investigate the impact of TAS2R14 on thyroid cancer cell behavior. We assessed TAS2R14 mRNA levels across a panel of cell lines, including a normal thyroid epithelial cell line (NTHY-ORI 3-1) and multiple thyroid cancer cell lines (TPC-1, KTC-1, WRO, B-CPAP, SW579, and FTC-133). As depicted in Figure 1A, a substantial reduction in TAS2R14 expression was observed in thyroid cancer cell lines relative to the normal thyroid cell line, as determined by PCR analysis.

To elucidate the role of TAS2R14 in thyroid cancer progression, we selected the TPC-1 cell line because of its minimal TAS2R14 expression and the WRO cell line because of its comparatively high expression. Lentivirus-mediated transfection was employed to overexpress TAS2R14 in TPC-1 cells and to downregulate its expression in WRO cells (Figure 1B,C). Notably, Transwell assay results indicated that TAS2R14 overexpression in TPC-1 cells led to a 50%–70% reduction in cell invasion, whereas its knockdown in WRO cells had the opposite effect (Figure 1D,E).

Furthermore, our findings indicated that TAS2R14 overexpression in TPC-1 cells was associated with decreased cell viability and increased apoptosis, whereas in WRO cells, TAS2R14 knockdown increased cell viability and reduced apoptosis (Figure 1F–H). To assess the effect on cell migration, we performed a wound healing assay. The results demonstrated that TAS2R14 overexpression in TPC-1 cells curtailed cell migration, whereas its knockdown in WRO cells facilitated cell migration (Figure 1I,J).

These results underscore the tumor-suppressive role of TAS2R14, highlighting its potential as a negative regulator of cell viability, invasion, and migration and a promoter of apoptosis in thyroid cancer cells.

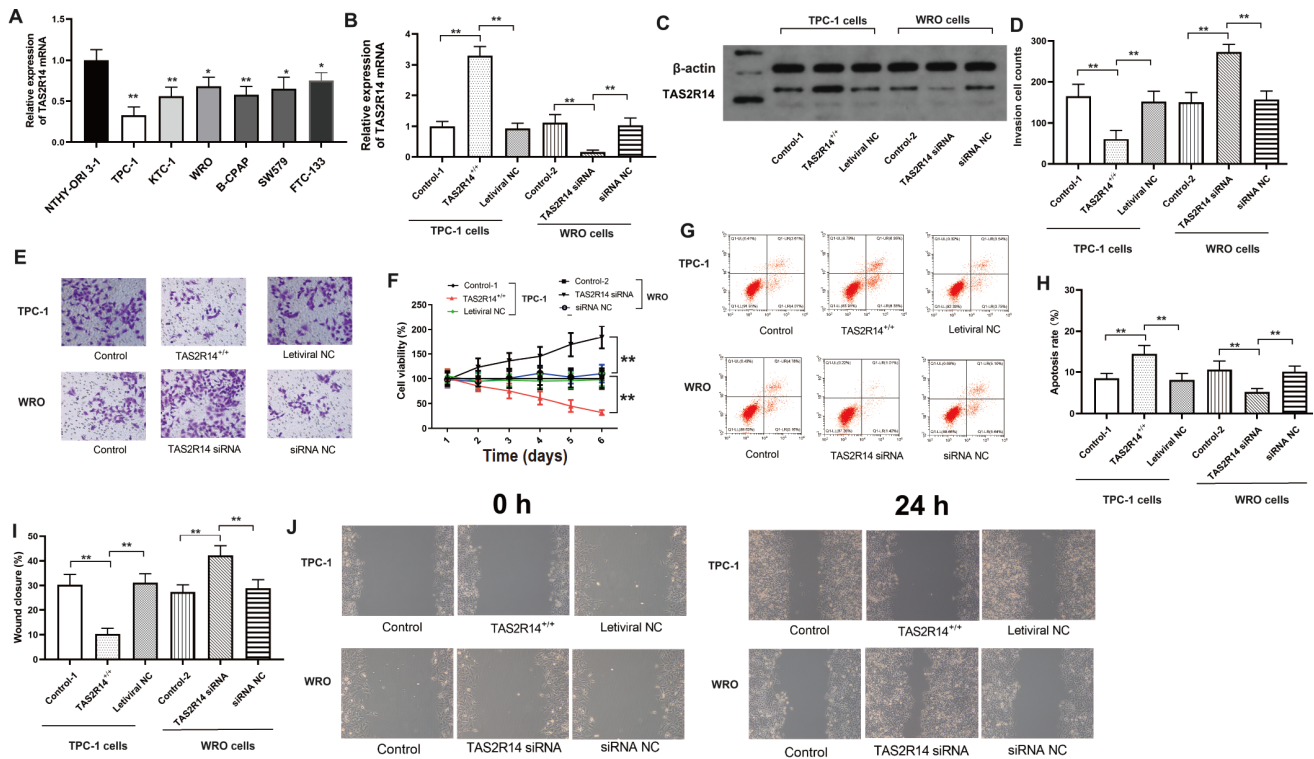


Figure 1. TAS2R14 inhibits cell proliferation, invasion, and migration in thyroid cancer cells (A) Expression levels of TAS2R14 in a normal thyroid cell line (NTHY-ORI 3-1) and thyroid cancer cell lines (TPC-1, KTC-1, WRO, B-CPAP, SW579, and FTC-133). * $P < 0.05$, ** $P < 0.01$ compared with NTHY-ORI 3-1. (B,C) Expression levels of *TAS2R14* in TPC-1 cells or WRO cells after *TAS2R14* overexpression or knockdown. *GAPDH* was used for normalization. ** $P < 0.01$. (D,E) Cell invasion in TPC-1 cells or WRO cells after *TAS2R14* overexpression or knockdown. ** $P < 0.01$. (F) Viability of TPC-1 and WRO cells after *TAS2R14* overexpression or knockdown. ** $P < 0.01$. (G,H) Cell apoptosis in TPC-1 cells or WRO cells after *TAS2R14* overexpression or knockdown. ** $P < 0.01$. (I,J) Wound healing assays in TPC-1 cells or WRO cells after *TAS2R14* overexpression or knockdown. ** $P < 0.01$. Data are presented as the mean $\pm$ SD of three independent experiments.

TAS2R14 enhances ferroptosis in thyroid cancer cells

Ferroptosis, characterized by its dependence on iron and reactive oxygen species (ROS), is a new pivotal mechanism in cancer cell death. The overexpression of TAS2R14 in TPC-1 cells markedly increased the levels of intracellular iron and ROS, which are key indicators of ferroptosis initiation (Figure 2A). Conversely, *TAS2R14* knockdown decreased these levels, suggesting its regulatory role in iron homeostasis. Further analysis of ferroptosis markers revealed that TAS2R14 overexpression upregulated MDA and downregulated GSH levels, indicative of increased lipid peroxidation and decreased antioxidant capacity, respectively (Figure 2B,C). The protein expression patterns of ferroptosis-related markers, including transferrin receptor (TRF), acyl-CoA synthetase long-chain family member 4 (ACSL4), and glutathione peroxidase 4 (GPX4), corroborated the proferroptotic effect of TAS2R14 (Figure 2D,E). The role of ferroptosis in TAS2R14-mediated cell proliferation was confirmed via the ferroptosis inhibitor Fer-1, which reversed the inhibitory effect of TAS2R14 on cell viability (Figure 2F). We detected ROS and 4-HNE for further verification (Figure 2G-I). These findings underscore the potential of TAS2R14 as a modulator of ferroptosis in thyroid cancer, offering insights into its tumor-suppressive function.

miR-450b-5p suppresses TAS2R14 expression in thyroid cancer cells

The regulation of the TAS2R family by miRNAs remains unexplored,

prompting our investigation into the potential involvement of TAS2R14 in thyroid cancer progression through miRNA interactions. We utilized the TargetScan database to identify miRNAs with predicted binding sites to TAS2R14 and assessed their expression in thyroid cancer tissues and adjacent normal tissues via microarray BeadChip and PCR analysis. This analysis revealed significant dysregulation of several miRNAs, with miR-450b-5p exhibiting the most pronounced upregulation ($P < 0.01$) in cancer tissues (Supplementary Figure S1A,B). The predicted binding sites between miR-450b-5p and *TAS2R14* are depicted in Supplemental Figure S1D.

Further quantification of miR-450b-5p expression in a normal thyroid epithelial cell line (NTHY-ORI 3-1) and thyroid cancer cell lines (TPC-1, KTC-1, WRO, B-CPAP, SW579, and FTC-133) confirmed an increase in miR-450b-5p levels, specifically in the cancer cell lines (Supplementary Figure S1C). To elucidate the interaction between miR-450b-5p and *TAS2R14*, TPC-1 and WRO cell lines were treated with miR-450b-5p mimics and antagonists. The overexpression of miR-450b-5p led to a significant decrease in TAS2R14 expression, which was reversed by anti-miR-450b-5p treatment in a dose-dependent manner (Supplementary Figure S2C-E).

To validate the direct interaction between miR-450b-5p and TAS2R14's 3' untranslated region (3'UTR), we designed mutations within the *TAS2R14* 3'UTR and conducted a luciferase reporter assay using plasmids containing both wild-type (WT) and mutant (MUT) *TAS2R14* 3'UTR sites in the 293T, TPC-1, and WRO cell lines

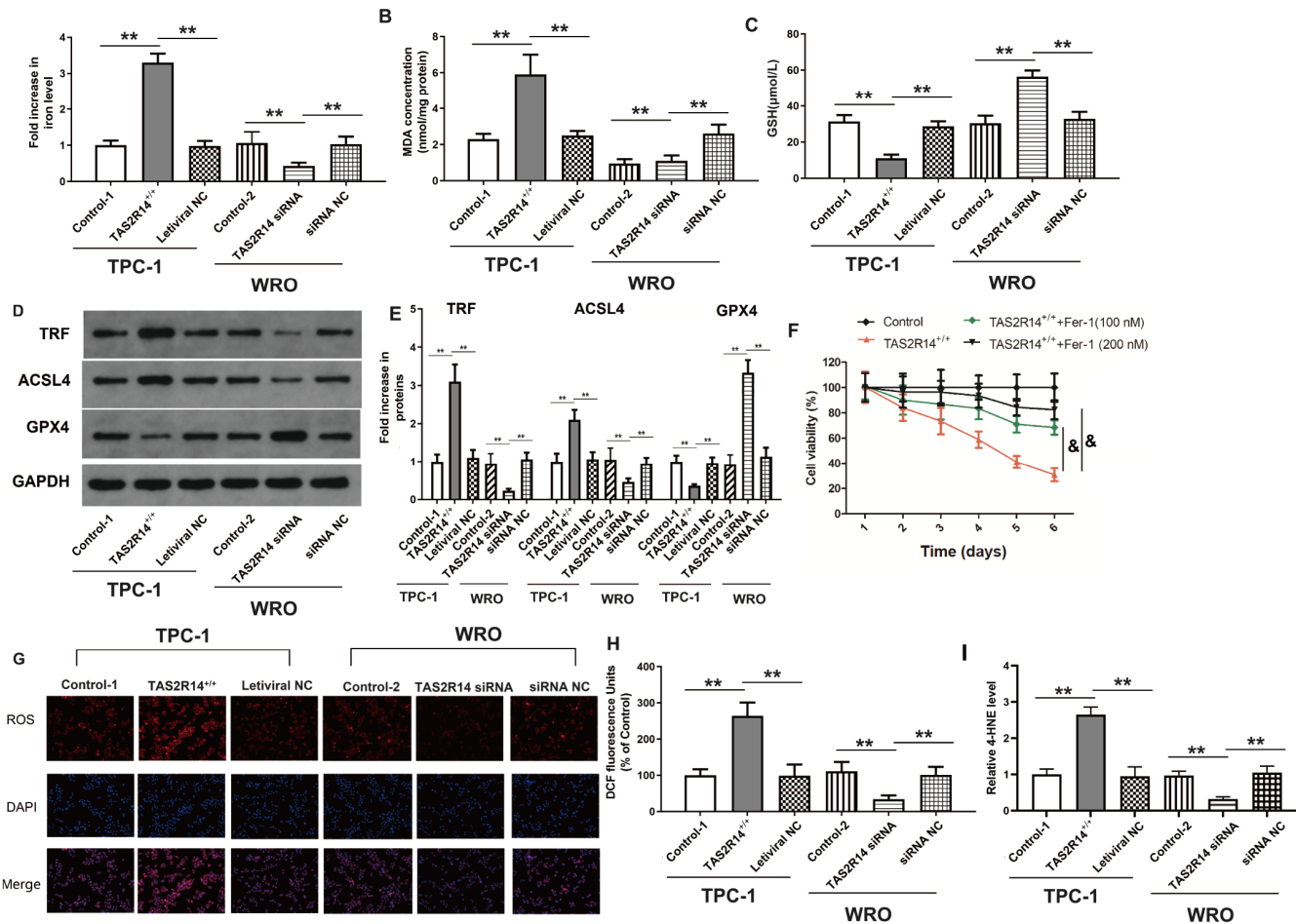


Figure 2. TAS2R14 promotes ferroptosis in thyroid cancer cells (A) Iron content in TPC-1/WRO cells after *TAS2R14* overexpression or knockdown. (B) MDA concentration in TPC-1/WRO cells after *TAS2R14* overexpression or knockdown. (C) GSH concentration in TPC-1/WRO cells after *TAS2R14* overexpression or knockdown. (D,E) Expression of ferroptosis-related markers (TRF, ACSL4, and GPX4) in TPC-1/WRO cells after *TAS2R14* overexpression or knockdown. GAPDH was used for normalization. (F) Viability of *TAS2R14*-overexpressing TPC-1 cells after treatment with Fer-1. (G,H) ROS content in *TAS2R14*-overexpressing TPC-1 cells and *TAS2R14*-knockdown WRO cells. (I) Relative changes in 4-HNE in TPC-1/WRO cells after *TAS2R14* overexpression or knockdown. TRF: transferrin receptor; ACSL4: acyl-CoA synthetase long-chain family member 4; GPX4: glutathione peroxidase 4; Fer-1: ferrostatin-1. Data are presented as the mean $\pm$ SD of three independent experiments. ** $P < 0.01$ compared with indicated groups. & $P < 0.05$ compared with indicated groups.

(Supplementary Figure S2F). Compared with control transfection, transfection with miR-450b-5p mimics resulted in a significant reduction in luciferase activity (Supplementary Figure S2G), confirming the direct binding and regulatory effects of miR-450b-5p on *TAS2R14*.

These findings collectively demonstrate that miR-450b-5p is upregulated in thyroid cancer and directly interacts with *TAS2R14*, suggesting a role for miR-450b-5p in the posttranscriptional regulation of *TAS2R14* in cancer cells.

LncRNA HCG11 downregulates miR-450b-5p expression in thyroid cancer cells

Given the known interactions between lncRNAs and miRNAs, we hypothesized that specific lncRNAs might orchestrate the expression of miR-450b-5p in thyroid cancer tissues. To identify potential lncRNA regulators, we employed bioinformatics analysis via the ENCORI database to predict lncRNAs whose binding sites are complementary to those of miR-450b-5p. Subsequent PCR analysis comparing normal thyroid cells (NTHY-ORI 3-1) with thyroid

cancer cell lines (TPC-1 and WRO) revealed that among several candidates, the lncRNAs NOP14-AS1, PURPL, and HCG11 were markedly under-expressed in TPC-1 cells (Supplementary Figure S3A). Compared with those in the normal cell line, quantitative PCR further confirmed the downregulation of these lncRNAs in both TPC-1 and WRO cells (Supplementary Figure S3B). To discern the specific role of these lncRNAs, we utilized small interfering RNAs (siRNAs) to downregulate their expression individually in the cell lines. Strikingly, only the knockdown of lncRNA HCG11 resulted in significant upregulation of miR-450b-5p, as depicted in Supplementary Figure S3C–E, suggesting a direct regulatory effect of lncRNA HCG11 on miR-450b-5p expression. To validate the direct interaction between lncRNA HCG11 and miR-450b-5p, we designed a luciferase reporter assay incorporating the 3'UTR of *HCG11* with both WT and MUT sequences in 293T, TPC-1, and WRO cells (Supplementary Figure S3F). The assay results demonstrated that the luciferase activity was specifically modulated by the WT lncRNA HCG11 in response to the miR-450b-5p level, whereas the MUT form did not (Supplementary Figure S3G and Figure 3H),

confirming the presence of a recognition sequence for miR-450b-5p within the lncRNA HCG11.

We then investigated the role of the lncRNA HCG11 in modulating the biological behavior of thyroid cancer cells. Our results demonstrated that overexpression of the lncRNA HCG11 significantly inhibited cell viability and invasion while promoting the apoptosis of thyroid cancer cells (Figure 3A–D). Furthermore, the upregulation of lncRNA HCG11 was associated with a reduction in cell migration (Figure 3E–J). Conversely, knockdown of lncRNA HCG11 led to increased cell viability, migration, and invasion, as well as decreased apoptosis, *in vitro*. To elucidate the underlying molecular mechanism, we treated lncRNA HCG11-overexpressing TPC-1 cells and WRO cells with miR-450b-5p (Figure 3). We observed that miR-450b-5p treatment effectively reversed the inhibitory effects of lncRNA HCG11 overexpression on the biological behavior of cells. Similarly, anti-miR-450b-5p treatment rescued the phenotype induced by lncRNA HCG11 knockdown, including cell proliferation, invasion, migration, and apoptosis, *in vitro* (Figure 3). These findings delineate a novel regulatory axis in thyroid cancer in which the lncRNA HCG11 directly targets miR-450b-5p, offering new insights into the intricate regulatory networks that govern gene expression in this disease.

To clarify the expression and clinical value of HCG11 in thyroid tumors, we conducted a comprehensive study and found that lncRNA HCG11 is significantly under-expressed in thyroid cancer tissues compared with normal tissues (Supplementary Figure S4A, F). Our research also revealed that the downregulation of lncRNA HCG11 is common across different stages of thyroid cancer, regardless of age, ethnicity, or subtype (Supplementary Figure S4B–E). Additionally, via the nuclear fragmentation method, we investigated the distribution of the lncRNA HCG11 within the nucleus and cytoplasm of cells (Supplementary Figure S4G, H). Our results revealed that the lncRNA HCG11 co-localized with Tubulin in TPC-1 and WRO cells, suggesting that it is located primarily in the cytoplasm. In summary, our findings collectively highlight the downregulation of the lncRNA HCG11 in thyroid cancer tissues and its potential role as a prognostic biomarker and therapeutic target for thyroid cancer.

STAT3 stimulates lncRNA HCG11 expression in thyroid cancer

In our analysis of RNA sequencing data from the GEPIA dataset, which included 512 thyroid cancer cases and 337 controls, we observed a downregulation of STAT3 in various tumor types, with a notable decrease in thyroid cancer (data not shown). Correlation and regression analyses revealed a significant positive correlation between STAT3 mRNA levels and lncRNA HCG11 expression in thyroid cancer tissues ($P < 0.0001$) (data not shown). This correlation was further validated via a PCR-based analysis of paired thyroid cancer and adjacent normal tissues, where STAT3 expression was found to be low and moderately correlated with lncRNA HCG11 levels in the collected tissues ($P = 0.0002$) (Figure 4A, B). Moreover, siRNA-mediated knockdown of STAT3 in TPC-1 and WRO cells resulted in decreased lncRNA HCG11 expression (Figure 4E), suggesting a regulatory link between STAT3 and lncRNA HCG11 in thyroid cancer.

To elucidate the molecular mechanisms underlying the downregulation of lncRNA HCG11, we conducted a detailed promoter analysis. The promoter sequence of the human lncRNA HCG11 was

retrieved from the UCSC Genome Browser. A series of luciferase activity assays were performed in TPC-1 cells to identify the proximal promoter region of lncRNA HCG11. The promoter activities of a 2000-base pair (bp) region upstream of the transcription start site (TSS) and six deletion constructs were assessed (Figure 4C). Compared with the pGL3-basic vector, the pGL3-2000/0 plasmid exhibited robust luciferase activity in 293T cells, with no significant differences observed between the full-length and deletion constructs (Figure 4D). Further truncation analyses revealed two critical fragments at -568 nt and -140 nt, which contained regulatory elements essential for lncRNA HCG11 transcription (Figure 4F). ChIP assays confirmed that STAT3 could directly bind to these regions within the lncRNA HCG11 promoter in TPC-1 and WRO cells (Figure 4G), indicating that STAT3 mediates the transcriptional regulation of lncRNA HCG11 in thyroid cancer. Subsequent experiments revealed that perturbation of STAT3 expression led to an increase in miR-450b-5p levels and a decrease in TAS2R14 expression (Figure 4H, I). These findings corroborate the notion that STAT3 exerts regulatory control over both miR-450b-5p and TAS2R14 through its influence on HCG11, which aligns with our initial hypotheses.

To investigate whether the functional relevance of this interaction is directly linked to miR-450b-5p/TAS2R14 regulation, rescue experiments (STAT3 knockdown + HCG11 overexpression) were performed to establish causal relationships. Figure 4K, L showed the viability of TPC-1/WRO cells after they were subjected to lncRNA HCG11 overexpression and siRNA against STAT3. The results showed that STAT3 knockdown significantly abolished the effect of HCG11 overexpression on cell viability. Figure 4M showed the apoptosis rate of TPC-1 cells after they were subjected to lncRNA HCG11 overexpression and siRNA against STAT3, which suggested that STAT3 knockdown significantly abolished the effect of HCG11 overexpression on cell apoptosis. Our research reveals a novel regulatory axis in thyroid cancer in which STAT3 acts as a transcriptional modulator of the lncRNA HCG11, providing insights into the intricate interplay between transcription factors and non-coding RNAs involved in tumor suppression.

lncRNA HCG11 regulates TAS2R14 expression via a miR-450b-5p-dependent mechanism

To elucidate the regulatory network involving the lncRNAs HCG11, miR-450b-5p, and TAS2R14, we conducted a detailed investigation. By performing linear regression and correlation analyses on thyroid cancer tissue samples, we discerned a significant moderate correlation between the mRNA levels of the lncRNAs HCG11 and TAS2R14 (Figure 5A). Through lentiviral transfection to over-express lncRNA HCG11 and siRNA knockdown in TPC-1 and WRO cells, we observed notable upregulation of TAS2R14, suggesting that lncRNA HCG11 can promote its expression in thyroid cancer cells (Figure 5B, C). Conversely, lncRNA HCG11 knockdown attenuated TAS2R14 expression, indicating a potential regulatory role of lncRNA HCG11 *in vitro* (Figure 5D, E). Intriguingly, the effects of lncRNA HCG11 overexpression on TAS2R14 were abrogated by the introduction of miR-450b-5p, highlighting a miR-450b-5p-dependent regulatory mechanism (Figure 5D, E). Furthermore, the suppressive effect of lncRNA HCG11 knockdown on TAS2R14 was counteracted by anti-miR-450b-5p treatment, reinforcing the interplay between these molecules (Figure 5F, G). To determine the direct interaction between miR-450b-5p and the

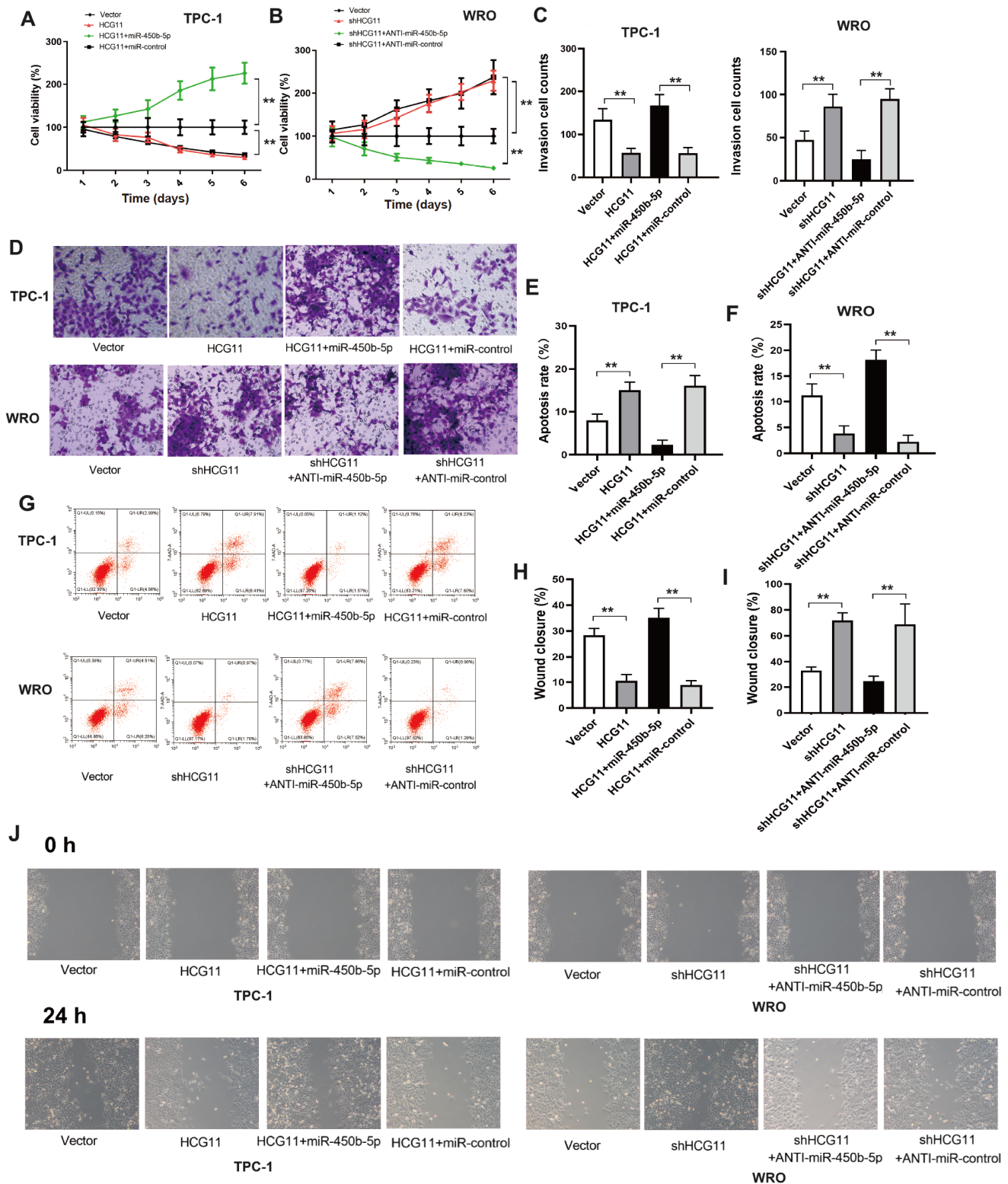


Figure 3. LncRNA HCG11 targets miR-450b-5p in thyroid cancer cells (A,B) Cell viability of lncRNA HCG11-overexpressing TPC-1 cells or lncRNA HCG11-knockdown WRO cells after incubation with miR-450b-5p or anti-miR-450b-5p. (C,D) Cell invasion of lncRNA HCG11-overexpressing TPC-1 cells or lncRNA HCG11-knockdown WRO cells after incubation with miR-450b-5p or anti-miR-450b-5p. (E-G) Apoptosis of lncRNA HCG11-overexpressing TPC-1 cells or lncRNA HCG11-knockdown WRO cells after incubation with miR-450b-5p or anti-miR-450b-5p. (H-J) Wound healing assay of lncRNA HCG11-overexpressing TPC-1 cells or lncRNA HCG11-knockdown WRO cells after incubation with miR-450b-5p or anti-miR-450b-5p. ** $P < 0.01$.

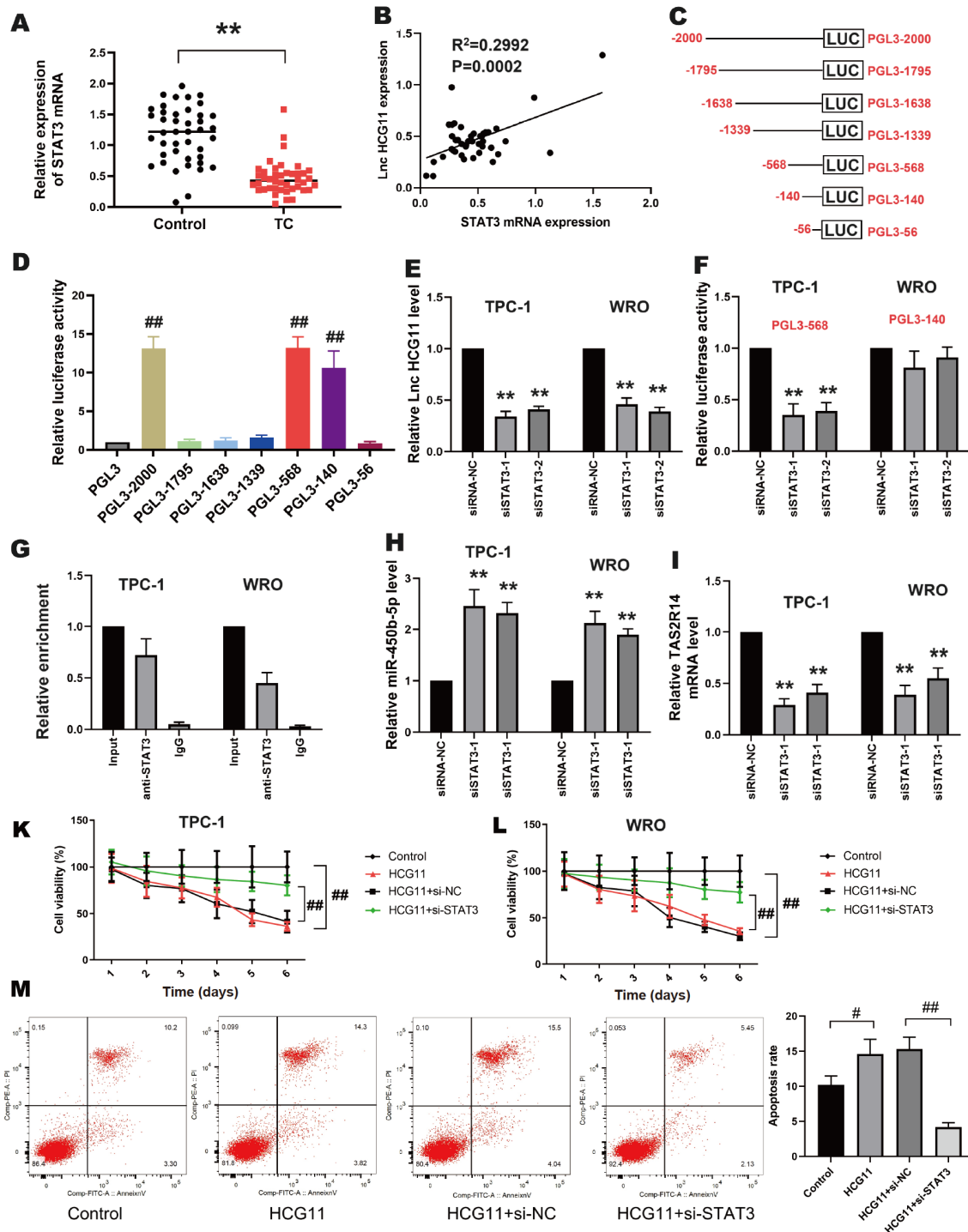


Figure 4. STAT3 regulates lncRNA HCG11 expression in thyroid cancer tissues and cells (A) Expression of STAT3 in thyroid cancer tissues and adjacent normal tissues. (B) Linear correlation between STAT3 expression and lncRNA HCG11 expression in thyroid cancer tissues and adjacent normal tissues. (C) Schematic diagram of the lncRNA HCG11 promoter fragments. These promoter fragments were subsequently cloned upstream of the firefly luciferase reporter gene in the pGL3-basic vector. (D) Transcriptional activity analysis of the potential lncRNA HCG11 promoter fragments in 293T cells. (E) Expression of lncRNA HCG11 in TPC-1 cells or WRO cells after STAT3 knockdown. *GAPDH* was used for normalization. (F,G) ChIP assay in TPC-1 cells or WRO cells, followed by quantitative PCR amplification of binding site 2 (–568 nt) within the lncRNA HCG11 promoter region. The genomic DNA input was 1%. (H) STAT3 can regulate the expression of miR-450b-5p. (I) STAT3 can regulate the expression of TAS2R14. (K) Viability of TPC-1 cells after they were subjected to lncRNA HCG11 overexpression and siRNA against STAT3. (L) Viability of WRO cells after they were subjected to lncRNA HCG11 overexpression and siRNA against STAT3. (M) The percentage of apoptotic TPC-1 cells after cells were subjected to lncRNA HCG11 overexpression and siRNA against STAT3. Data are presented as the mean $\pm$ SD of three independent experiments. ** $P < 0.01$ compared with siRNA-NC; # $P < 0.05$ compared with indicated groups; ## $P < 0.01$ compared with indicated groups.

lncRNA HCG11/TAS2R14 axis, we performed a biotin-coupled miRNA pull-down assay using streptavidin-coated beads. The cells were transfected with 3' end-biotin-labelled miR-450b-5p (Bi-has-miR-450b-5p), with control cells receiving a biotinylated *C. elegans* miRNA (Bi-cel-miR67). At 48 h post-transfection, we observed a significant enrichment of lncRNA HCG11 and TAS2R14 in the Bi-miR-450b-5p pull-down samples compared with the control samples, with lncRNA HCG11 showing over 3-fold enrichment and TAS2R14 showing approximately 4-fold enrichment (Figure 5H,I).

These findings demonstrate that lncRNA HCG11 modulates TAS2R14 expression, at least in part, through a miR-450b-5p-dependent mechanism by sharing miRNA response elements with TAS2R14. This regulatory interplay provides novel insights into the molecular underpinnings of thyroid cancer pathophysiology.

Validation of the effects of TAS2R14, lncRNA HCG11, and miR-450b-5p on thyroid tumor growth in a nude mouse model

To extend our findings *in vivo*, we established xenograft tumor

models via the overexpression or knockdown of TAS2R14 in TPC-1 cells and found that TAS2R14 inhibited tumor growth (Figure 6A, B). Furthermore, we established xenograft tumor models using TPC-1 cells overexpressing lncRNA HCG11 and WRO cells with lncRNA HCG11 knockdown. The mice were treated with miR-450b-5p or anti-miR-450b-5p every three days for a total of six treatments. On day 30, the mice were euthanized, and the tumors were harvested. As expected, lncRNA HCG11 knockdown was associated with increased tumor size and weight, while its overexpression attenuated tumor growth (Figure 6C–H). Notably, miR-450b-5p was found to counteract the tumor-suppressive effects of lncRNA HCG11 overexpression. Moreover, anti-miR-450b-5p administration mitigated the tumor growth induced by lncRNA HCG11 knockdown. In brief, these nude mouse models validated the effects of TAS2R14, lncRNA HCG11, and miR-450b-5p on thyroid tumor growth.

Collectively, these results underscore the role of STAT3-mediated upregulation of lncRNA HCG11 in inhibiting thyroid cancer progression by modulating miR-450b-5p and, consequently, reg-

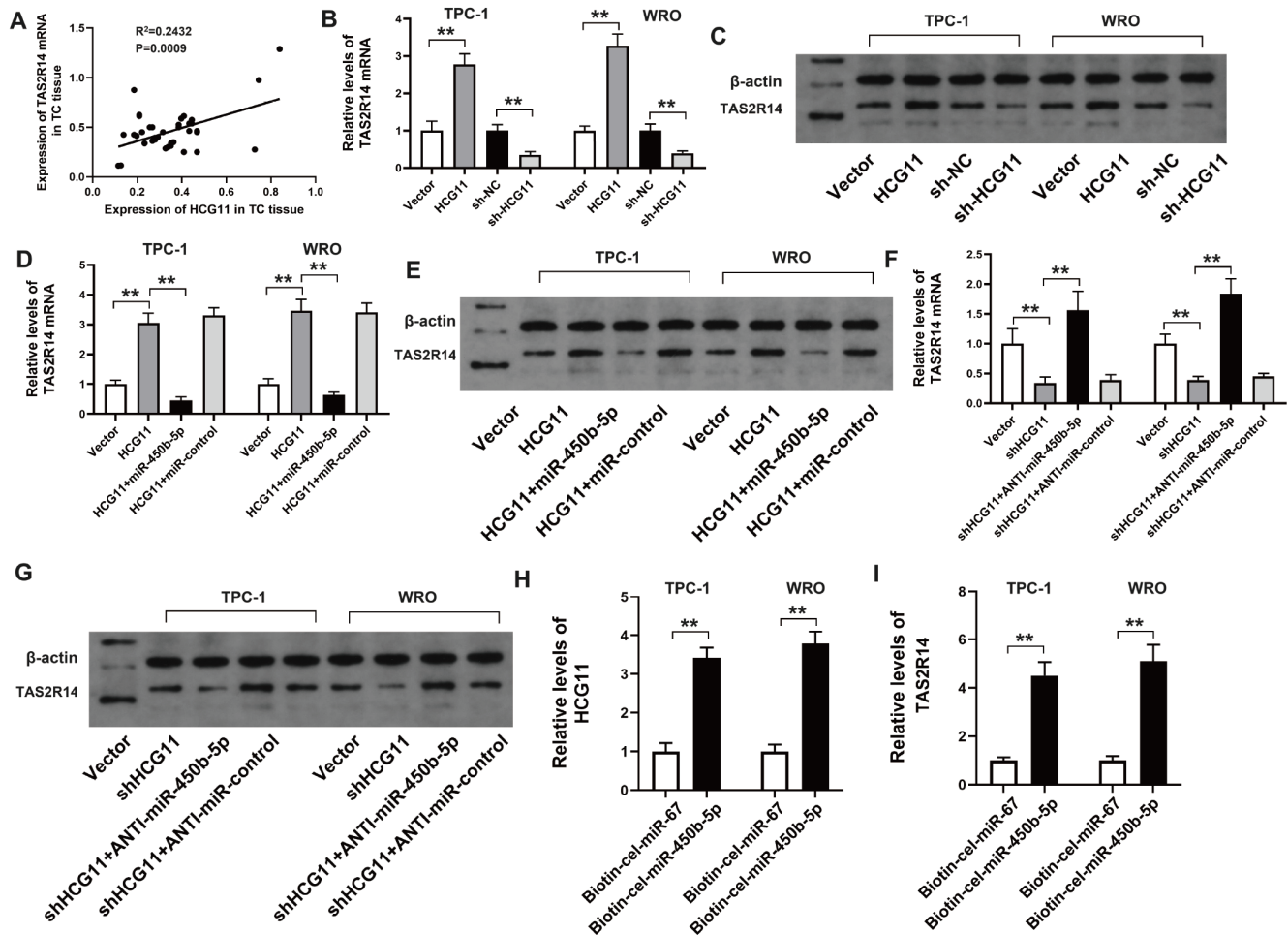


Figure 5. lncRNA HCG11 regulates TAS2R14 expression through a miR-450b-5p-dependent mechanism (A) Linear correlation between TAS2R14 expression and lncRNA HCG11 expression in thyroid cancer tissues and adjacent normal tissues. (B,C) Expression of TAS2R14 in TPC-1 cells or WRO cells after overexpression of lncRNA HCG11 or knockdown of lncRNA HCG11. *GAPDH* was used for normalization. (D,E) Expression of TAS2R14 in lncRNA HCG11-overexpressing TPC-1 cells or WRO cells after incubation with the miR-450b-5p mimic or anti-miR-450b-5p. *GAPDH* was used for normalization. (F,G) Expression of TAS2R14 in lncRNA HCG11-knockdown TPC-1 cells or WRO cells after incubation with the miR-450b-5p mimic or anti-miR-450b-5p. *GAPDH* was used for normalization. (H,I) The mRNA levels of lncRNA HCG11 or TAS2R14 were detected by biotin-coupled miR-450b-5p pull-down in TPC-1 cells and WRO cells. Data are presented as the mean $\pm$ SD of three independent experiments. $^{**}P < 0.01$.

ulating TAS2R14 (Supplementary Figure S5). This study provides a novel perspective on the intricate regulatory networks involved in thyroid cancer and highlights the potential therapeutic implications of targeting the lncRNA HCG11/miR-450b-5p/TAS2R14 axis.

Discussion

The TAS2Rs, a subset of the G-protein coupled receptor family, are integral to the perception of bitter compounds, with a total of 25 distinct TAS2Rs identified in humans that are crucial for this sensory function [28,29]. The interplay between bitter taste receptors and the process of oncogenesis has recently attracted significant attention, as indicated by an expanding corpus of scientific literature [30]. Although the correlation between TAS2R genes and a diminished susceptibility to cancer has been a contentious issue [31–34], the majority of studies converge on the notion that TAS2Rs play a tumor-suppressive role, albeit with the important proviso that these findings are still in their nascent stages. In addition to their sensory functions, TAS2Rs may also have broader implications for the transcriptomic landscape and behavior of acute myeloid leukaemia cells [35]. The involvement of TAS2Rs in the modulation of cell survival has been noted in the context of ovarian cancer [36]. Notably, TAS2R8 and TAS2R10 have shown anti-cancer stemness and anti-invasive properties in human neuroblastoma cells [37], whereas the activation of TAS2R4 and TAS2R14 has been correlated with the inhibition of proliferation and migration in breast cancer cells [38]. In our study, we advanced this nascent line of inquiry to include thyroid cancer, revealing that TAS2R14 exerts a suppressive influence on the proliferation, invasion, and migration of thyroid cancer cells. Notably, TAS2R14 facilitates ferroptosis (Figure 2), an emerging form of regulated cell death, within these cells. Our research further revealed a novel regulatory pathway in which the expression of TAS2R14 is modulated by the STAT3-regulated lncRNA HCG11 through a miR-450b-5p-dependent mechanism. This represents a ground-breaking revelation that the TAS2R family is subject to miRNA regulation and marks the pioneering discovery that the TAS2R family is influenced by lncRNAs. The complex interplay among TAS2R14, STAT3, lncRNA HCG11, and miR-450b-5p offers a compelling area for future investigations, with profound implications for the development of targeted therapeutics that could interrupt the pathogenesis of thyroid cancer.

Non-coding RNAs, encompassing lncRNAs and miRNAs, have emerged as pivotal regulators of various malignancies and are increasingly recognized as prognostic biomarkers in oncology [39]. The lncRNA HCG11, in particular, has been implicated in the modulation of an array of cancers, including non-small cell lung cancer [40], salivary adenoid cystic carcinoma [41], breast cancer [42], colorectal cancer [43], ovarian cancer [44], glioma [45], and osteosarcoma [46], among others. Similarly, miR-450b-5p has been identified as a participant in tumorigenesis and is involved in hepatocellular carcinoma [47], colorectal cancer [48], non-small cell lung cancer [49], nasopharyngeal carcinoma [50], glioblastoma [51], and cervical cancer [52]. In our study, we delineated a complex lncRNA-miRNA-mRNA regulatory network, within which lncRNA HCG11 appears to exert a significant influence on the development of thyroid cancer through its interactions with miR-450b-5p, thereby regulating the expression of TAS2R14. Our research revealed that the lncRNA HCG11 is under-expressed in thyroid cancer tissues and is subject to regulation by STAT3.

Furthermore, we elucidated that lncRNA HCG11 modulates the expression of TAS2R14 in a miR-450b-5p-dependent manner. Additionally, the upregulation of the lncRNA HCG11, which is mediated by STAT3, inhibits the progression of thyroid cancer by sequestering miR-450b-5p and, in turn, modulating TAS2R14. These findings suggest that the non-coding RNA-regulated TAS2R14-centered network may play a crucial role in the prognosis and pathogenesis of thyroid cancer, opening new avenues for the development of targeted therapeutic strategies for patients with this disease.

STAT3 is an intrinsically latent transcription factor pivotal for transducing extracellular signals from cytokines and growth factors via engagement with polypeptide receptors on the cell surface [53]. Its transformation to an active state is predominantly instigated by tyrosine phosphorylation. Upon activation, STAT3 dimerizes, translocates to the nucleus, and binds with specific DNA sequences, thereby initiating the transcription of downstream target genes [54]. Historically, STAT3 has been implicated in fostering the survival and proliferation of tumor cells within the context of RET/PTC tumorigenesis via NIH3T3 or TP53G199V signaling in anaplastic thyroid cancer cell lines. However, an emerging paradigm suggests that STAT3 can also function as a tumor suppressor across various thyroid cancer subtypes [55,56]. Notably, a recent study revealed that while shRNA-mediated knockdown of *STAT3* did not influence the *in vitro* growth of thyroid cancer cell lines with high STAT3 activity, it paradoxically led to the formation of larger tumors when STAT3-depleted cells were xenografted into mice. This finding is corroborated by another study demonstrating that STAT3 deficiency accelerates tumorigenesis in a murine model of B-RafV600E-induced papillary thyroid cancer [57]. In the context of tumor suppression, STAT3 has been shown to augment the transcription of the tumor suppressor insulin-like growth factor binding protein 7, thereby negatively regulating aerobic glycolysis and attenuating energy metabolism in cancer cells [57]. These findings underscore the dualistic role of STAT3 in the context of thyroid cancer. In our study, we elucidated that STAT3 is indispensable for the lncRNA HCG11-mediated suppression of thyroid cancer. Our findings underscore that STAT3 modulates the expression of lncRNAs in thyroid cancer tissues and cells. The induction of lncRNA HCG11 by STAT3 results in diminished expression of miR-450b-5p and the promotion of TAS2R14, consequently leading to the suppression of thyroid cancer. In essence, the STAT3-mediated upregulation of the lncRNA HCG11 impedes the progression of thyroid cancer by sequestering miR-450b-5p and regulating TAS2R14, offering novel insights into the targeting of this transcription factor to curb thyroid cancer progression. In addition to the STAT3–HCG11–miR-450b-5p–TAS2R14 axis, we also revealed other molecular mechanisms underlying thyroid carcinogenesis. TRMT13, a tRNA methyltransferase, has been shown to inhibit papillary thyroid cancer growth by targeting ANAPC4, a component of the APC/C complex involved in cell cycle regulation [58]. Interestingly, both TAS2R14 and TRMT13 are downregulated in thyroid cancer and exert tumor-suppressive effects through distinct yet potentially complementary pathways. Moreover, the tumor microenvironment (TME) plays a critical role in thyroid cancer progression. A recent study constructed a 5-gene prognostic model based on endothelial cell heterogeneity, highlighting genes such as PLAT that inhibit angiogenesis and tumor migration [59]. Although our study focused on cancer cell-intrinsic mechanisms, it would be worthwhile to investigate whether TAS2R14 expression influences TME remodelling, particularly

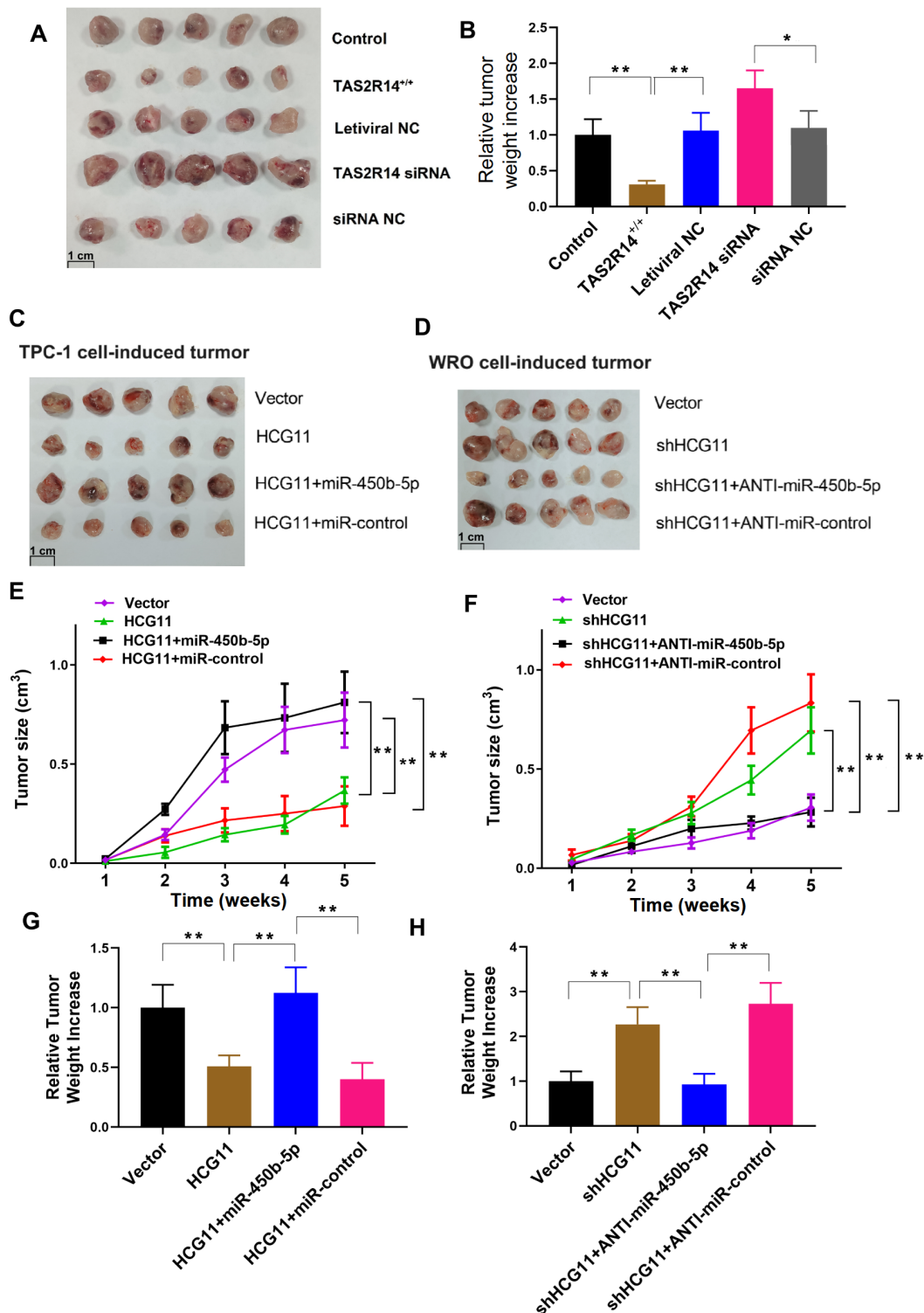


Figure 6. Subcutaneous tumor xenograft experiment in nude mice to validate the effects of TAS2R14, lncRNA HCG11, and miR-450b-5p on thyroid tumor growth (A,B) After intervention with TAS2R14 expression, the mice were euthanized at week 5, the tumors were excised and photographed, and the tumor weights were measured. (C,D) After simultaneous intervention with lncRNA HCG11 and miR-450b-5p expression, the mice were treated at week 5, and the tumors were excised and photographed. (E,F) After simultaneous intervention with lncRNA HCG11 and miR-450b-5p expression, the volume of subcutaneous tumors was measured weekly. (G,H) After simultaneous intervention with lncRNA HCG11 and miR-450b-5p expression, the mice were treated at week 5, and the tumors were excised and weighed. * $P < 0.05$, ** $P < 0.01$, $n = 5$.

angiogenesis or immune infiltration, and whether it is correlated with the endothelial-based prognostic signature. Integrating TME-focused biomarkers with cancer cell-specific markers such as TAS2R14 may increase prognostic accuracy and inform combination therapies targeting both compartments.

As a result, our study illuminates a novel aspect of function and signaling within the bitter taste receptor family that was previously unrecognized, enhancing our understanding of their role in disease mechanisms (Supplementary Figure S5). However, we acknowledge that systemic induction of ferroptosis could lead to off-target effects and toxicity, given the crucial role of iron metabolism and redox balance in normal physiology. Specifically, non-specific activation of TAS2R14 might affect bitter taste perception or other extra-gustatory functions of TAS2Rs in various tissues. As a result, we may need to develop tissue-specific delivery systems (e.g., nanoparticle-based targeting or thyroid-specific promoters) to minimize systemic exposure. Second, we want to emphasize the importance of developing highly specific TAS2R14 agonists that do not cross-react with other TAS2Rs or GPCRs. Third, developing combination therapies that lower the required doses of TAS2R14 agonists by synergizing with other ferroptosis inducers or standard chemotherapeutic agents may be a better idea. Notably, further preclinical studies in appropriate animal models are essential for evaluating the efficacy and safety of TAS2R14-targeted therapies. In addition, anaplastic thyroid carcinoma (ATC) is a highly aggressive and lethal form of thyroid cancer with limited treatment options and a poor prognosis. Given that TAS2R14 promotes ferroptosis (a form of regulated cell death) and inhibits proliferation and migration in differentiated thyroid cancer models, it is plausible that a loss or alteration of TAS2R14 function could contribute to the aggressive phenotype of ATC. The STAT3/HCG11/miR-450b-5p axis we identified might also be dysregulated in ATC. Investigating the expression and tumor-suppressive function of TAS2R14, as well as the feasibility of inducing ferroptosis through this receptor in ATC models, represents a critical and immediate direction for our future research.

In summary, our study highlights the pivotal role of TAS2R14 in thyroid carcinogenesis. Our findings indicate that TAS2R14 suppresses cell proliferation, invasion, and migration while simultaneously promoting ferroptosis in thyroid cancer cells. Furthermore, we elucidated that the expression of TAS2R14 is modulated by the STAT3-driven lncRNA HCG11-miR-450b-5p regulatory axis. This research diverges from established paradigms by revealing the involvement of the TAS2R family in the induction of ferroptosis in cancer cells. Consequently, our investigation sheds new light on a previously unappreciated function and signaling pathway within the bitter taste receptor family, thereby broadening our comprehension of their contribution to disease pathophysiology.

Supplementary Data

Supplementary data is available at *Acta Biochimica et Biophysica Sinica* online.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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