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TSH Increases Iodine Uptake by Thyroid Cancer Cells During BRAF Silencing

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Abstract

BACKGROUND—The BRAF^{V600E} mutation is present in 62% of radioactive iodine resistant (RAIR) thyroid tumors and is associated with down-regulation of the sodium-iodide-symporter (NIS) and TSH-receptor (TSHr). We sought to evaluate the combined effect of BRAF inhibition and TSH supplementation on ¹³¹I uptake of BRAF^{V600E}-mutant human thyroid cancer cells.

MATERIALS AND METHODS—WRO cells (a BRAF^{V600E}-mutant follicular-derived papillary thyroid carcinoma cell line) were transfected with siRNA targeting BRAF for 72 hours in a physiologic TSH environment. NIS and TSHr expression were then evaluated at three levels: gene expression, protein levels, and ¹³¹I uptake. These three main outcomes were then reassessed in TSH-depleted media and media supplemented with supratherapeutic concentrations of TSH.

RESULTS—*NIS* gene expression increased 5.5-fold 36 hours after transfection ($p=0.01$) and *TSHr* expression increased 2.8-fold at 24 hours ($p=0.02$). NIS and TSHr protein levels were similarly increased 48 and 24 hours post-transfection, respectively. Seventy two hours after BRAF inhibition, ¹³¹I uptake showed was unchanged in TSH-depleted media, increased by 7.5-fold ($p<0.01$) in physiologic TSH media, and increased by 9.1-fold ($p<0.01$) in supratherapeutic TSH media.

CONCLUSIONS—The combined strategy of BRAF inhibition and TSH supplementation results in greater ¹³¹I uptake than when either technique is utilized alone. This represents a simple and feasible approach that may improve outcomes in patients with RAIR thyroid carcinomas for which current treatment algorithms are ineffective.

Keywords

Papillary thyroid cancer; BRAF(V600E) mutation; radioactive iodine; radioactive iodine resistance; thyroid stimulating hormone; sodium-iodine symporter

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INTRODUCTION

The loss of radioactive iodine (RAI) uptake in differentiated thyroid carcinomas accounts for significant morbidity and mortality of this increasingly common disease [1, 2]. Roughly 25% of primary well-differentiated thyroid tumors and 50% of metastases are RAI resistant (RAIR) and effective adjuvant medical therapies following thyroidectomy are limited [3].

The efficacy of RAI ablation following thyroidectomy is dependent on active intracellular transport and trapping of iodine by the sodium-iodide symporter (NIS) [4, 5]. NIS is a basolateral membrane protein that is regulated by a host of iodine metabolizing proteins such as the TSH receptor (TSHr), which controls transcription and posttranslational modification of NIS [6, 7]

A rapidly growing body of literature has implicated members of the MAPK signaling pathway, of which BRAF is the strongest activator, with tumorigenesis of aggressive thyroid carcinomas [8]. The BRAF^{V600E} mutation is present in 40–50% of all papillary thyroid cancers (PTC) and is associated with aggressive features such as extrathyroidal extension, lymph node metastases, advanced tumor stage, and radioactive iodine resistance [9–11]. It is thought that the loss of responsiveness to ¹³¹I is due to loss of function of iodine metabolizing proteins such as NIS and TSHr [3, 12, 13]. Tumor cells harboring this mutation have decreased *NIS* and *TSHr* gene expression compared to similar cells without the mutation [12, 14–17]. Several recent *in vitro* and *in vivo* mouse studies have demonstrated that BRAF inhibition with small-molecule MAPK pathway inhibitors restores expression of iodine metabolizing proteins and increases susceptibility to RAI [18, 19].

It has also been shown that increasing circulating TSH to supratherapeutic levels prior to treatment with RAI increases its potency [20–22]. It is believed that TSH enhances the function of the sodium-iodide symporter and results in increased ¹³¹I uptake [7]. For this reason, many centers routinely administer human recombinant TSH or withhold thyroid hormone prior to ¹³¹I therapy in order to achieve supratherapeutic TSH levels at the time of treatment [22–24].

Thus, it has been independently demonstrated that both BRAF inhibition and TSH supplementation can increase RAI uptake in RAIR tumors, however the additive potential of implementing both strategies simultaneously has not been reported. We hypothesized that supplementing BRAF^{V600E}-mutant human thyroid cancer cells with supratherapeutic concentrations of TSH while transiently inhibiting BRAF would result in greater ¹³¹I uptake compared to BRAF inhibition in lower TSH environments.

MATERIALS AND METHODS

Patient selection and analysis

First, to evaluate the prevalence of the BRAF^{V600E} mutation and its effect on tumor behavior and levels of *NIS* and *TSHr*, a representative cohort of patients who underwent surgery for papillary thyroid carcinoma between 2003 and 2010 was selected. After obtaining approval from our Institutional Review Board and written informed consent from each patient, tumor and representative normal tissue samples from the contralateral lobe were collected. All samples were snap-frozen in liquid nitrogen and stored at –80°C until analysis. A retrospective review of a prospectively maintained patient database was performed for age, sex, and histopathologic features. All surgical specimens were reviewed by an endocrine pathologist.

Cell culture

The BRAF^{V600E}-mutant human follicular thyroid carcinoma-derived cell line WRO was used in this study. Of note, both BRAF^{V600E}-mutant and wild-type WRO cells have been reported and it is accepted that two distinct cell lines have been distributed [25]. The WRO cells used in this report were confirmed to be the BRAF^{V600E} variety. These cells were a generous gift from Dr. James A. Fagin (Memorial Sloan-Kettering, NY, USA) and were validated using short tandem repeat and single nucleotide polymorphism array analysis in 2008 [25].

Cell culture media preparation

In order to investigate the role of TSH in iodine uptake, cells were maintained in three different types of media: H6 (physiologic TSH concentration), H5 (no TSH), and supratherapeutic TSH media. H6 medium was made using HamsF12:DMEM supplemented with insulin (10 µg/ml), human transferrin (5 µg/ml), somatostatin (1 mg/ml), glycyl-L histidyl-L-lysine acetate (2 ng/ml), hydrocortisone (0.36 ng/ml), TSH (10 mU/ml), penicillin G (100 IU/ml), streptomycin sulfate (100 µg/ml), and amphotericin B (0.25 µg/ml). H5 medium contained the same components as H6 except without TSH. Supratherapeutic TSH media also contained the same components as H6 except for supplementation with 500 mU/ml of TSH. This concentration is similar to that which is achieved after administration of recombinant TSH prior to RAI ablation.

Small interfering RNA plasmids and transfection

Two oligonucleotides targeting an area of the *BRAF* gene outside of the V600E mutation site consisting of ribonucleosides with the presence of 2'-deoxyribonucleosides at the 3' end, 5'-(CGAGACCGAUCCUCAUCAG)d(TT)-3' and 5'-r(CUGAUGAGGAUCGGUCUCG)d(TT)-3' were synthesized and annealed as previously described (Qiagen, Valencia, CA, USA) [26]. Cells were transfected using Lipofectamine (HiPerFect Transfection Reagent, Qiagen, Valencia, CA, USA). Transfection experiments were performed using Fast-Forward Transfection protocol according to the manufacturer. 3.5×10^5 cells were seeded into a 6-well plate. Cells were exposed to 10 nmol, 20 nmol and 40 nmol of siRNA for up to 96 hours. Positive controls and transfection efficiency were measured with AllStars Hs Cell Death Control siRNA (Qiagen, Valencia, CA, USA). Negative control siRNA transfection was performed using a nonsilencing siRNA (AllStars Negative Control siRNA, Qiagen, Valencia, CA, USA).

RNA extraction, reverse transcription and real-time PCR

RNA was extracted from cells and frozen tissue by homogenization in RNeasy Lysis Buffer (Buffer RLT, Qiagen, Valencia, CA, USA) using the manufacturer's instructions (RNeasy Mini Kit; Qiagen, Valencia, CA, USA). RNA purity was confirmed by spectrophotometry.

First-strand cDNA synthesis was performed using one microgram of each RNA sample primed with SuperScriptTM First-Strand Synthesis system, Oligo (dT)¹²⁻¹⁸ primer, random hexamers and superscript II reverse transcriptase (Invitrogen, Carlsbad, CA). A reaction mixture containing 2.5 µl of cDNA template, 12.5 µl TaqMan Universal PCR master mix (Applied Biosystems, Foster city, CA, USA), and 1.25 µl primer probe mixture was amplified using the following thermal cycler parameters: incubation at 50°C for 2 minutes and denaturing at 95°C for 10 minutes, then 40 cycles of the amplification step (denaturation at 95°C for 15 seconds and annealing/extension at 60°C for 1 minute). *BRAF*, *NIS*, and *TSHr* gene expression were measured in triplicate and normalized relative to the house-keeping gene β -glucuronidase (Applied Biosystems, Foster city, CA, USA). The mean of the

reference normalized expression measurements (ΔC_t) in triplicate was used for statistical analysis. Gene expression values were calculated according to the $\Delta\Delta C_t$ method.

BRAF^{V600E} mutation analysis

gDNA was extracted from cells and tumor samples using the QIAamp DNA Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. The following primers adapted from those previously reported [27] were used as a template for a standard PCR reaction: forward 5'-TGCTTGCTCTGATAGGAAAATG-3', reverse 5'-GACTTTCTAGTAACTCAGCAGC-3'. The PCR product was purified using the QIAquick PCR Purification Kit (Qiagen, Valencia, CA, USA) and visualized on a 2% agarose gel, which confirmed the presence of a single band of approximately 238 base pairs. The PCR product was then direct-sequenced using an Applied Biosystems Automated 3730xl DNA analyzer (Biotechnology Resource Center of Cornell University, Ithaca, NY, USA).

Western Blots

Protein was extracted with Radioimmunoprecipitation assay (RIPA) Lysis buffer (Santa Cruz Biotechnology, Santa Cruz, CA, USA) containing protease inhibitors. Protein concentration was determined by the Pierce BCA assay method according to the manufacturer's protocol (Thermo Scientific, Waltham, MA, USA). Fifty μ g of protein per lane was loaded on a 12% SDS-PAGE gel. After a semi-dry transfer onto nitrocellulose membrane, blots were incubated in a 5% milk in 0.05% Tris buffer saline solution with tween (TSBT) blocking solution for 1 hour. Membranes were probed overnight at 4°C with primary antibodies targeting NIS (1:200), TSH (1:1000), or phospho-ERK (1:200) in 5% milk in 0.05% TBST (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Blots were then incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies (Santa Cruz Biotechnology, Santa Cruz, CA, USA) as appropriate for 1 hour at room temperature. Immunoreactive bands were visualized using Amersham ECL Enhanced Chemiluminescence System (GE Healthcare, Piscataway, NJ, USA). Blots were then stripped with 10 mL of Restore PLUS Western Blot Stripping Buffer (Thermo Scientific, Rockford, IL, USA) and re-probed for either GAPDH (Abcam, Cambridge, MA, USA, 1:10000) or β -actin (Cell Signaling, 1:1000) as loading controls.

Radionucleotide Uptake *in vitro*

Radioactive iodine uptake was measured according to the protocol used by Haddad *et al* with slight modifications [28]. ^{131}I was measured at 24, 48, 72 and 96 hours after siRNA transfection. Cells were trypsonized and 5×10^5 cells were transferred into each well of a 12-well plate in 1 ml of medium. One μCi of ^{131}I was added to each well and incubated for 30 minutes. After completion of incubation time, 0.5 ml of fresh medium was added into each well. Cells were then centrifuged and the supernatant was transferred into a second test tube at which point the cells were washed with PBS three times. Tubes were then weighed (#1 containing the pellet of cells, #2 containing media, #3 containing wash 1, and #4 containing wash #2) and radiation was recorded by a Wizard scintillation well counter. A Pierce BCA protein assay was performed in parallel in order to measure protein concentration. Results were normalized to protein milligrams and expressed as cpm/mg.

Statistical analysis

Data was presented as mean $\pm$ standard deviation (normally distributed continuous variables), median and range (non-normally distributed continuous variables), % change, or fold-change of gene expression. Significance was assessed using Fisher's exact test, student *t*-test, or Wilcoxon rank sum test as appropriate. Statistical significance was set at $p=0.05$.

Statistical analysis was performed using SPSS 18.0 statistical software (Cornell University, NY).

RESULTS

BRAF^{V600E} MUTATION AND THYROID IODINE METABOLIZING GENE EXPRESSION IN A PATIENT POPULATION

A representative cohort of 47 patients who underwent a thyroidectomy for cancer was selected (Table 1). The BRAF^{V600E} mutation was detected in 24 of the 47 (51%) of the malignant tumors. The presence of extra-thyroidal extension was significantly higher (68% vs. 32%, $p=0.020$) and the prevalence of the follicular variant of papillary thyroid carcinoma (fvPTC) was significantly lower (18.2% vs. 81.8%, $p=0.017$) among tumors carrying the BRAF^{V600E} mutation. There was no association between the presence of the BRAF^{V600E} mutation and age, sex, tumor size, lymph node metastases, angiolymphatic invasion, and multifocality.

Mean gene expression of *NIS* and *TSHr* was then measured from 10 of the tumors that carried the BRAF^{V600E} mutation and 10 BRAF^{WT} tumors. Tumors carrying the BRAF^{V600E} mutation had significantly lower gene expression of *NIS* ($\Delta\text{CT}\pm\text{SD } 0.27\pm 0.03$ vs. 0.78 ± 0.09 , $p < 0.01$) and *TSHr* ($\Delta\text{CT}\pm\text{SD } 0.42\pm 0.09$ vs. 0.71 ± 0.02 , $p < 0.01$) compared to BRAF^{WT} tumors (Table 1).

EFFECT OF BRAF KNOCKDOWN IN HUMAN THYROID CANCER CELLS IN A PHYSIOLOGIC (CONTROL) TSH ENVIRONMENT

Transient knockdown of BRAF in human thyroid cancer cells—First, to establish the effectiveness of siRNA transfection for inhibiting BRAF and restoring *NIS* and *TSHr* expression, transfections were carried out in a physiologic (control) TSH environment using three different siRNA doses (10 nmol, 20 nmol, and 40 nmol). Significant differences in *BRAF*, *NIS*, and *TSHr* expression were noted with all three concentrations compared to negative controls, but there were no significant differences among the different concentrations. For simplicity, only the results of the 20 nmol transfections are presented (results of the 10 nmol and 40 nmol transfections are available upon request).

BRAF gene expression was decreased by an average of 51% ($p < 0.01$) 24 hours post-transfection (Figure 1A). Inhibition was first detected at 12 hours and returned to near-baseline levels at 36 hours. To measure *BRAF* activity at the protein level, Western blots for P-ERK (the main downstream actuator of the MAP/MEK/ERK cascade) were performed at baseline, 24, 48, and 72 hours after siRNA transfection. A transient 27% decrease in P-ERK protein expression occurred at 24 hours and returned to slightly above baseline by 72 hours (Figure 1B).

Restoration of *NIS* and *TSHr* gene expression and protein levels following siRNA transfection—To evaluate the effect of BRAF inhibition on iodine metabolizing proteins, *NIS* and *TSHr* gene expression and protein levels were measured pre- and post-transfection. *NIS* gene expression increased by an average of 3.9 fold ($p=0.01$) 36 hours after siRNA transfection relative to non-transfected cells and returned to near-baseline by 48 hours (Figure 2A). *NIS* Western blots performed at 24, 48, and 72 hours post-transfection revealed a band at 50 kDa (non-glycosylated *NIS*) and an additional wide band of approximately 87–150 kDa (glycosylated *NIS*), as has been described previously [29]. *NIS* protein levels increased by 50% 48 hours and returned to baseline by 72 hours (Figure 2B)

TSHr gene expression increased by an average of 2.5 fold ($p=0.02$) 24 hours post-transfection (Figure 2C). TSHr protein levels similarly increased at 24 hours compared to pre-transfection levels and returned to baseline by 48 hours (Figure 2D).

EFFECT OF TSH WITHDRAWAL AND SUPPLEMENTATION ON IODINE UPTAKE FOLLOWING BRAF INHIBITION

Effect of TSH withdrawal on iodine metabolizing genes—WRO cells grown in H5 media (no TSH) were found to have significantly lower *NIS* gene expression 36 hours after transfection than cells grown in H6 media (physiologic TSH concentration) ($\Delta\text{CT}\pm\text{SD}$ 2.6 ± 0.02 vs. 4.7 ± 0.07 , $p=0.02$) (Figure 3A). Similarly, *TSHr* gene expression was significantly lower in cells grown in H5 media compared to cells grown in H6 media 24 hours after transfection in WRO ($\Delta\text{CT}\pm\text{SD}$ 1.4 ± 0.01 vs. 2.2 ± 0.07 , $p=0.03$) (Figure 3B).

Effect of TSH concentration on ^{131}I uptake after BRAF inhibition—TSH concentration was found to have a significant dose-dependent effect on ^{131}I uptake following siRNA transfection (Figure 4). WRO cells grown in H5 media (no TSH) showed no increase in ^{131}I uptake 72 hours after siRNA transfection compared to non-transfected cells, while WRO cells grown in H6 media (physiologic TSH) showed a 7.5-fold ($p<0.01$) increase at the same time-point. Furthermore, supplementing the media with supratherapeutic TSH resulted in an even greater 9.1-fold increase of ^{131}I uptake ($p<0.01$) at 72 hours compared to non-transfected cells. The maximal ^{131}I uptake that was achieved in the supratherapeutic TSH environment was 1.7-fold higher than the maximum uptake achieved in physiologic TSH conditions ($p<0.01$). ^{131}I uptake was not significantly changed when cells grown in any of the three conditions were not transfected or were transfected with non-silencing siRNA (Figure 4).

DISCUSSION

RAIR thyroid cancers present a deadly dilemma for patients and clinicians, as currently alternate medical treatments for local recurrences and metastases are limited [24]. Since the $\text{BRAF}^{\text{V600E}}$ mutation is present in the majority of RAIR tumors, it is an attractive candidate for the development of targeted molecular therapies.

In the first part of this study, we demonstrated in a small representative patient population that the $\text{BRAF}^{\text{V600E}}$ mutation is associated with aggressive clinical features and decreased expression of *NIS* and *TSHr*. Both of these observations have been reported previously [30–34] and are included to provide a clinical context for the subsequent *in vitro* data.

The main aim of this study was to determine if the combination of *BRAF* inhibition and TSH supplementation results in greater cellular iodine uptake than implementation of each strategy independently. This multimodal approach utilizes two strategies that already have been established or are currently under clinical investigation independently and thus offers a simple and feasible strategy to restore susceptibility of RAIR tumors to ^{131}I . To inhibit *BRAF*, we elected to use siRNA against the *BRAF* gene because of the highly specific inhibition of *BRAF* that is achieved. The same specificity is not achieved with similar strategies that use tyrosine kinase inhibitors or small molecules MAPK pathway inhibitors such as PLX4720.

To quantify activity of the RAF/MEK/ERK pathway at the protein level following *BRAF* inhibition, we elected to measure P-ERK levels rather than BRAF. P-ERK lies downstream of BRAF and activates hundreds of other transcription factors during activation of the cascade [35, 36]. Therefore, measuring this step of the pathway provides the most direct assessment of its overall activity. In addition, Western blots for P-ERK are more reliable and

more reproducible than Western blots for BRAF and therefore, P-ERK is more suitable for use in this study. Although a profound inhibitory effect on BRAF and P-ERK was not observed with siRNA transfection, the inefficiency of knockdown of this pathway has previously been reported. Galabova-Kovacs and Matzen *et al* demonstrated that knocking out *BRAF* in mouse embryos resulted in virtual absence of P-ERK in the placenta but only a 60% reduction in the embryo [37]. Pritchard *et al* similarly observed an incomplete reduction of P-ERK levels in fibroblasts of *BRAF*-knockout mice [38]. These findings have led to the conclusion that a complex array of cell signaling and kinase activity occurs along multiple steps of the RAF/MEK/ERK pathway that can circumvent BRAF-dependent kinase activity [39]. Thus, targeting a single step along this pathway in an attempt to abrogate downstream activity has proven to be a difficult and often inefficient endeavor.

Increasing expression of iodine metabolizing genes by knocking down BRAF is not, by itself, a novel concept. In 2007, Liu *et al* observed restoration of *TSHr* and *NIS* expression in *BRAF*^{V600E}-mutant human papillary thyroid cancer cells after transfection with U0126, a direct MEK-pathway inhibitor [40]. More recently, Chakravarty *et al* demonstrated in mice engineered with doxycycline-inducible *BRAF*^{V600E} expression that activation of the BRAF mutation results in near-complete abolition of several iodine metabolizing genes including *NIS* and *TSHr* (23). Expression of these genes as well as ¹²⁴I thyroid uptake was partially recovered by switching off the *BRAF*^{V600E} mutation and also by treating the mice with PLX4720, a small molecule BRAF inhibitor. Interestingly, dox-induction of *BRAF*^{V600E} also rendered the mice hypothyroid and significantly increased plasma TSH concentrations at the time of the iodine-uptake assays. Although the mice returned to a euthyroid state two weeks after doxycycline withdrawal, iodine uptake was not reassessed at that time and, therefore, the influence of the elevated TSH is unclear from this study.

There are two main limitations to this paper. First, the siRNA utilized in this study targets a region of the BRAF gene outside of the V600E mutation site and thus silences BRAF wild-type and mutant cells equally. It is therefore unclear if these results are unique to *BRAF*^{V600E} mutants or could be observed in BRAF wild-type cells as well. Second, the effect of the siRNA transfection on BRAF gene expression was modest, and the observed changes at the protein level were even smaller. As noted above, the intricacies of the RAF/MEK/ERK pathway are not yet completely understood and it is believed that multiple escape pathways exist involving other members of the RAF family of kinases to continue MEK and ERK phosphorylation despite BRAF inhibition.

In conclusion, we have demonstrated that the simultaneous implementation of two independent treatment strategies for RAI thyroid tumors (BRAF inhibition and TSH supplementation) increases ¹³¹I uptake more effectively than when either modality is implemented alone. This promising approach represents a substantial advancement in the treatment of RAI thyroid cancers and may offer a new option for patients who have failed our current treatment algorithms.

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Figure 1.

Knockdown of BRAF expression following transfection of siRNA in WRO human thyroid cancer cells. (A) Total RNA was quantified by qPCR at four different time points following transfection with 20 nmol of siRNA and compared to non-transfected cells and transfection with non-silencing siRNA. WRO cells exhibited a 51% ($p < 0.01$) decrease in *BRAF* gene expression 24 hours post-transfection. This inhibitory effect was first observed at 12 hours and returned to near-baseline by 48 hours. (B) Western blot of P-ERK (a downstream actuator of the RAF/MEK/ERK signal pathway) showing a 27% reduction of protein levels when normalized to GAPDH 24 hours post-transfection and recovery of pre-transfection levels by 48 hours.

Figure 2.

Changes in NIS and TSHr gene expression and protein levels following transfection of 20 nmol of siRNA in WRO cells compared to non-transfected cells and transfection with non-silencing siRNA in a physiologic (control) TSH environment. (A) *NIS* gene expression increased by 3.9 fold ($p=0.01$) 36 hours after transfection with 20 nmol of siRNA. (B) Western blot of NIS showing a 50% increase of protein levels 48 hours post-transfection that returned to baseline by 72 hours. (C) *TSHr* gene expression increased by 2.5 fold ($p=0.02$) 24 hours post-transfection with 20nmol of siRNA. (D) Western blot of TSHr showing increased protein levels at 24 hours post-transfection which returned to baseline by 48 hours.

Figure 3.

The effect of ambient TSH on *NIS* and *TSHr* gene expression following BRAF silencing. A) WRO cells grown in H6 media (physiologic TSH concentration) were found to have significantly increased *NIS* recovery 36 hours after transfection with siRNA than cells grown in H5 (TSH-free) media (4.7 ± 0.07 vs. 2.6 ± 0.02 , $p=0.02$). B) *TSHr* gene expression recovery was also significantly higher in cells grown in H6 media compared to cells grown in H5 media 24 hours after transfection in WRO cells (2.2 ± 0.07 vs. 1.4 ± 0.01 , $p=0.03$).

Figure 4.

^{131}I uptake of WRO cells following transfection of 20 nmol of siRNA^{BRAF} when grown in three different TSH environments: no TSH (H5), physiologic TSH (H6), and media supplemented with 50x the physiologic concentration of TSH (suprathreshold). ^{131}I uptake did not increase in TSH-free media, increased by 7.5-fold ($p<0.01$) in physiologic TSH media, and increased by 9.1 fold ($p<0.01$) in suprathreshold TSH media 72 hours after siRNA transfection. Non-transfected WRO cells and WRO cells transfected with non-silencing siRNA grown in all three media conditions are shown as negative controls.

Table 1

Patient Demographics, Histopathologic Features, and Iodine Metabolizing Gene Expression.

	Total (N=47)	BRAF ^{V600E} (N=24)	BRAF ^{wt} (N=23)	P-value*
PATIENT DEMOGRAPHICS				
Age (mean±SD)	44.2±13.9	46.0±15.2	42.2±12.5	0.35
Gender				
Male (N=12)	12 (25.5%)	7 (58.3%)	5 (41.7%)	0.74
Female (N=35)	35 (74.5%)	17 (48.6%)	18 (53.3%)	
HISTOPATHOLOGIC FEATURES				
Tumor size in cm [median (range)]	1.5 (0.7–8.0)	1.9 (0.8–8.0)	1.2 (0.7–4.5)	0.12
Histologic Subtypes				
Papillary carcinoma (PTC)	24 (51.1%)	13 (54.2%)	11 (45.8%)	0.77
Follicular variant of PTC	11 (23.4%)	2 (18.2%)	9 (81.8%)	0.02
Tall cell variant of PTC	6 (12.8%)	5 (83.3%)	1 (16.7%)	0.19
Follicular carcinoma	2 (4.3%)	0 (0%)	2 (100%)	0.23
Poorly differentiated	4 (8.5%)	4 (100%)	0 (0%)	0.11
Extrathyroidal extension	25 (53.2%)	17 (68%)	8 (32%)	0.02
Lymphovascular invasion	11 (23.4%)	7 (63.4%)	4 (36.4%)	0.49
Multifocality	18 (38.3%)	9 (50.0%)	9 (50.0%)	1.00
Lymph node metastases	20 (42.6%)	11 (55.0%)	9 (45.0%)	0.77
IODINE METABOLIZING GENE EXPRESSION**				
<i>NIS</i>	0.53 ± 0.35	0.27 ± 0.03	0.78 ± 0.09	<0.01
<i>TSHr</i>	0.62 ± 0.05	0.42 ± 0.09	0.71 ± 0.02	<0.01

*p-values were derived using student's *t*-test (age and gene expression), Wilcoxon rank-sum test (tumor size), and Fisher's exact chi-squared (all other variables).

** Gene expression given as $\Delta\Delta$ CT±SD.