

Title: Inverse agonist of estrogen-related receptor gamma (ERR γ) enhances sodium iodide symporter function through mitogen-activated protein kinase signaling in anaplastic thyroid cancer cells

Running title: GSK5182 modulates NIS function in ATC cells

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Abstract

Anaplastic thyroid cancer (ATC), a rare thyroid cancer with poor prognosis, is associated with insufficient function of the sodium iodide symporter (NIS). Estrogen-related receptor gamma (ERR γ) is a member of the orphan nuclear receptors, with important functions in cell development and homeostasis. However, there are no reports that demonstrate whether ERR γ is related with NIS function. Here, we evaluated the role of ERR γ on the regulation of NIS function in ATC cells by using GSK5182, an inverse agonist of ERR γ .

Methods

Two ATC cell lines, BHT-101 and CAL62, were incubated with the GSK5182 at various time points and dosages. Serial assessment of the NIS function was performed in the ATC cells by their uptake of radioiodine. The effects of GSK5182 on ERR γ and the mitogen-activated protein (MAP) kinase pathway as well as on NIS protein were evaluated by immunoblot assay. To examine whether the GSK5182-induced NIS functional activity can be affected by inhibition of the MAP kinase pathway, the MAP kinase activity and levels of radioiodine uptake were determined following treatment of an MEK inhibitor to GSK5182-treated cells. Finally, the cytotoxic effect of ^{131}I was determined by clonogenic assay.

Results

Treatment with GSK5182 resulted in dose- and time-dependent increases in iodide uptake in ATC cells, which were accompanied by both the down-regulation of ERR γ protein and the activation of extracellular signal-regulated kinase (ERK)-1/2. Both the increased radioiodine uptake and ERK-1/2 activation of ATC cells were completely inhibited by the specific MEK

inhibitor. GSK5182 treatment enhanced the membrane localization of NIS in both ATC cells. Accordingly, pre-exposure to GSK5182 resulted in enhanced cytotoxic effects of ^{131}I treatment in ATC cells.

Conclusion

These findings suggest that the inverse agonist of $\text{ERR}\gamma$ enhances the responsiveness of radioiodine therapy by modulating NIS function in ATC cells via the regulation of $\text{ERR}\gamma$ and the MAP kinase signaling pathway.

Key Words: Sodium iodide symporter (NIS); Anaplastic thyroid cancer; Estrogen-related receptor gamma ($\text{ERR}\gamma$); Radioiodide therapy; MAP kinase signaling

Introduction

Anaplastic thyroid cancer (ATC) is one of the most aggressive and lethal cancers known to affect humans (1, 2). ATC rapidly metastasizes from the thyroid glands to the lungs, bone, regional lymph nodes, and brain (3). This is in contrast to the benign nature of well-differentiated thyroid cancer that accounts for most thyroid cancers. Treatments of ATC with surgery, radiotherapy, and chemotherapy alone or in combination have shown little or no effect on patient survival (4-7). Consequently, the development of novel therapeutic approaches is urgently required.

The sodium iodide symporter (NIS) is a plasma membrane glycoprotein that mediates the active influx of iodide into cells (8). In thyroid cancer, endogenous NIS accommodates the widespread application of radioiodine therapy in the clinical situation, which has been shown to be an effective therapeutic method of eliminating malignant cells with minimal adverse effects over the years. Poorly differentiated cancer cells, including ATC cells, tend to exhibit a progressive dedifferentiation that leads to a decrease in the levels of NIS (9, 10). This results in the inability of ATC cells to accumulate a high iodine concentration and hence their resistance of radionuclide therapy, which finally leads to poor prognosis (11). Thus, many attempts have been conducted to restore the NIS function in ATC cells, using several methods such as gene delivery (12, 13) and epigenetic modulation with epigenome-modifying drugs, etc. (14), but few satisfactory outcomes have been acquired until now.

Estrogen-related receptors ($ERR\alpha$, $ERR\beta$, and $ERR\gamma$) are constitutively active nuclear receptors that bear high levels of sequence identity to estrogen receptors (ERs) (15). The ERR isoforms are primarily expressed in several organs, such as the heart, brain, kidney, pancreas, and liver (16, 17). Recently, several studies have shown that $ERR\gamma$ is involved in several metabolic

diseases, such as type 2 diabetes mellitus, alcohol-induced oxidative stress, liver injury, and microbial infection through impaired hepatic gluconeogenesis (18, 19), hepatic insulin signaling (20), and iron metabolism (21).

Crystal structure studies have demonstrated that ERRs are constitutively active without their ligand, and several small-molecule ligands can either activate or repress the functional activity of the ERRs (22). Among them, GSK5182 (a 4-hydroxy tamoxifen analogue) is a selective inverse agonist of ERR γ that does not interact with another nuclear receptor. Several lines of evidence have revealed that GSK5182 not only alleviates diabetes symptoms through the inhibition of hepatic gluconeogenesis in a PGC-1 α -dependent manner (19, 23) but also shows antimicrobial effects by the reduction of ERR γ -mediated hepcidin mRNA expression (21). Although the biological effects of ERR γ have been extensively investigated in various disease models, the role of ERR γ on NIS function in ATC remains to be clearly determined.

Among various nuclear receptors, the retinoic acid receptors RARs through its activators retinoic acids (RAs), have been proven to be effective in restimulating iodide uptake in non-thyroidal cancer and thyroid cancer *in vitro* and *in vivo* (24, 25). We speculated that the ERR γ of the nuclear receptor family might also be involved in controlling NIS function in thyroid cancer, similar to the biological roles of RA and RAR on NIS function.

To explore the effects of ERR γ on NIS function in ATC cells, we adopted GSK5182 as a specific inverse agonist of ERR γ and investigated the changes of endogenous ERR γ protein and MAP kinase signaling by this agent. The effects of GSK5182 on the functional activity and expression level of the NIS protein in ATC cells were also assessed. Finally, the enhanced cytotoxic effects of ¹³¹I from GSK5182 treatment were evaluated in ATC cells.

MATERIALS AND METHODS

Cells

Anaplastic thyroid cancer cell lines, BHT-101 and CAL-62, were purchased from Deutsche Sammlung von Mikroorganismen und Zellkulturen. Both cell lines were maintained in DMEM high supplemented with 10% FBS, 1% antibiotic-antimycotic (Hyclone) at 37 °C in a 5% CO₂ atmosphere.

¹²⁵I Uptake Assay

Cells were plated in 24-well plates for 24h and then treated with GSK5182 synthesized by Daegu-Gyeongbuk Medical Innovation Foundation (DGMIF, Daegu, Korea and prepared as a 100mM stock solution in DMSO and stored at -80°C) for 24 h. After aspirating drug-containing medium, cells were washed with 1mL HBSS and incubated with 500μL of Hank's balanced salt solution (HBSS) containing 0.5% bovine serum albumin (bHBSS), 3.7 kBq carrier-free ¹²⁵I (Perkin-Elmer) and 10 μmol/L sodium iodide (specific activity of 740 MBq/mmol) at 37°C for 30 min. The cells were then washed twice with ice-cold bHBSS and were lysed with 500μl of 2% sodium dodecyl sulfate (SDS). The radioactivity was measured using a gamma counter (Cobra II; Canberra Packard, Packard Bioscience). The radioactivity of the cells was normalized using total protein concentrations determined by a BCA kit (Pierce Protein Biology). Cells were pre-incubated with 300 μM KClO₄ (as a specific inhibitor for NIS) for 30 min to inhibit the iodide uptake, and followed by ¹²⁵I uptake test as described above.

Clonogenic Assay

Cells were plated into 6-well plates and left for 24 h. After treatment with 25 μ M GSK5182 for 24h, drug-containing medium was discarded and cells were washed twice with PBS. The medium was then replaced with DMEM in the presence or absence of 50 μ Ci 131 I (KIRAMS, Korea) for 6 h. Cells were washed with cold bHBSS and left in regular culture medium for the time corresponding to six doublings. Finally, cells were fixed in 4% Paraformaldehyde (PFA) solution and stained with 0.05% crystal violet. Control and 131 I treated colonies with over than 50 cells were counted.

Western Blot

The cells were treated with or without GSK5182 for 24 h and washed twice with cold PBS and lysed with RIPA buffer containing complete protease inhibitor cocktail (Roche). In case of plasma membrane protein for NIS, samples were prepared with protein biotinylation kit (EZ-Link™ Sulfo-NHS-Biotin, Thermo Scientific) according to manufacturer's instruction. Briefly, either untreated- or treated-cells were washed twice with ice-cold PBS/CM (PBS containing 0.1 mM calcium chloride and 1 mM magnesium chloride, pH 7.3) and incubated with EZ link NHS-Sulfo-SS-biotin (1 mg/mL) in PBS/CM for 30 min at 4 °C. The reaction was quenched by 2 washes with cold 100 mM glycine in PBS/CM and further incubation with 100 mM glycine in PBS/CM at 4 °C for 20 min. Cells were then quickly washed two times with PBS/CM before lysis with RIPA buffer containing protease inhibitors cocktail and phosphatase inhibitors (Roche) for 1 h at 4 °C with constant shaking. Lysates were centrifuged at 16,000g for 30 min at 4 °C. A portion of the supernatant was used for total cell protein immunoblots. The remaining sample was used to obtain membrane protein by incubation with 100 μ L streptavidin beads (Thermo Scientific) for 1 h at room temperature. Beads were washed 3 times with RIPA buffer, and bound

proteins were eluted with 50 μ L of Laemmli buffer (62.5 M Tris, pH 6.8; 20% glycerol; 2% SDS; 5% b-mercaptoethanol; and 0.01% bromphenol blue) for 30 min at room temperature. Equal amounts of total and biotinylated plasma membrane protein were loaded in each lane and resolved by 4-12% gradient Bis-Tris gel (Invitrogen). Proteins were transferred to 0.2- μ m PVDF membrane (Invitrogen). Membranes were incubated overnight at 4°C with primary mouse monoclonal human NIS-specific antibody (dilution, 1:1000, Thermo Scientific, Catalog#: MS-1653-P1, Clone: FP5A), followed by incubation with HRP-conjugated secondary antibody at room temperature. ECL-Plus (Amersham Pharmacia) was used to detect peroxidase activity according to the manufacturer's protocol.

Similarly, for other protein also, equal amounts of protein were loaded in each lane and resolved by 4-12% gradient Bis-Tris gel (Invitrogen). Proteins were transferred to 0.2- μ m PVDF membrane (Invitrogen). Membranes were incubated overnight at 4°C with primary antibodies and then incubated with the appropriate HRP-conjugated secondary antibody at room temperature. ECL-Plus was used to detect peroxidase activity according to the manufacturer's protocol. Band densities were determined by ImageJ software.

Statistical Analysis

All data are expressed as the means \pm SD, and statistical significance was determined using an unpaired Student's test of GraphPad Prism 5. P values < 0.05 were considered statistically significant.

Results

Enhanced radioiodine uptake in ATC cells by the inverse agonist of ERR γ

Treatment with GSK5182 resulted in a significant increase of radioiodine uptake in both ATC cell lines, in a dose-dependent fashion (Fig. 1A). The maximum relative fold increase of iodide uptake was 2.3 and 2.9 in the BHT-101 and CAL-62 cells, respectively, compared with the vehicle group, which was obtained at a concentration of 50 μ M GSK5182. Accordingly, 50 μ M GSK5182 was mainly used for the further iodide uptake tests.

To examine whether the increased radioiodine uptake is related to modulation of NIS function by GSK5182, KClO₄, a specific inhibitor of NIS, was co-incubated with GSK5182-treated BHT-101 and CAL-62 cells and the change of radioiodine uptake levels was determined. The level of radioiodine uptake between vehicle-treated cells and combined vehicle and KClO₄-treated cells was not different in both ATC cell, indicating that these ATC cells have negligible NIS-mediated iodide uptake activity (Supplemental Fig. 1). KClO₄ completely blocked the enhanced radioiodine uptake in both types of GSK5182-treated cells (Fig. 1B), suggesting that the augmentation of iodide uptake is directly involved with the improved functional activity of NIS mediated by GSK5182.

We assessed the radioiodine uptake level in GSK5182-treated BHT-101 and CAL-62 cells according to different time points. An increase of radioiodine uptake was observed in both treated cells within as early as 2 h post-treatment, and it reached its peak at 24 h (Supplemental Fig. 2).

Down-regulation of the endogenous ERR γ by GSK5182 in ATC cells

To determine the effect of GSK5182 on ERR γ protein levels in ATC cells,

immunoblotting analysis was conducted using an ERR γ -specific antibody. Both BHT-101 and CAL-62 cells showed endogenous expression of the ERR γ protein. Treatment with GSK5182 resulted in a marked reduction of ERR γ protein in both cells, with a relative decrease of 3.6-fold and 2.4-fold in the BHT-101 and CAL-62 cells, respectively (Fig. 2).

Increase of membrane-localized NIS in ATC cells through activation of MAP kinase signaling

A significant increase of phosphorylated MAP kinase levels, such as p44 and p42 ERK, was found in both ATC cells treated with GSK5182 (Fig. 3A). The relative increase of the phosphorylated forms of ERK1 and ERK2 was 1.6-fold and 2.1-fold, respectively, in BHT-101 cells. GSK5182 treatment resulted in 5.8-fold and 2.2-fold increases in phosphorylated p44 and p42 ERK, respectively, in CAL-62 cells, and it was completely inhibited by the selective MEK inhibitor, PD98059 (Fig. 3B).

To determine the effect of GSK5182 on NIS protein status, we examined the change in levels of membranous and total NIS proteins collected from GSK5182-treated ATC cells using plasma membrane biotinylation kit, followed by immunoblotting with NIS-specific antibody. As illustrated in Fig. 4A, GSK5182 induced drastic increases in plasma membrane-localized NIS protein with an immature form in both ATC cells, compared with control cells. Quantification analysis of the band intensity revealed 3.8-fold and 6.0-fold increases of membrane NIS protein in the BHT-101 and CAL-62 cells, respectively (Supplemental Fig. 3). However, there was no significant change in the levels of NIS protein in total cell lysates of untreated and GSK5182-treated ATCs. A radioiodine uptake test with PD98059 treatment demonstrated that the GSK5182-induced iodide uptake reverted to the basal level in both ATC cell lines, whereas

incubation of cells with PD98059 alone did not affect the iodide uptake (Fig. 4B).

Amelioration of ^{131}I -mediated cytotoxicity by GSK5182 in ATCs

As shown in Fig. 5 and Supplemental Fig. 4, clonogenic assay with ^{131}I demonstrated minimal cytotoxic effects in the ATC cells treated with either GSK5182 or ^{131}I alone. A relative colony-forming ability of ^{131}I or GSK5182 groups was $92.9 \pm 5.8\%$ and $94.5 \pm 10.8\%$ in CAL-62 cells, respectively. Similar with CAL-62 cells, a relative colony-forming ability of ^{131}I or GSK5182 groups was $95.2 \pm 4.2\%$ and $93.2 \pm 5.5\%$ in BHT-101, respectively. However, the combination of GSK5182 with ^{131}I resulted in a marked reduction of the colony-forming ability to approximately $58.5 \pm 7.4\%$ and $72.8 \pm 2.4\%$ in CAL-62 and BHT-101, respectively.

Discussion

We have demonstrated that GSK5182, an inverse agonist of $\text{ERR}\gamma$, reduces the endogenous $\text{ERR}\gamma$ protein level in ATC cells and leads to the activation of MAP kinase signaling. Importantly, treatment of GSK5182 results in enhanced radioiodine uptake in a dose- and time-dependent fashion in ATC cells. Both the GSK5182-induced ERK-1/2 activation and increase of radioiodine uptake are completely inhibited by PD98059, a selective MEK inhibitor. Further examination showed that GSK5182 increases NIS plasma membrane protein without affecting levels of total NIS protein. Furthermore, the enhancement of NIS function modulated by GSK5182 leads to the increased susceptibility of ATC cells to radioiodine therapy.

Recently, many studies have shown interesting therapeutic outcomes from GSK5182-mediated $\text{ERR}\gamma$ regulation in various metabolic and cardiac diseases (18, 20, 21, 26), but no studies have unraveled the involvement of GSK5182-modified $\text{ERR}\gamma$ on regulating NIS function

in thyroid cancer, especially anaplastic thyroid cancer. In an attempt to determine the effects of GSK5182 on radioiodine uptake in ATC cells, we selected two different anaplastic thyroid cancer cells, BHT-101 and CAL-62, which have characteristic mutations of the K-RAS and BRAF genes, respectively. These cell lines have been used to evaluate the therapeutic efficacy of several drugs to induce cytotoxic effects directly as well as to modulate NIS function (14, 27). Interestingly, the incubation of ATC cells with GSK5182 led to dose- and time-dependent increases of radioiodine uptake, but negligible uptake was shown in vehicle-treated cells. From these findings, we questioned whether the increased iodide uptake was associated with modulated NIS function. Thus, an iodide uptake test was further performed by introducing a specific inhibitor of NIS protein, potassium perchlorate (KClO₄), to GSK5182-treated cells. KClO₄ has been widely used to validate the functional activity of NIS protein (8). Iodide uptake analysis with KClO₄ clearly showed the inhibition of enhanced radioiodine uptake to basal level, revealing that increased iodide uptake is related with the GSK5182-induced modulation of NIS function. Next, it was speculated that the enhanced iodide uptake might be involved with a change in endogenous ERR γ protein by GSK5182. We observed that both ATC cell lines exhibited a substantial and similar level of expression of ERR γ protein, and the treatment of GSK5182 significantly reduced the ERR γ protein level. Taken together, these results suggest that the GSK5182-induced reduction of endogenous ERR γ protein may affect the NIS function and finally lead to increased radioiodine uptake in ATC cells.

MAP kinases are evolutionarily conserved enzymes that transfer signals from cell surface receptors to critical intracellular molecules (28). Among the MAP kinases, p38 MAP kinase has been identified to be related to maintaining the normal basal expression of serotonin transporters (29) and improving the activation of norepinephrine transporters (30). Katherine et

al. has reported that PI3K activation induce the underglycosylated intracellular NIS protein expression in breast cancer (MCF-7) cells and leads to iodide uptake ability (31). Another report has shown that MEK inhibition with MEK inhibitor result in the decrease of NIS protein levels through lysosome-mediated NIS degradation in human breast cancer cells, suggesting MEK activation act as an important role in maintain NIS protein stability in human breast cancers (32). More recently, Lee et al. reported that the activation of protein kinase C signaling with phorbol 12-myristate 13-acetate down-regulated the iodide uptake in NIS-expressing non-thyroidal cancer cells, and that EGF-mediated MAP kinase activation reversely enhanced the radioiodine uptake. Based on these studies, it was presumed that the enhanced radioiodine uptake of ATC cells might be linked with the up-regulation of these kinase pathways. From immunoblotting analysis with phosphor-ERK1/2-specific antibody, we were able to find that the ERK signaling is constitutively active in both ATC cell types and that treatment of GSK5182 increases the phosphorylated MAP kinase level. Conversely, PD98059, a selective inhibitor of MAP kinase, inhibits the increased phosphorylation of ERK1/2 MAP kinase as well as the iodide uptake induced by GSK5182. It would therefore be reasonable to postulate that the enhanced radioiodine uptake of ATC cells may be associated with the activation of MAP kinase signaling that results from the GSK5182-mediated reduction of ERR γ protein.

The total amount of NIS protein as well as plasma-membrane-localized NIS protein is crucial for the iodide transport capacity within thyroid cells. Even though sufficient expression of NIS protein is observed in thyroid cancer cells, adequate iodide uptake to generate an effective radioiodine therapy cannot occur owing to intracellular retention of NIS. The NIS transporter needs to be translocated to the plasma membrane to be fully functionalized in thyroid cells. Thyroid-stimulating hormone is needed to generate the membrane retention of NIS, and its

depletion leads to an acute decrease of iodide uptake in thyroid cells (33). Furthermore, breast cancer cells with sufficient endogenous expression of NIS exhibited a deficient iodide uptake ability, which partially resulted from intracellular localization of the NIS protein (24, 34).

Based on these reports, we speculated that both the down-regulation of ERR γ protein level and activation of MAP kinase by GSK5182 may affect the total and plasma membrane NIS protein status. We could detect the endogenously expressed total NIS protein without increase of membrane NIS protein in vehicle-treated cells. Although several reports has demonstrated that ATC cells do not express NIS protein (35), we can evidently detect the NIS protein expression in both ATC cells in this study. The discrepancy may be due to the development of anti-NIS specific antibody with high sensitivity and specificity as well as better experimental conditions. Surprisingly, biotinylation experiments with GSK5182-treated ATC cells revealed the increase of immature NIS protein at the plasma membrane. But there is no change of NIS protein in total cell lysate protein. The exact mechanism responsible for the GSK5182-mediated increase of iodide transport is not yet clear. Since the general posttranscriptional mechanism to affect both the localization and function of many transporters includes the glycosylation and phosphorylation processes (36), it could be possible that similar processes may be involved in the NIS-regulating process.

Successful radioiodine therapy against thyroid cancer depends mainly on an effective accumulation of the therapeutic radioiodine. It would be reasonable to postulate that the enhanced radioiodine uptake by GSK5182 may induce potential cytotoxic effects against ATC cells. Notably, in our study, GSK5182 treatment was sufficient to enhance the killing effects of therapeutic radioiodine on ATC cells, but treatment of ^{131}I alone was not able to induce sufficient cytotoxic effects on ATC cells. These findings propose that ERR γ -modulated NIS function can

provide a reasonable approach to achieve a radioiodine accumulation that allows for acceptable therapeutic outcomes with clinically used doses of ^{131}I . Further preclinical investigations should be required to evaluate the effects of GSK5182-mediated MAP kinase activation on the efficacy of radioiodine accumulation in ATC cancer of living mice, using nuclear medicine imaging instruments such as SPECT or PET.

Conclusion

Our findings propose a mechanism that $\text{ERR}\gamma$ -regulated MAP kinase pathways may play important roles in regulating NIS function (Fig. 6). These findings also suggest that the appropriate regulation of these signaling pathways to enhance NIS function to allow radioiodine therapy is realistically feasible, uncovering new therapeutic strategies for anaplastic thyroid cancer. In future, the $\text{ERR}\gamma$ inverse agonist as adjuvant can be introduced to the development of new therapeutic protocols for patients with ATCs. Our current study guarantees further investigation for the discovery of new drug candidates or GSK5182 derivatives to regulate the $\text{ERR}\gamma$ activity in order to mediate a more effective re-induction of radioiodine uptake in ATC cells. Since lack of iodide organification is a limiting factor for effective radioiodine therapy in ATC cells, further study should be required to uncover its effects of GSK5182 on iodide organification in ATC cells.

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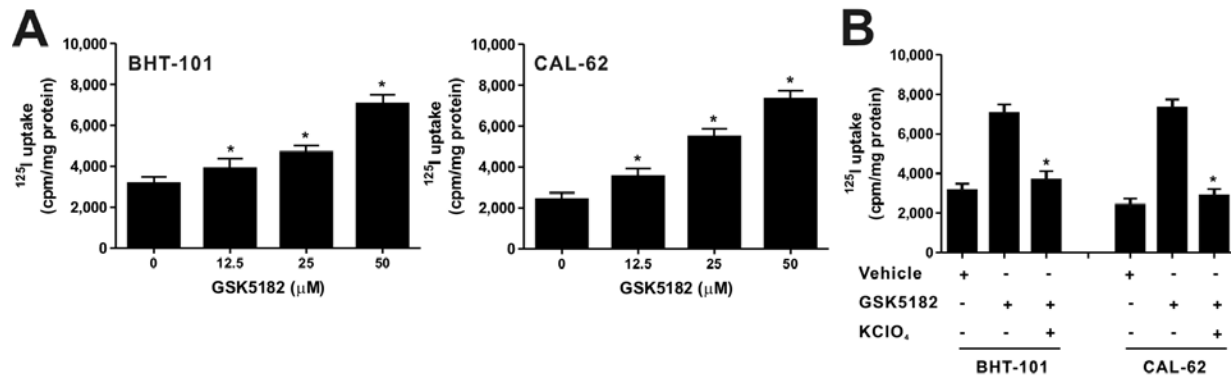


FIGURE 1. Effects of GSK5182 on the kinetics of iodide uptake in anaplastic thyroid cancer cells. (A) Increase of iodide uptake in a GSK5182 dose-dependent manner in ATC cells. Each cell was treated with various dose of GSK5182 for 24h and the iodide uptake levels of both ATCs were confirmed. (B) Inhibition of ^{125}I uptake after treatment of 300 μM of KClO_4 for 30 min in both ATCs. *, $P < 0.05$; when compared with GSK5182. All data are expressed as mean \pm SD; $n=3$.

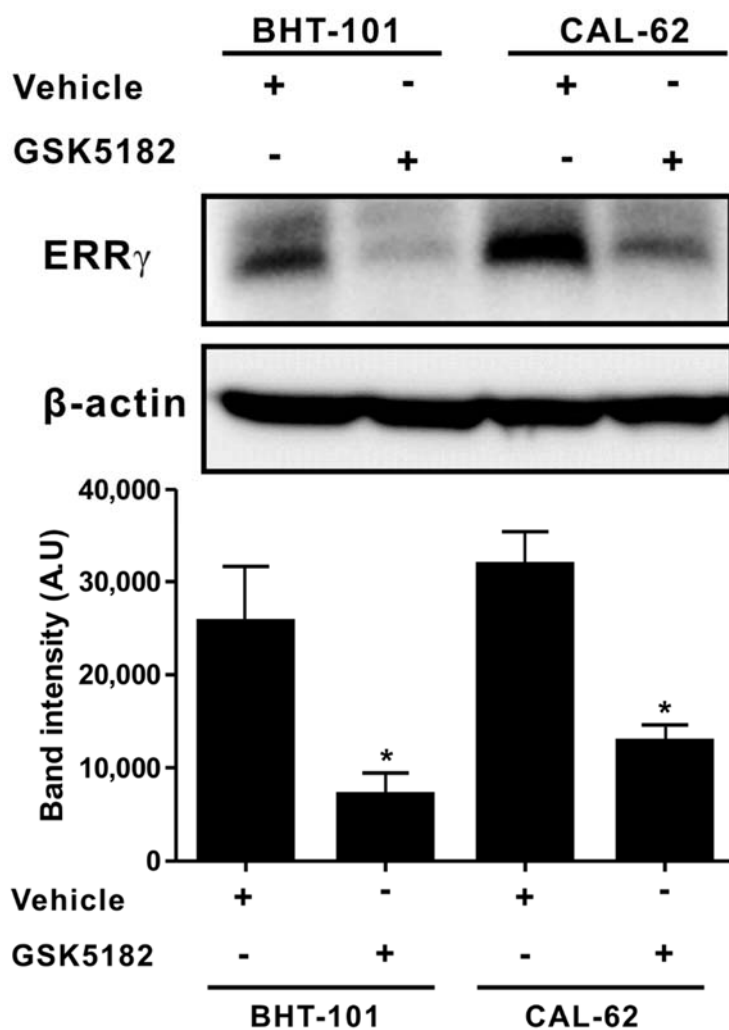


FIGURE 2. Effects of GSK5182 on endogenous ERR γ protein in anaplastic thyroid cancer cells. Decrease of endogenously expressed ERR γ protein levels by GSK5182 in ATC cells. Each cell was treated with 50 μ M GSK5182 for 24 h and immunoblotting was done with ERR γ -specific antibody. Quantitative analysis of ERR γ protein levels by calibrated densitometer. Data mean \pm SD of 3 samples per group expressed in arbitrary units. *, P < 0.05; when compared with untreated cells.

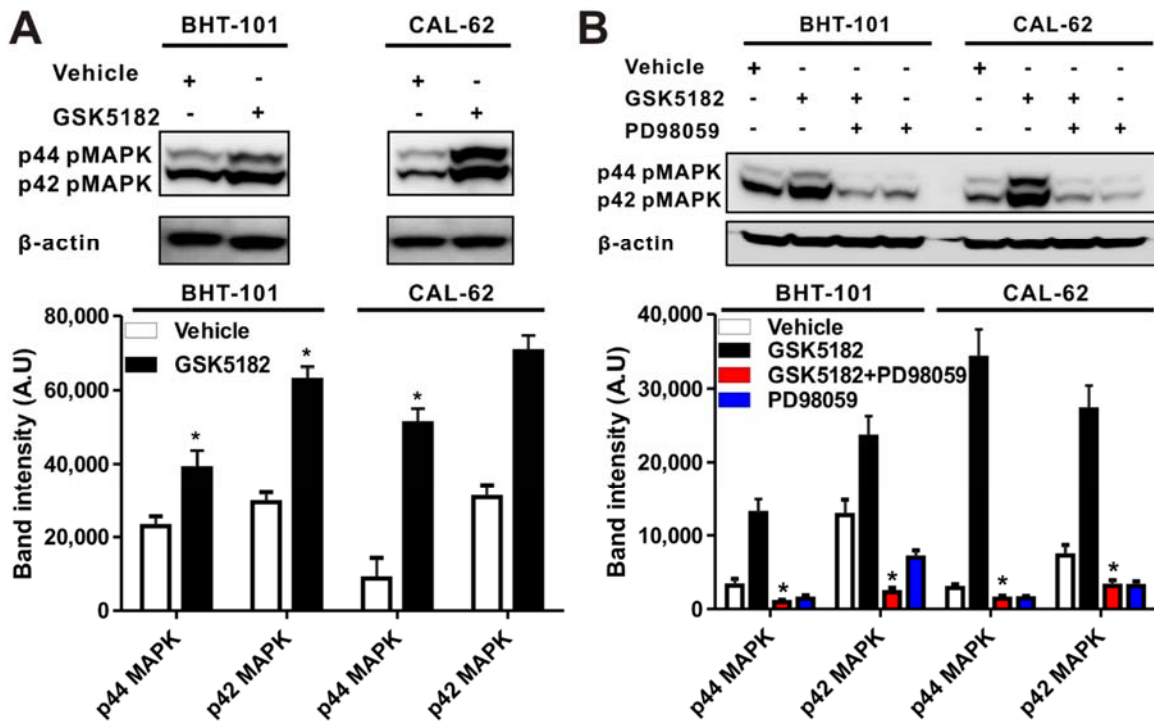


FIGURE 3. GSK5182-induced MAP Kinase activation in anaplastic thyroid cancer cells. (A) Activation of MAP kinase signaling in ATC cells by GSK5182 (Left panel). Each cells were incubated with 50 μ M GSK5182 for 24 h and phosphorylated ERK1/2 levels was determined with phospho-p44/42 specific antibody. Quantitative analysis of phosphorylated p44/42-ERK levels by scanning densitometry (Right panel). (B) Reversal of activated MAK kinase signaling by PD98059 (Left panel). Cells were co-treated with both 20 μ M PD98059 and GSK5182, and phosphorylated ERK1/2 levels were determined with immunoblotting. Quantitative analysis of phosphorylated p44/42-ERK levels by scanning densitometry after co-treatment with both 20 μ M PD98059 and GSK5182 (Right panel). Data mean \pm SD of 3 samples per group expressed in arbitrary units. *P< 0.05; when compared with untreated cells.

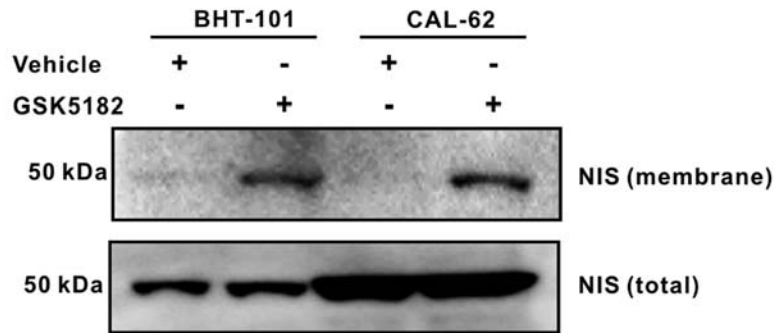
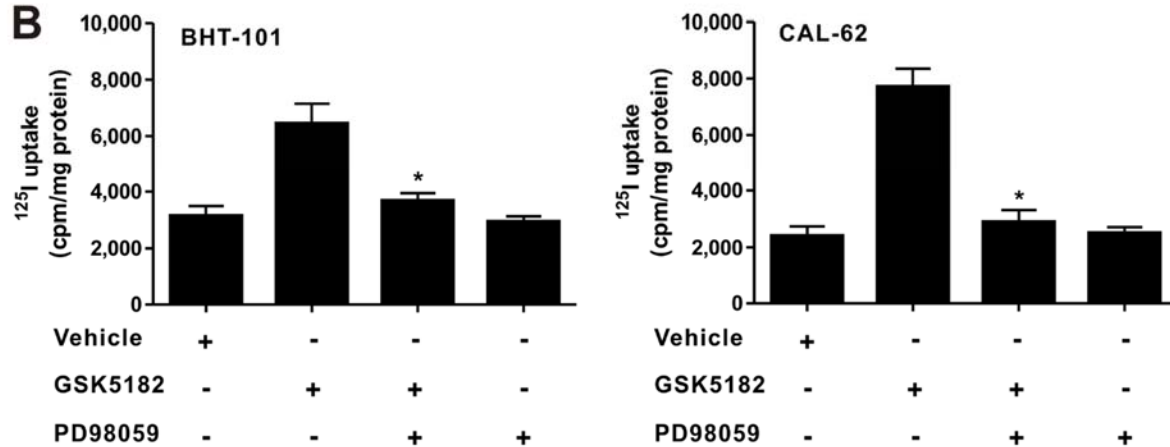
A**B**

FIGURE 4. Enhancement of membrane-localized NIS protein level by GSK5182. (A) Detection of membrane and total NIS protein. The membranous fraction of cells treated with or without GSK5182 were collected and immunoblotting analysis was done with NIS-specific antibody. (B) Inhibition of increased iodide uptake by PD98059 in GSK5182-treated ATC cells. Cells were co-treated with both 20 μ M PD98059 and GSK5182, and iodide uptake levels were determined. Data are expressed as mean \pm SD; n=3, * P<0.05; when compared with GSK5182.

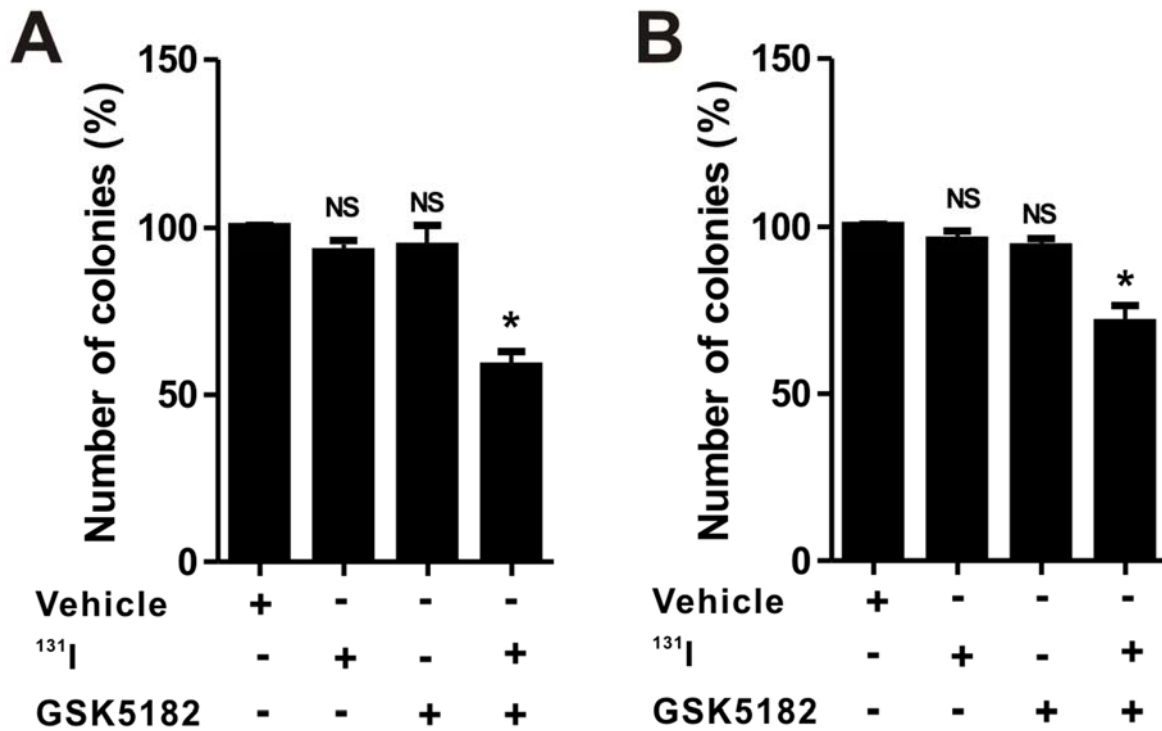


FIGURE 5. Increased cytotoxicity of ^{131}I by GSK5182 against anaplastic thyroid cancer cells. Either (A) CAL-62 or (B) BHT-101 cells were pre-treated with or without GSK5182 prior to day 1. After washing cells, they were further co-incubated with $50\mu\text{Ci } ^{131}\text{I}$ for 6h. Quantification of percentage of colonies number after ^{131}I treatment. All the data are expressed as mean \pm SD; n=3. * $P < 0.05$; NS=Not significant when compared with untreated cells.

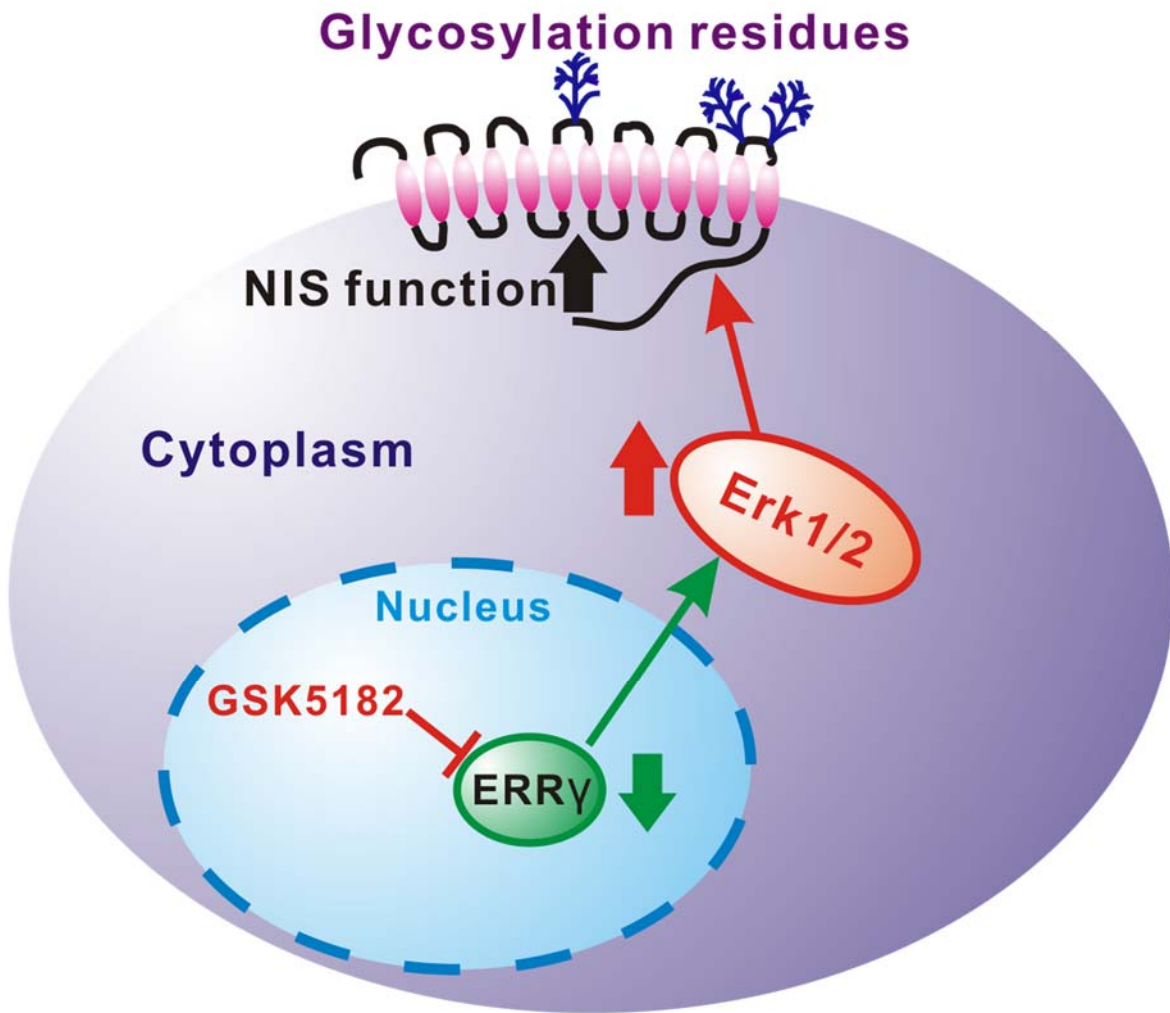


FIGURE 6. Proposed mechanism of GSK5182 in anaplastic thyroid cancer cells. Schematic representation of GSK5182-induced modulation of NIS function through MAP kinase pathway.