CYP2S1 is a synthetic lethal target in BRAF$^{V600E}$-driven thyroid cancers

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BRAF$^{V600E}$ is the most common genetic alteration and has become a major therapeutic target in thyroid cancers; however, intrinsic feedback mechanism limited clinical use of BRAF$^{V600E}$ specific inhibitors. Synthetic lethal is a kind of interaction between two genes, where only simultaneously perturbing both of the genes can lead to lethality. Here, we identified CYP2S1 as a synthetic lethal partner of BRAF$^{V600E}$ in thyroid cancers. First, we found that CYP2S1 was highly expressed in papillary thyroid cancers (PTCs) compared to normal thyroid tissues, particularly in conventional PTCs (CPTCs) and tall-cell PTCs (TCPTCs), and its expression was positively associated with BRAF$^{V600E}$ mutation. CYP2S1 knockdown selectively inhibited cell proliferation, migration, invasion and tumorigenic potential in nude mice, and promoted cell apoptosis in BRAF$^{V600E}$ mutated thyroid cancer cells, but not in BRAF wild-type ones. Mechanistically, BRAF$^{V600E}$-mediated MAPK/ERK cascade upregulated CYP2S1 expression by an AHR-dependent pathway, while CYP2S1 in turn enhanced transcriptional activity of AHR through its metabolites. This AHR/CYP2S1 feedback loop strongly amplified oncogenic role of BRAF$^{V600E}$ in thyroid cancer cells, thereby causing synthetic lethal interaction between CYP2S1 and BRAF$^{V600E}$. Finally, we demonstrated CYP2S1 as a potential therapeutic target in both BRAF$^{V600E}$-drived xenograft and transgenic mouse models by targetedly delivering CYP2S1-specific siRNA. Altogether, our data demonstrate CYP2S1 as a synthetic lethal partner of BRAF$^{V600E}$ in thyroid cancers, and indicate that targeting CYP2S1 will provide a new therapeutic strategy for BRAF$^{V600E}$ mutated thyroid cancers.

INTRODUCTION
The incidence rate of thyroid cancer increased rapidly all over the world since the 1970s, until now it has become the most common endocrine malignancy.1 Mitogen-activated protein kinase/extracellular signal-regulated protein kinase (MAPK/ERK) signaling is highly selected in thyroid cancers, particularly in papillary thyroid cancers (PTCs). Among MAPK/ERK pathway-related genetic alterations, BRAF$^{V600E}$ mutation is the most common in PTCs.2 However, unlike melanoma, which is known as another kind of BRAF$^{V600E}$ mutation dominated malignancy, patients diagnosed with BRAF$^{V600E}$-mutated thyroid cancers seldom benefit from BRAF$^{V600E}$ specific small-molecule inhibitors because of the existence of HER3 feedback activation.3 Thus, it is pressing to develop an effective therapeutic strategy for this type of thyroid cancer.

Synthetic lethal is a kind of interaction between two genes, where only simultaneously perturbing both of the genes can lead to lethality. This will provide an alternative paradigm to target "undruggable but important" targets in human cancers.4 Given that metabolic alterations act as a hallmark of cancer, thus approaches utilize metabolic properties specific in distinct oncogenic backgrounds can be considered to achieve synthetic lethality in cancer cells.5,6 A previous study has identified eight potential metabolic-related synthetic lethality partners of BRAF$^{V600E}$ in melanoma through a high-throughput screening.7 We thus analyzed the expression of these genes in PTCs using The Cancer Genome Atlas (TCGA) dataset, and found increased expression of CYP2S1, an orphan cytochrome P450 (CYP) enzyme,8 in PTCs compared to control subjects. In addition, its expression was strongly associated with BRAF$^{V600E}$ mutation, suggesting that CYP2S1 may be selectively essential in BRAF$^{V600E}$-mutated thyroid cancers.

The CYP superfamily includes the genes that code phase I enzymes metabolism both endogenous and exogenous substrates.9 There is evidence reporting that dioxin can induce CYP2S1 expression by an aryl hydrocarbon receptor (AHR)-dependent pathway, and CYP2S1 may be involved in metabolic activation of carcinogens which bring about harmful effects.10,11 The ability of CYP2S1 to metabolism endogenous substrates is still controversial, and current evidence suggests that CYP2S1 is capable of metabolize cyclooxygenase-derived and lipxygenase-derived eicosanoids.12 Considering that the eicosanoids are "double edged sword" for carcinogenesis13 and increased expression of CYP2S1 in PTCs, thus there is a high possibility that CYP2S1 participates in malignant phenotypes of thyroid cancer cells, particularly in BRAF$^{V600E}$ mutated ones.

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RESULTS

CYP2S1 is identified as a potential synthetic lethal partner of BRAFV600E in thyroid cancer cells.

Eight metabolism-related genes including HMGCL, HMGCS1, CYP39A1, CYP2C9, CYP2E1, CYP2J2, CYP2S1, and CSGLCA-T have been screened to be potential synthetic lethal partners of BRAFV600E in melanomas, and HMGCL was finally validated as a synthetic lethal target of BRAFV600E in melanomas.

Similar to melanomas, there is a high prevalence of BRAFV600E mutation in thyroid cancers.14,15 Thus, we first analyzed the expression of these eight genes in PTCs from TCGA database. The results showed that the expression of HMGCL, CYP2J2, CYP2S1, and CSGLCA-T was significantly elevated in PTCs, especially the latter three genes (Fig. 1a, Supplementary Fig. 1) compared to control subjects. Of them, the expression of CYP2S1 and CSGLCA-T was positively associated with BRAFV600E mutation, particularly the former (Fig. 1b, Supplementary Fig. 2).

Considering that BRAFV600E mutation is also found in other types of cancer such as melanomas, colon cancers, and lung adenocarcinomas,16 we also investigated the association of CYP2S1 expression with BRAFV600E mutation in the above cancers. As shown in Supplementary Fig. 3, CYP2S1 expression was not clearly associated with BRAF wild-type ones (Fig. 2b), also supported by colony formation assays (Fig. 2c). Next, we tested the effect of CYP2S1 depletion on cell apoptosis. As expected, CYP2S1 knockdown strongly induced cell apoptosis in BRAFV600E-mutated thyroid cancer cell lines, but not in BRAF wild-type ones (Fig. 2d). Besides, our data also showed that CYP2S1 knockdown selectively decreased migration and invasion abilities of BRAFV600E-mutated thyroid cancer cells compared to the control (Fig. 2e).

Validation of CYP2S1 as a synthetic lethal partner of BRAFV600E in xenograft tumor models

To validate the above conclusions in vivo, we established tumor xenografts in nude mice by injecting 8305C cells harboring BRAFV600E or C643 cells harboring wild-type BRAF. The results indicated that, compared to the controls, CYP2S1 depletion led to a significant reduction in the growth rate and weight of 8305C cell-derived xenograft tumors (Fig. 3a), while almost did not affect the growth of C643 cell-derived xenograft tumors (Fig. 3b). At the end of experiments, we performed western blot and IHC assays to confirm the inhibition of CYP2S1 expression in xenograft tumors (Fig. 3c, Supplementary Fig. 4). Next, we determined the effect of CYP2S1 depletion on cell proliferation in xenograft tumors by staining Ki-67. The results showed that, relative to the controls, CYP2S1 depletion remarkably decreased the percentage of Ki-67 positive cells in 8305C cell-derived xenograft tumors (Fig. 3d), while almost did not change Ki-67 levels in C643 cell-derived xenograft tumors (Fig. 3e), further supporting the above in vitro findings.
BRAFV600E-mediated MAPK/ERK cascade increases CYP2S1 expression in thyroid cancer cells.

The above results showed that CYP2S1 was significantly upregulated in BRAFV600E-mutated PTCs relative to BRAF wild-type ones. This observation suggests that BRAFV600E-mediated MAPK/ERK signaling may be involved in regulating CYP2S1 expression in thyroid cancers. First, we ectopically expressed human wild-type BRAF and BRAFV600E in NIH3T3 cells. The results showed that, compared to empty vector, wild-type BRAF almost did not affect the activity of MAPK/ERK signaling and CYP2S1 expression, while BRAFV600E strongly enhanced ERK phosphorylation and increased CYP2S1 expression at both protein and mRNA levels (Fig. 4a, b).

On the other hand, we treated BRAFV600E-mutated thyroid cancer lines BCPAP and 8505C with 1 μM PLX4720 (a selective inhibitor of BRAFV600E) alone or in combination with 500 nM GSK1120212 (MEK1/2 inhibitor) for 24 h. The results showed that both PLX4720 and GSK1120212 effectively suppressed ERK phosphorylation and decreased protein expression of CYP2S1.
particularly a combined treatment of these two drugs (Fig. 4c), also supported by qRT-PCR results that mRNA expression of CYP2S1 was significantly inhibited by PLX4720 in combination with GSK1120212 (Fig. 4d).

To further determine whether activated ERK promotes CYP2S1 expression by stabilizing mRNA or enhancing its transcription, we used actinomycin D to monitor the mRNA decay when BCPAP and 8505C cells were treated with PLX4720 and GSK1120212 or DMSO. As shown in Fig. 4e and Supplementary Fig. 5, the treatment of BCPAP and 8505C cells with PLX4720 and GSK1120212 almost did not affect the mRNA stabilization of CYP2S1. In addition, a combined treatment of PLX4720 and GSK1120212 significantly inhibited the activity of CYP2S1 promoter compared to the control in BCPAP and 8505C cells (Fig. 4f). These data indicate that BRAFV600E enhances CYP2S1 transcription at least partially through activating MAPK/ERK signaling pathway.

Fig. 3  Validation of CYP2S1 as a synthetic lethal partner of BRAFV600E in vivo. a, b Establishment of xenograft tumor models by subcutaneous inoculation of 8305C and C643 cells stably knocking down CYP2S1 or control cells (n = 5 per group). Left panels show time courses of tumor growth. Representative images of xenograft tumors and statistical analysis of tumor weight were shown in right panels. c Validation of CYP2S1 knockdown in xenograft tumor tissues by western blot analysis (left panels). GAPDH was used as a loading control. The density of CYP2S1 proteins on western blot was normalized to GAPDH, and statistical analysis was shown in right panel. d, e The levels of Ki-67 proteins in the indicated xenograft tumors by IHC assay (left panels). Statistical analysis of the percentage of Ki-67 positive cells was shown in right panels. Scale bars, 200 µm. Error bars represent SD. ***P < 0.001
BRAFV600E promotes CYP2S1 expression via an AHR-dependent pathway

It should be noted that although CYP2S1 is classified into the CYP2 family based on its amino acid sequence identity, it exhibits typical features of CYP1 family members such as induced by dioxin via AHR. Thus, we suppose that BRAFV600E regulates CYP2S1 expression in thyroid cancer cells probably by AHR. First, we analyzed AHR expression using TCGA database, and found that its expression pattern was similar to that of CYP2S1 (Fig. 5a–c). AHR expression was significantly upregulated in CPTCs and TCPTCs compared to control subjects (Fig. 5b). Moreover, BRAF V600E-mutated PTCs exhibited higher expression of AHR than BRAF wild-type ones (Fig. 5c). The IHC results further confirmed high expression of AHR in PTCs compared to control subjects (Fig. 5d).

Notably, we found a close relationship between mRNA expression of AHR and CYP2S1 in PTCs from TCGA dataset (Fig. 5e), suggesting that AHR may be involved in regulating CYP2S1 transcription. To prove this, we ectopically expressed AHR in BCPAP and 8505C cells, and found that ectopic expression of AHR strongly increased CYP2S1 expression (Fig. 5f). Conversely, AHR knockdown in these cells decreased CYP2S1 expression (Fig. 5g). To determine whether AHR directly regulates the activity of CYP2S1 promoter, we constructed luciferase reporter plasmid containing CYP2S1 promoter. As expected, ectopic expression of AHR significantly enhanced the expression of CYP2S1 promoter in 293T and 8505C cells (Fig. 5h). These data indicate that CYP2S1 is a potential downstream target of AHR.

Considering a positive association of BRAFV600E mutation with AHR expression, we thus speculate that BRAFV600E-mediated MAPK/ERK signaling can upregulate AHR expression, thereby enhancing CYP2S1 transcription. First, we analyzed AHR expression using TCGA database, and found that its expression pattern was similar to that of CYP2S1 (Fig. 5a–c). AHR expression was significantly upregulated in CPTCs and TCPTCs compared to control subjects (Fig. 5b). Moreover, BRAF V600E-mutated PTCs exhibited higher expression of AHR than BRAF wild-type ones (Fig. 5c). The IHC results further confirmed high expression of AHR in PTCs compared to control subjects (Fig. 5d).

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Considering a positive association of BRAFV600E mutation with AHR expression, we thus speculate that BRAFV600E-mediated MAPK/ERK signaling can upregulate AHR expression, thereby enhancing CYP2S1 transcription. First, we ectopically expressed human wild-type BRAF and BRAFV600E in NIH3T3 cells, and found that BRAFV600E clearly increased ERK phosphorylation and AHR expression compared to empty vector and wild-type BRAF (Fig. 5i). Meanwhile, we also observed that BRAFV600E significantly upregulated a well-known downstream target of AHR, Cyp1b1, relative to empty vector and wild-type BRAF (Supplementary Fig. 6a). Conversely, we treated BCPAP and 8505C cells with 1 μM PLX4720 in combination with 500 nM GSK1120212 for 24 h. This caused a strong inhibition of ERK phosphorylation and a decreased expression of AHR and its target CYP1B1 (Fig. 5j, Supplementary Fig. 6b).

To figure out whether BRAFV600E induces CYP2S1 expression via AHR, we knocked down AHR expression in NIH3T3 cells ectopically
expressing BRAF<sup>V600E</sup>. The results expectedly showed that BRAF<sup>V600E</sup> dramatically increased the expression of AHR and CYP2S1 compared to empty vector; however, BRAF<sup>V600E</sup>-mediated Cyp2s1 upregulation could be reversed by AHR depletion (Fig. 5k), indicating that BRAF<sup>V600E</sup> increases CYP2S1 expression via an AHR dependent pathway.

A positive feedback loop between CYP2S1 and AHR
There is evidence for the existence of a positive regulatory loop between CYP1 and AHR by the metabolic substrates<sup>21</sup>, we thus suppose that it exists similar regulatory loop between CYP2S1 and AHR. To validate this, we constructed luciferase reporter plasmid containing CYP1B1 promoter, which is a downstream target of...
Fig. 5  AHR-dependent upregulation of CYP2S1 by BRAF<sup>V600E</sup> mediated MAPK/ERK signaling.  

**a** High expression of AHR in PTCs compared to normal thyroid tissues (N) from TCGA database.  
**b** CYP2S1 expression in different histological subtypes of PTCs. CPTC, conventional PTC; FVPTC, follicular variants PTC; TCPTC, tall-cell PTC.  
**c** High expression of AHR in BRAF<sup>V600E</sup> mutated PTCs (V600E) compared to BRAF wild-type ones (WT).  
**d** Evaluation of AHR expression in 10 PTCs and their matched noncancerous tissues (N) by IHC staining. Representative IHC images were shown in left panel. Scale bars, 200 µm. Quantitative illustration of AHR proteins was shown in right panel.  
**e** The relationship between mRNA expression of AHR and AHR in PTCs from TCGA database. Western blot analysis was performed to evaluate the effects of ectopic expression of AHR (f) and AHR knockdown (g) on CYP2S1 expression in BCPAP and 8505C cells. GAPDH was used as a loading control.  
**h** Dual-luciferase reporter system was used to evaluate the effect of ectopic expression of AHR on promoter activity of CYP2S1 in 293T and 8505C cells. Renilla luciferase was used as an internal control.  
**i** Western blot analysis was performed to evaluate the effect of ectopic expression of wild-type BRAF and BRAF<sup>V600E</sup> on ERK phosphorylation (p-ERK) and AHR expression in NIH3T3 cells. Tubulin was used as a loading control.  
**j** BCPAP and 8505C cells were treated with a combination of 1 µM PLX4720 and 500 nM GSK1120212 for 24 h, and western blot analysis was then performed to determine whether BRAF<sup>V600E</sup> regulated CYP2S1 expression via AHR. Tubulin was used as a loading control. Error bars represent SD. **P < 0.01; ***P < 0.001

Fig. 6  AHR/CYP2S1 feedback loop causing synthetic lethal interaction between CYP2S1 and BRAF<sup>V600E</sup>.  
**a** Dual-luciferase reporter system was used to evaluate the effect of CYP2S1 knockdown on promoter activity of a downstream target of AHR, CYP1B1, in BCPAP and 8505C cells. Renilla luciferase was used as an internal control.  
**b** qRT-PCR was performed to evaluate the effect of CYP2S1 knockdown on mRNA levels of CYP1B1. 18S rRNA was used as a reference gene.  
**c** HPLC was used to analyze 12-HHT levels in the supernatant of BCPAP and 8505C cells.  
**d** The effect of CYP2S1 knockdown and 12-HHT restoration on the proliferation of BCPAP and 8505C cells was monitored by MTT assay. Error bars represent SD. Each experiment was performed in triplicate. **P < 0.01; ***P < 0.001
AHR, and performed dual-luciferase reporter assays. The results showed that CYP2S1 knockdown in BCPAP and 8505C cells significantly reduced promoter activity of CYP1B1 compared to the control (Fig. 6a). Accordingly, we also observed that downstream target of AHR, CYP1B1, was significantly downregulated upon CYP2S1 depletion (Fig. 6b). These results support the existence of the CYP2S1-AHR feedback loop in the BRAF V600E-mutated thyroid cancer cells.

Next, we choose one of the classical CYP2S1 metabolic substrates, 12-HHT, and tested its biological role in BRAFV600E-mutated thyroid cancer cells. Using high performance liquid chromatography (HPLC), we demonstrated that CYP2S1 knockdown significantly reduced 12-HHT levels in the supernatants of BCPAP and 8505C cells (Supplementary Fig. 7a, Fig. 6c), while CYP2S1 knockdown in C643 cells almost did not affect 12-HHT levels (Supplementary Fig. 7b). As expected, the restoration of 12-HHT could partially reverse inhibitory effect of CYP2S1 knockdown in cell viability by adding 12-HHT to the supernatants of BCPAP and 8505C cells knocking down CYP2S1 (Fig. 6c, d). However, the restoration of 12-HHT did not affect transcriptional activity of AHR (data not shown), indicating that 12-HHT is not an endogenous AHR ligand. Considering that metabolic substrates of CYP2S1 are not well identified, endogenous ligands of AHR need to be further investigated in the future. Collectively, our data indicate the existence of the CYP2S1-AHR feedback loop, contributing to malignant phenotypes of BRAFV600E-driven thyroid cancers, and also make rationalization of the synthetic lethal interaction between CYP2S1 and BRAFV600E (Fig. 7a).

CYP2S1 is a potential therapeutic target in BRAFV600E-driven thyroid cancers

The above findings indicate that CYP2S1 may be a potential therapeutic target in BRAFV600E-driven thyroid cancers. To prove this, we first established a collaurum-based siRNA delivery system, which has been demonstrated to be an effective strategy for cancer therapy in vitro and in vivo. Based on our design, Au-si-RNAs can be effectively accumulated in tumor sites by enhanced permeability and retention (EPR) effect, and enter cancer cells via endocytosis. Under intracellular glutathione (GSH), si-CYP2S1 can be released to target and downregulate CYP2S1 (Fig. 7b). As shown in Supplementary Fig. 8a and b, successful synthesis of Au-si-RNAs were verified by dynamic light scattering and ultraviolet-visible spectra absorption. Their size is appropriate to extravasate the leaky pore of tumor vasculature via EPR effect. Expectedly, Au-si-CYP2S1 could effectively suppress CYP2S1 expression in both BRAFV600E-mutated and BRAF wild-type thyroid cancer cells compared to the control (Supplementary Fig. 8c). Similarly, Au-si-CYP2S1 selectively inhibited cell proliferation in...
BRAFV600E-mutated thyroid cancer cells, but not in BRAF wild-type ones (Fig. 8a). Besides, Au-si-CYP2S1 also enhanced the antitumor efficacy of the doxorubicin (DOX), a traditional chemotherapeutic drug, compared to the control (Supplementary Fig. 9).

Next, to further validate therapeutic potential of Au-si-CYP2S1 in vivo, we established xenograft and transgenic mouse models of thyroid cancer. As expected, compared to Au-si-NC, Au-si-CYP2S1 remarkably inhibited the growth and weight of 8305C cell-derived xenograft tumors, but not C643 cell-derived xenograft tumors (Fig. 8b). This was further supported by Ki-67 and CYP2S1 staining (Fig. 8c, Supplementary Fig. 10). Besides, we observed that Au-si-NC treatment significantly decreased body weight of mice compared to Au-si-CYP2S1 treatment in 8305C cell-derived xenograft models, but not in C643 cell-derived xenograft models (Supplementary Fig. 11).

Fig. 8 Targeting CYP2S1 is an effective therapeutic strategy for BRAFV600E mutated thyroid cancer cells. a Time courses of cell proliferation upon CYP2S1 knockdown in the indicated cell lines were determined by MTT assay. b Xenograft tumor models were established by subcutaneous inoculation of 8305C (5 × 10⁶) or C643 (3 × 10⁶) cells, and mice were then randomized to two groups (Au-si-CYP2S1 and Au-si-NC; n = 5/group), respectively. Left panels show time courses of tumor growth in mice with the indicated treatments. Representative images of xenograft tumors and statistical analysis of tumor weight were shown in right panels. c Evaluation of CYP2S1 and Ki-67 expression in the indicated xenograft tumors by IHC assay. Scale bars, 200 µm. d Transgenic mouse models of thyroid cancer were established, and similarly treated with as mentioned above. Representative images of thyroid tumors in the indicated groups were shown in left panel. Right panel represents mean tumor weight (n = 3/group). e Evaluation of CYP2S1 and Ki-67 expression in the indicated tumor tissues by IHC assay. Scale bars, 200 µm. Error bars represent SD. *P < 0.05; **P < 0.01; ***P < 0.001.
We also designed Au-si-Cyp2s1 to target Cyp2s1 in NIH3T3 cells and transgenic model of thyroid cancer. The results showed that Au-si-Cyp2s1 effectively suppressed CYP2S1 expression in NIH3T3 cells compared to the control (Supplementary Fig. 12). In addition, Au-si-Cyp2s1 obviously inhibited tumor growth and weight in transgenic model of thyroid cancer (Fig. 8d), also supported by Ki67 and CYP2S1 staining (Fig. 8e, Supplementary Fig. 13). Importantly, Au-si-RNAs almost did not cause obvious side effects on the liver or kidney of mice in both xenograft and transgenic mouse models (Supplementary Fig. 14). Taken together, our data indicate that CYP2S1 is an effective therapeutic target in BRAFV600E-driven thyroid cancers.

**DISCUSSION**

BRAFV600E mutation occurs exclusively in PTCs and some ATCs arising from PTCs, and it is now recognized as the most common genetic alteration in thyroid cancers. The tumorigenic role of BRAFV600E mutation had been proved through thyroid-specific knock-in of BRAFV600E, resulting in aggressive PTC. Thyroid cancers carrying BRAFV600E mutation always indicate poor clinical outcomes and are more likely to resistant to traditional therapies. Although the MAPK/ERK signaling rebound following the applying of BRAFV600E specific inhibitors limits their clinical use, targeting BRAFV600E is still promising.

In this study, we found that CYP2S1 was highly expressed in PTCs relative to control subjects, particularly in CPTCs and TCPTCs, and its expression was strongly associated with BRAFV600E mutation. By a series of in vitro and in vivo functional studies, we demonstrated that CYP2S1 knockdown selectively inhibited cell proliferation, colony formation, migration, invasion, and tumorigenic potential in nude mice, and induced cell apoptosis in BRAFV600E-mutated thyroid cancer cells, but not in BRAF wild-type ones. These results indicate that CYP2S1 may serve as a potential synthetic lethal partner of BRAFV600E in thyroid cancers.

As a member of the CYP family, it is possible that oncogenic role of CYP2S1 is dependent on its metabolic capacity. Current knowledge of CYP2S1 in endogenous function is largely related to eicosanoids metabolism, and some of its metabolic products are well-known downstream target CYP1B1, indicating that CYP2S1 in thyroid cancers.

We found that BRAFV600E-mediated MAPK/ERK cascade increases CYP2S1 expression via an AHR dependent pathway, while certain metabolites of CYP2S1 may induce the transcriptional activity. BRAFV600E-mutated thyroid cancer cells, while almost did not affect the growth of BRAFV600E wild-type ones. This raised the possibility that targeting CYP2S1 may be an effective strategy for BRAFV600E-mutated thyroid cancers.

In summary, we find that BRAFV600E-mediated MAPK/ERK cascade increases CYP2S1 expression via an AHR dependent pathway, while certain metabolites of CYP2S1 may induce the transcriptional activity. This AHR/CYP2S1 feedback loop strongly amplies oncogenic role of BRAFV600E in thyroid cancer cells, thereby causing synthetic lethal interaction between CYP2S1 and BRAFV600E. Thus, our data demonstrate that CYP2S1 is a potential synthetic lethal partner of BRAFV600E and also provide an alternative therapeutic strategy for BRAFV600E-mutated thyroid cancers by targeting CYP2S1.

**MATERIALS AND METHODS**

Clinical samples

With the approval of the Institutional Review Board and Human Ethics Committee of the First Affiliated Hospital of Xi'an Jiaotong University School, ten paraffin-embedded primary PTCs and their matched noncancerous thyroid tissues (control subjects) were obtained from this hospital. Informed consent was provided to all patients prior to conducting this study. None of the patients received any therapeutic intervention before the surgery. All of the excised tissues were histologically examined by two senior pathologists at the Department of Pathology of the Hospital based on World Health Organization (WHO) criteria.

Cell culture and drug treatments

Human thyroid cancer cell lines, such as BCPAP, 8305C, 8505C, K1, and TPC-1 were kindly provided by Dr. Haixia Guan (The First Affiliated Hospital of China Medical University, Shenyang, China). Human thyroid cancer cell line C643 was provided by Dr. Lei Ye (Ruijin Hospital, Shanghai, China). Human embryonic kidney cell line 293T and mouse fibroblast cell line NIH3T3 were obtained from ATCC (Rockville, MD). All cell lines were routinely cultured at 37 °C in RPMI 1640 or DMEM medium with 10% fetal bovine serum. In some experiments, cells were treated with 1 μM BRAF kinase inhibitor PLX4720 (Selleck Chemicals, TX, USA), 500 nM MEK1/2 inhibitor GSTK1120212 (Selleck Chemicals, TX, USA), 5 mg/mL actinomycin D (Selleck Chemicals, TX, USA), 30 nM DOX (Selleck Chemicals, TX, USA) for the indicated times. All inhibitors were dissolved in dimethylsulfoxide (DMSO), aliquoted and stored at –80 °C until use. The same volume of DMSO was used as control.
RNA interference, lentivirus transfection, and expression plasmids were treated with si-CYP2S1 or Au-si-NC at the indicated concentrations for 48 h, and cells were then harvested and subjected to further experiments.

Animal studies
Four to five-week-old female athymic nude mice purchased from SLAC laboratory Animal Co., Ltd. (Shanghai, China) were housed in a specific pathogen-free environment, and then randomly divided into four groups (n = 5/group). Tumor xenografts were established by subcutaneous inoculation of 8305C (5 × 10⁶) and C643 (3 × 10⁶) cells stably knocking down CYP2S1 or control cells into flanks of nude mice. Tumor size was measured by a Vernier caliper every other day since 5 days after injection, and tumor volumes were calculated by the formula: Tumor volume = length × width² × 0.5. The mice were sacrificed at 21 days after injection and tumors were then weighted and harvested for further examinations.

Immunohistochemistry (IHC)
IHC was used to evaluate the expression of CYP2S1, AHR and Ki-67 in xenograft tumor sections as described previously.23 Protein expression was quantitated by integral optical density using Image-pro plus 6.0 (Media Cybernetics, USA). Each stained section was evaluated under the same magnification, light brightness and exposure intensity. The evaluation of the percentage of Ki-67 positive cells was conducted by calculating the number of the positive cells in ten microscopic fields from each group.

Statistical analysis
All statistical analyses were conducted using the SPSS statistical package (16.0, SPSS Inc. Chicago, IL). Unpaired student’s t test was used to compare the means of two groups of data. One-way analysis of variance (ANOVA) followed by Bonferroni’s multiple comparison test was used to compare differences between groups. All values were expressed as the mean ± standard deviation. All values with P < 0.05 were considered significantly.

DATA AVAILABILITY
The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

ACKNOWLEDGEMENTS
We thank Dr. Haixia Guan (The First Affiliated Hospital of China Medical University, Shenyang, China) and Lei Ye (Ruijin Hospital, Shanghai, China) for kindly providing human thyroid cancer cell lines. We also would like to thank Prof. Tyler Jacks (Massachusetts Institute of Technology, USA) and Prof. Martin McMahon (University of California, USA). Tp53^fl/fl mouse strain was purchased from The Jackson Laboratory (stock no. 008462). Thyroid cancer mouse model was similarly established as previously described.46 Au-siRNAs were administered 8 weeks following tumor induction with the same dose schedule as xenograft model. The mice were sacrificed after a 5-week treatment, and tumors were then weighted and harvested for further examination.

Transgenic mouse strains TPO-CreERT and Braf^CA were kindly provided by Profs. Tyler Jacks (Massachusetts Institute of Technology, USA) and Martin McMahon (University of California, USA). Tp53^fl/fl mouse strain was purchased from The Jackson Laboratory (stock no. 008462). Thyroid cancer mouse model was similarly established as previously described.46 Au-siRNAs were administered 8 weeks following tumor induction with the same dose schedule as xenograft model. The mice were sacrificed after a 5-week treatment, and tumors were then weighted and harvested for further examination.

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The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

ACKNOWLEDGEMENTS
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