Excess iodine promotes papillary thyroid carcinoma through the AKT/mTOR pathway

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Abstract

The incidence of thyroid cancer in the world is increasing year by year, among which PTC accounts for more than 80%. Iodine is an essential trace element for thyroid hormone synthesis, but the relationship between excessive iodine and thyroid cancer is not clear. This study assumes that high iodine intake is related to the occurrence of thyroid cancer, and may affect the cell cycle through AKT/mTOR signaling pathway and promote the progress of cancer. The human PTC cell line TPC-1 was treated with different concentrations of potassium iodide (KI) to evaluate its effect on cell proliferation and migration. The results showed that an appropriate concentration of KI (such as 10^{-3} mM) could significantly enhance the proliferation of TPC-1 cells, and a high concentration of KI(≥1 mM) might inhibit cell proliferation. In addition, the expression of key proteins in the AKT/mTOR signaling pathway (such as p-AKT, p-mTOR, p-P706k) was up-regulated in TPC-1 cells treated with high iodine, indicating that the AKT/mTOR signaling pathway was activated. After the AKT/mTOR signaling pathway inhibitor LY294002 was used, the cell proliferation and migration ability decreased significantly, and cell cycle analysis showed that more cells treated with high iodine entered the S phase, while the proportion of cells in the G1 phase increased after LY294002 treatment. To sum up, this study provides preliminary evidence that an appropriate amount of KI affects cell proliferation and migration by activating the AKT/mTOR signaling pathway and inducing Cyclin D1 overexpression, thus promoting the development of PTC cell line TPC-1. This study is of great significance for understanding the relationship between excessive iodine intake and thyroid cancer and developing new prevention and treatment strategies.

Introduction

In 2020, there are 586,000 cases of thyroid cancer in the world, and the incidence of thyroid cancer ranks ninth in the world; among the 19.3 million new cancer cases in the world, thyroid cancer accounts for 3.0%; among the 10 million cancer deaths worldwide, thyroid cancer accounts for 0.4% (Sung et al., 2021). The age-standardized incidence rate of thyroid cancer has increased significantly in China in recent years, and the incidence rate of thyroid cancer is the third highest among all cancers (Han et al., 2024). Meanwhile, thyroid cancer has the highest incidence rate among all endocrine system cancers and head and neck cancers, as well as the highest incidence rate among people aged 18 to 33 years (Araque et al., 2017). Currently, differentiated thyroid cancers include papillary thyroid carcinoma (PTC), follicular thyroid carcinoma (FTC), and oncocytic thyroid carcinoma (OTC); of these, PTC accounts for more than 80% of all thyroid cancer cases (Aschebrook-Kilfoy et al., 2011; Chen et al., 2023). Although rapid advancements in diagnostic techniques and overdiagnosis are major contributors to the rapid increase in thyroid cancer incidence, they are not the only reasons (Kitahara and Sosa, 2016). Environmental factors are important in the high incidence of thyroid cancer.

Iodine is an essential trace element for the synthesis of thyroid hormone (TH) in the body and plays a crucial role in human growth and development (Aschebrook-Kilfoy et al., 2011). the relationship between iodine excess and human health, especially the relationship between iodine excess and thyroid cancer, still requires further study, as there is currently a lack of definitive conclusions (Winder et al., 2022).

Some studies (Kanno et al., 1992; Sosonkina et al., 2014; Wang et al., 2022) suggest that iodine excess is not a risk factor for PTC and may even be a protective factor. However, more and more results of some epidemiologic investigations have shown that iodine excess is closely related to PTC. Lind et al (Lind et al., 1998)investigated the relationship between types of thyroid cancer and water iodine content in several countries and found that PTC incidence was higher and metastatic in areas with iodine excess. Several experiments (Kanno et al., 1992) have also concluded that iodine excess is a risk factor for the development and progression of thyroid cancer. Therefore, we believe that high iodine is associated with thyroid cancer and that research on iodine and thyroid cancer is imperative. Moreover, the mechanism of the relationship between iodine and thyroid cancer is unclear.

Thyroid carcinogenesis may be associated with multiple signaling pathways (Amjad et al., 2024; Deng et al., 2024; Gao et al., 2024; Guo et al., 2024; Luo et al., 2024), and the protein kinase B (AKT)/mammalian target of rapamycin (mTOR) signaling pathway is one of the most critical intracellular signaling pathways controlling cellular function. Hyperactivation of the AKT/mTOR signaling pathway, which regulates the cell cycle and influences cell proliferation and migration, has been observed in almost all solid tumors (Steelman et al., 2011; Yu et al., 2024). Activation of the AKT/mTOR signaling pathway usually begins with a receptor on the cell surface, which binds to the corresponding ligand, triggering a series of cascade reactions that activate the associated P70S6K is also a serine/threonine kinase that, when phosphorylated, promotes protein synthesis (p-P70S6K) through a variety of pathways and is involved in a variety of intracellular biological processes including cell growth, proliferation, metabolism, survival and autophagy, which have been implicated in cancer development (Glaviano et al., 2023; Xu et al., 2004). Activation of the AKT/mTOR pathway upregulates the cell cycle and promotes cancer progression (Zhang et al., 2015). We suggest that the AKT/mTOR pathway may be involved in the effect of high iodine on thyroid cancer development.

Currently, areas of waterborne iodine overdose exist in more than 30 countries, such as China (Ma et al., 2022), Somalia (Kassim et al., 2014), and Kenya (Cui et al., 2024; Watts et al., 2020). The Chinese Center for Disease Control and Prevention (CDC) found that the median water iodine concentration (WIC) in 920 townships in China was greater than 100 μg/L (Hou et al., 2023) and that more than 30 million people live in areas of iodine overdose in water sources (Shen et al., 2011). Exploring the relationship between high iodine and thyroid cancer has important public health implications. In hence, the present study was designed to explore the mechanism of the effect of high iodine on the development of thyroid cancer and to verify that high iodine affects the cell cycle through the AKT/mTOR signaling pathway by examining the key factors of the AKT/mTOR pathway (p-)AKT, (p)-mTOR, p-P70S6K, PTEN, and the key factor of the cell cycle Cyclin D1, which influence the proliferation and migration of the PTC cell line TPC-1, which promotes cancer progression by affecting cell cycle through AKT/mTOR signaling pathway.

Materials and methods

Chemicals and Solutions

Ly294002 (CAS No. : 154447-36-6), a specific inhibitor for the AKT/mTOR pathway, was purchased from MCE (purity > 99.9%) and dissolved in Dimethyl Sulfoxide (DMSO, Solarbio, D8371) to yield the working concentrations. It was well-mixed and well-distributed in DMSO before and throughout dosing. KI was purchased from Aladdin (P433794) and is chemical analysis grade.

Cell Lines and Cell Culture

The human PTC cell line TPC-1 was purchased from Wuhan Procell Life Technology Co., Ltd. The cells were cultured in a prepared RMPI 1640 complete medium and placed in a constant temperature sterile incubator at 37 ℃ with a carbon dioxide concentration of 5%. A water tray was placed at the bottom of the incubator to maintain a certain humidity in the incubator.

Cell Proliferation Assay

Cells were plated in 96-well dishes at a density of 3000 cells per well, incubated overnight, and then treated with test compounds diluted in a complete growth medium. The viability of the cells was assessed using the Cell Counting Kit-8 and Ethynyldeoxyuridine assay, following the instructions provided by the manufacturers.

Cell Cycle Assay

TPC-1 cell was seeded into 6 well plates (10^6 cells/well), cultured through 48h, and exposed to test agents in a complete medium. Cell cycle distribution was subsequently analyzed by Cell Cycle and Apoptosis Analysis Kit (Beyotime Biotechnology, C1052) according to the manufacturer’s protocol. The cellular DNA content was measured by flow cytometry (Becton Dickinson, BD), and the percentage of cells in each phase of the cell cycle was analyzed using the FlowJo software.

Cell Migration Assay

Draw three fine lines parallel to the long edge of the 6-well plate at a certain interval on the back of each well of the 6-well plate using a marker pen. Seed the cell suspension into the 6-well plate at a density of 800,000 cells per well and incubated overnight in a cell incubator. After the cells have covered the bottom of the well plate, use a 200 μL yellow tip to simultaneously create scratches perpendicular to the well plate and the fine lines in each well of the 6-well plate with uniform force. Add 2 mL of medium containing a very low serum concentration of 0.5% to each well to reduce the impact of cell proliferation on the results. Take photographs under a fluorescent inverted microscope at 0 h and 48 h, respectively, and measure the scratch area in the photos at 0 h and 48 h using ImageJ software.

Western Blot Analysis

Prepare a solution by mixing cell lysis buffer, PMSF, protease inhibitor, and phosphatase inhibitor in a ratio of 100:1:1:1, and add it to the cell culture dish. Repeatedly scrape the cells off using a cell scraper and transfer them to a pre-labeled 1.5 mL EP tube, allowing them to digest for 30 minutes undisturbed. Pre-chill a low-temperature high-speed centrifuge set to 4 ℃, 12,000 rpm, centrifuge for 20 minutes, sonicate for 30 seconds, and place in the centrifuge again. After centrifugation, transfer the supernatant to a new EP tube, take a small amount of protein sample, dilute it 10 times, and measure its concentration. Based on the measured concentration, add different volumes of loading buffer to dilute each protein sample to the same concentration. Then, separate them using Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) and transfer them electrophoretically to a Polyvinylidene Fluoride (PVDF) microporous membrane. After blocking quickly in blocking solution at room temperature for 1 hour, incubate the membrane overnight at 4°C with AKT, p-AKT, mTOR, p-mTOR, p-P70S6K, PTEN, and Cyclin D1 antibodies. After incubating with the secondary antibody (diluted 1:5000; CST) for 1 hour, wash the protein bands with TBST, observe them using Enhanced Chemiluminescence (ECL) detection reagent, and detect them with a chemiluminescent gel imaging system (Bio-Rad).

Statistical Analysis

Data was summarized and organized using Excel 2019, then imported into SPSS 24 software for data analysis and plotting. The results were expressed as mean ± standard deviation (SD). The comparison of means between two groups was analyzed using an independent two-sample t-test, while the comparison of means among multiple groups was conducted using One-Way ANOVA. Tukey's method was employed for pairwise comparisons between groups. Statistical significance was considered when $p < 0.05$.

Results

Effects of high iodine on the proliferation and migration ability of papillary thyroid carcinoma cells

TPC-1 cells were treated with different concentrations of KI solution (10^{-6}-10 mmol/L) for 12, 24, and 48 hours, and the changes in their proliferation ability were determined by CCK-8. The results showed that after 12 h of treatment of TPC-1 cells with KI solution, only the highest concentration of 10 mM group showed statistically different proliferative ability from that of the control group ($P > 0.05$), which was mainly manifested in the decrease of proliferative ability of TPC-1 cells caused by ultra-high concentration of KI solution. However, after 24 h of treatment with 10^{-5}, 10^{-3}, and 10^{-2} mM KI solutions, TPC-1 cells exhibited a significant change ($P < 0.05$) in proliferative capacity compared to the control group. Similarly, after 48 hours of treatment with 10^{-5}, 10^{-3}, and 10^{-1} mM KI solutions, TPC-1 cells showed significantly enhanced proliferation capacity compared to the control ($P < 0.05$). It is worth noting that too high concentration of KI (≥ 1 mM) may inhibit cell proliferation (see Table 1 and Fig. 1A). Based on the above experimental results, TPC-1 cells were selected to be cultured with 10^{-5}, 10^{-3}, 10^{-1}, and 10 mM KI concentrations for 48 h for proliferation experiments to validate the EDU-488 results, which showed that the real-time proliferative ability of cells was significantly stronger than that of the

control group under the treatment of 10^{-5} and 10^{-3} mM KI concentrations, and the difference was statistically significant ($P < 0.05$) (see Fig. 1B-C). Based on the above experimental results, TPC-1 cells were selected to be cultured at 10^{-3} mM KI concentration for 48 h. It was observed that the high concentration of KI promoted the migration ability of papillary thyroid cancer cells, and the difference was statistically significant ($P < 0.05$) (see Fig. 1D-E).

Table 1
Effects of different concentrations of KI on cell proliferation ability at various time points.

KI(mmol/L)	12 h	24 h	48 h
0	100.44 ± 1.31	100.32 ± 1.88	100.76 ± 0.88
10^{-6}	103.95 ± 4.37	99.61 ± 10.77	107.82 ± 3.36
10^{-5}	107.83 ± 8.60	117.10 ± 7.97*	118.60 ± 1.96*
10^{-4}	104.65 ± 8.98	113.63 ± 9.86	111.60 ± 6.33
10^{-3}	105.42 ± 6.82	117.99 ± 6.74*	129.57 ± 1.67*
10^{-2}	100.86 ± 13.75	117.24 ± 6.09*	110.52 ± 1.76
10^{-1}	94.21 ± 8.85	109.71 ± 12.91	123.04 ± 3.06*
1	91.53 ± 16.44	104.14 ± 11.82	94.34 ± 8.04
10	86.45 ± 15.92*	100.51 ± 16.82	83.72 ± 2.43*
Note: * $P < 0.05$ compared to the 0 mM KI group.			

Effects of high iodine on proliferation and migration ability of papillary thyroid cancer cells via AKT/mTOR signaling pathway

After the dose-response relationship between LY294002 and TPC-1 cells was detected by CCK-8, 10 μM was selected as the subsequent experimental dose (see Fig. 2A).EDU assay of cell proliferation ability revealed that, after 48 h of co-treatment by LY294002 and 10^{-3} mM KI compared with treatment with only 10^{-3} mM KI solution, the proliferation ability of TPC-1 cells was significantly reduced, even lower than the DMSO control group, and the differences were all statistically significant ($P < 0.05$) (see Fig. 2B-C). The scratch experiment revealed that after the inhibition of AKT/mTOR pathway expression, the migration ability of TPC-1 cells treated with 10^{-3} mM KI solution was significantly reduced compared with that of the undisturbed pathway, and even lower than that of the DMSO control group, and the differences were statistically significant ($P < 0.05$) (see Fig. 2D-E). The above findings suggest that inhibition of AKT/mTOR pathway expression reduces the proliferation and migration ability of papillary thyroid cancer cells.

Effect of high iodine on the expression of AKT/mTOR pathway in papillary thyroid cancer cells

Western Blot assay was performed to assess the activation level of the AKT/mTOR pathway. p-AKT, AKT, p-mTOR, mTOR, p-P70S6K, and PTEN protein expression was increased in the 10^{-3} mM KI group as compared to the control group, and the difference was statistically significant ($P < 0.05$). LY294002 combined with 10^{-3} mM KI The expression of p-AKT, AKT, p-mTOR, mTOR, p-P70S6K, and PTEN proteins was decreased in the treatment group, and the difference was statistically significant ($P < 0.05$) compared with that in the 10^{-3}mM KI group (see Fig. 3A-G).

Effect of high iodine on the cell cycle of papillary thyroid carcinoma

Compared with the control group, more TPC-1 cells entered the S phase under 10^{-3} mM KI treatment, implying that more cells entered the proliferation phase, which was also consistent with the previous proliferation assay results; after LY294002 inhibited the expression of the AKT/mTOR signaling pathway, compared with the 10^{-3} mM KI group, significantly fewer cells entered the S and G2 phases, and more cells were blocked into the G1 phase, and the difference was statistically significant ($P < 0.05$) (see Fig. 4A-B). It was detected that Cyclin D1 expression was increased in the 10^{-3} mM KI group, and the difference was statistically significant ($P < 0.05$) compared with the control group; compared with the 10^{-3} mM KI group, Cyclin D1 expression was decreased in the group co-treated with LY294002 and 10^{-3} mM KI, and the difference was statistically significant ($P < 0.05$) (see Fig. 4C-D).

Discussion

The incidence of thyroid cancer in men and women of all age groups has shown a significant upward trend in most countries over the past decade (Huang et al., 2023). The total age-adjusted incidence rate of thyroid cancer in Chinese females increased from 1.93/100,000 in 1983–1987 to 12.18/100,000 in 2008–2012, while that of males increased from 0.77/100,000 in 1983–1987 to 3.89/100,000 in 2008–2012, and this increasing trend will continue for another 20 years. This increasing trend will continue for another 20 years, with more than 3.7 million new cases expected between 2028 and 2032 (Li et al., 2021). From 1990 to 2013, the age-standardized incidence of thyroid cancer increased by 20% globally, with a higher relative increase in low-income countries (33%) than in high-income countries (19%) (Fitzmaurice et al., 2015). A breakdown of thyroid cancer by WHO region shows that the Western Pacific region has the highest thyroid cancer diagnosis rate of 279,035 in 2020, one of the 9 most common cancer types in the region; it is estimated that from 2020 to 2040, global thyroid cancer incidence will increase by 29.9% and thyroid mortality will increase by 67% in both men and women (Shank et al., 2022). Therefore, exploring potential environmental and lifestyle-related factors that may contribute to

the rising trend of thyroid cancer is a pressing scientific issue in the field of thyroid cancer prevention and treatment at present.

With the success of iodine deficiency prevention, many challenges have emerged, including the continued presence of areas with high water iodine concentrations (Chen et al., 2024a). Iodine is a trace element essential for normal growth and development of the human body and is the basis of thyroid hormones. Chronic iodine deficiency leads to insufficient synthesis and secretion of thyroid hormones, which can lead to various health problems, such as the increased risk of fetal miscarriage, stillbirth, congenital malformations, and perinatal death, impeded neurodevelopment and physical developmental delays in children and adolescents, as well as goiter and even thyroid cancer in all age groups (Zimmermann et al., 2008). According to an epidemiological survey in 2019, the median urinary iodine levels in Chinese schoolchildren and adults were 199.75 μg/L and 177.89 μg/L, respectively, and the goiter rates were 3.50% and 1.17%, suggesting that iodine levels are adequate in the Chinese population and that iodine deficiency disorders have been eliminated (Li et al., 2020). However, studies have shown that the relationship between iodine intake and human health has a 'U'-shaped curve, which implies that both excessive and insufficient iodine intake can be harmful (Jin et al., 2020; Laurberg et al., 2001; Wang et al., 2019). Some studies have shown that high iodine intake is associated with the development of thyroid cancer, and excessive intake increases the incidence of papillary thyroid cancer (PTC) (Lin and Wu, 2023; Wang et al., 2020). Part of China is still a high iodine area, and some residents drink high-iodine water sources, excessive iodine intake can affect their health. Although several studies have explored the relationship between high iodine and PTC, the findings are inconsistent and the underlying mechanisms remain unclear (Chen et al., 2024b; Fuziwara and Kimura, 2014; Lee et al., 2017; Xing et al., 2024; Yao et al., 2022). Therefore, it is crucial to investigate whether high iodine intake affects the development of PTC.

Our research shows that the effect of potassium iodide on the proliferation and migration of PTC cells has an inverted "U" relationship with iodine concentration. It has the potential to promote the development of TPC-1 cancer at 10^{-3} mM KI. Previous studies have demonstrated that increased phosphorylation of AKT and mTOR is a key marker of activation of the AKT/mTOR pathway, which is closely associated with the development of various cancers. aKT is a core protein of the pathway (KEGG, 2024), which can be activated by PIP3 to become p-AKT, which then phosphorylates downstream target proteins and regulates the cell cycle, autophagy, immune response, and inflammation. Cell cycle protein D1 is a key protein in cell cycle regulation and is closely related to cancer development. Its overexpression is often observed in various cancers (Gao et al., 2004; Jirstrom et al., 2005; Kaminagakura et al., 2011; Wang et al., 2023), and activation of the AKT/mTOR pathway promotes its overexpression (Qie and Diehl, 2016). Based on existing studies and our findings, we tentatively conclude that high iodine (10^{-3} mM) promotes the development of PTC by activating the AKT/mTOR signaling pathway and inducing Cyclin D1 overexpression.

Previous studies have shown that high iodine may affect thyroid cancer development through multiple pathways: (1) By decreasing the expression of SLC4A4 and further activating the JNK/P38 MAPK

signaling pathway, high iodine promotes cell growth, proliferation, invasion, and migration, increasing the risk of PTC metastasis (Lee et al., 2017; Li et al., 2024)[51]. (2) High iodine enhances miR-200c expression and promotes PTC proliferation and metastasis through YWHAG (Zhou et al., 2023). (3) High iodine promotes cell proliferation and migration by decreasing the expression of oncogene RSK4 and activating the MAPK signaling pathway (Chen et al., 2019). (4) High iodine increases the expression of β-catenin, c-myc, and Cyclin D1, activates the Wnt/β-catenin signaling pathway, and promotes thyroid cancer cell proliferation and migration (Zhang et al., 2019). Although our study and previous studies agree that high iodine promotes the development of thyroid cancer, there are differences in the underlying mechanisms or optimal concentrations of this effect. These differences may arise from a variety of factors, such as the use of different iodine compounds (e.g. KI or KIO3), variations in thyroid cancer cell types with different mutations or rearrangements, different methods and durations of cell processing, and the possibility that multiple signaling pathways may simultaneously affect thyroid cancer development and that there may be signaling crosstalk.

In conclusion, our study provides preliminary evidence that a certain dose of KI (10^{-3} mM) can promote the development of the PTC cell line TPC-1 by activating the AKT/mTOR signaling pathway and inducing Cyclin D1 overexpression, thereby affecting cell proliferation and migration.

Declarations

Author contributions Conceptualization: SL, YC, HL. Data curation: SL, YC. Formal analysis: SL. Funding acquisition: YC, HL. Investigation: SL, YC, XY, ZX, PD. Methodology: YC, HL. Project administration: YC, HL. Resources: YC, HL. Supervision: YC, HL. Validation: SL, YC, HL. Visualization: YC. Writing – original draft: SL. Writing – review & editing: SL. All authors read and approved the final manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest.

Informed consent The study was approved by the “Medical Ethics Committee of Tianjin Centers for Disease Control and Prevention” and has been carried out in accordance with the ethical guidelines set out in the Declaration of Helsinki. All the selected children agreed to participate with consent from their guardians, and the pregnant women consented to participate.

Consent to Participate All participants have consented to participate in this study.

Consent for Publication The participants have consented to the submission of the manuscript to the journal.

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Figures

Figure 1.

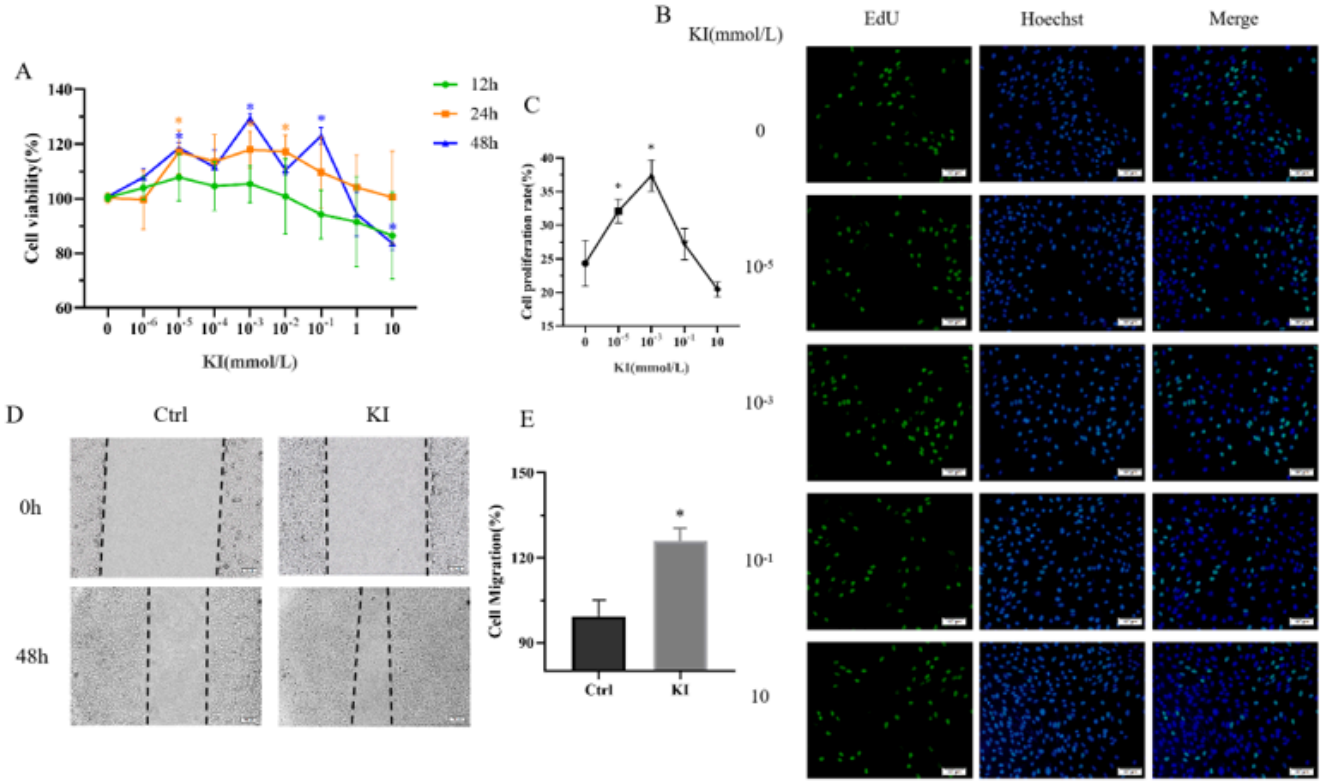

Figure 1

Effects of high iodine on the proliferation and migration ability of papillary thyroid carcinoma cells. (A) TPC-1 cells were treated with graded concentrations of high iodine for 12, 24, and 48 h. Cell proliferation was assessed by CCK-8 assay. (B) TPC-1 cells were treated with 10^{-5}、10^{-3}、10^{-1} and 10 mmol/L KI for 48 h. Cell viability was assessed by EdU assay. (C) Quantitative analysis of cell viability by EDU assay. (D) Scratch assay to detect the alteration of migration ability of TPC-1 cells cultured at 10^{-3} mmol/L KI concentration. (E) Quantitative analysis of scratch assay. $^{*}P<0.05$.

Figure 2.

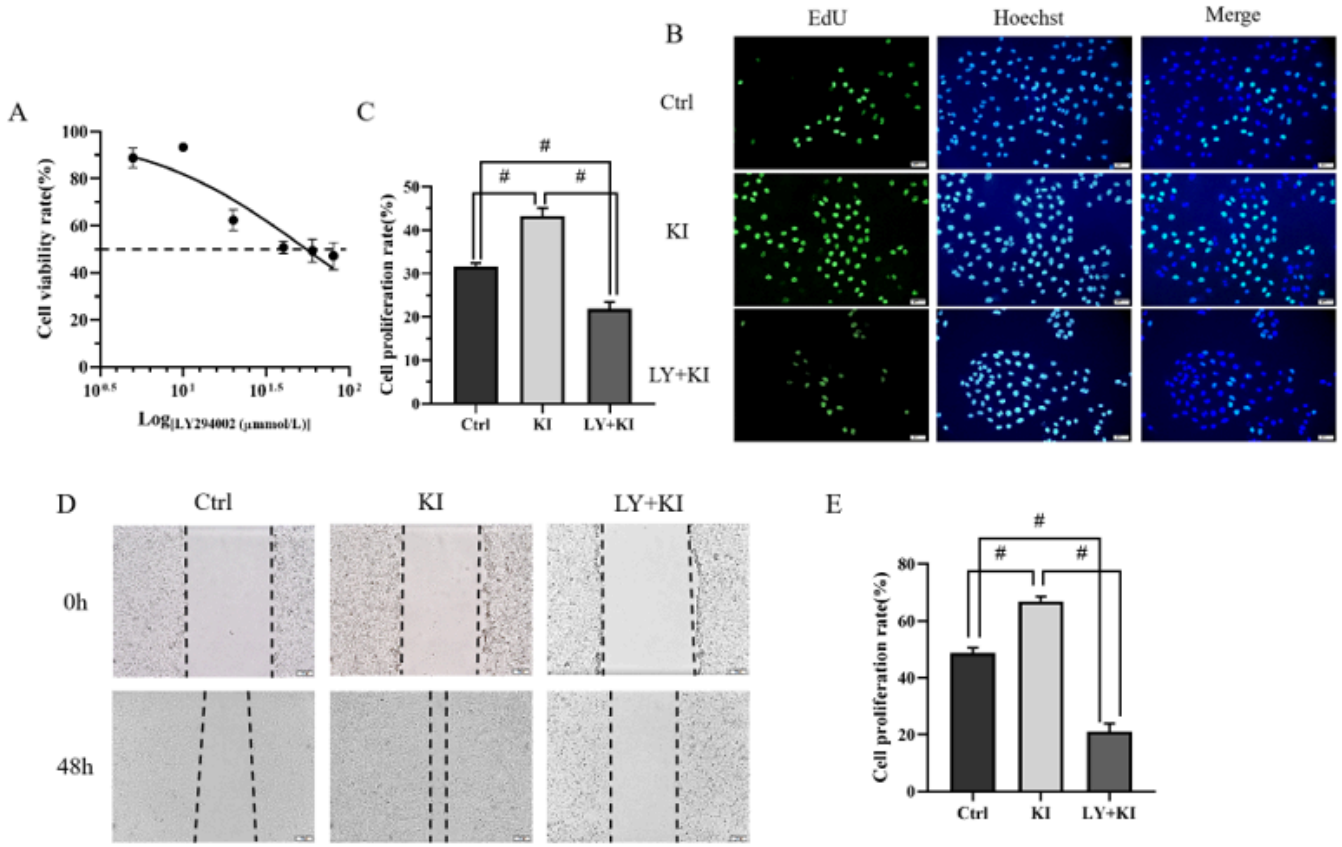

Figure 2

Effects of high iodine on proliferation and migration ability of papillary thyroid cancer cells via AKT/mTOR signaling pathway. (A) Dose-response relationship between LY294002 and TPC-1 cell viability. (B) TPC-1 cells were co-treated with or without 10 μmmol/L LY294002 with 10^{-3} mmom/L KI for 48 h. The control was a DMSO control group, and the 10^{-3} mmom/L KI-treated group was also supplemented with the same proportion of DMSO, and the concentration of both was <1%. Cell viability was assessed by EdU assay. (C) Quantitative analysis of cell viability by EDU assay. (D) Scratch assay was performed to detect changes in cell migration ability observed in TPC-1 cells co-treated with or without 10 μmmol/L LY294002 with 10^{-3} mmom/L KI for 48h. The control was a DMSO control group, and the same proportion of DMSO was also added to the 10^{-3} mmom/L KI-treated group, and the concentrations were all <1%. (E) Quantitative analysis of the scratch assay. $^{*}P<0.05$.

Figure 3.

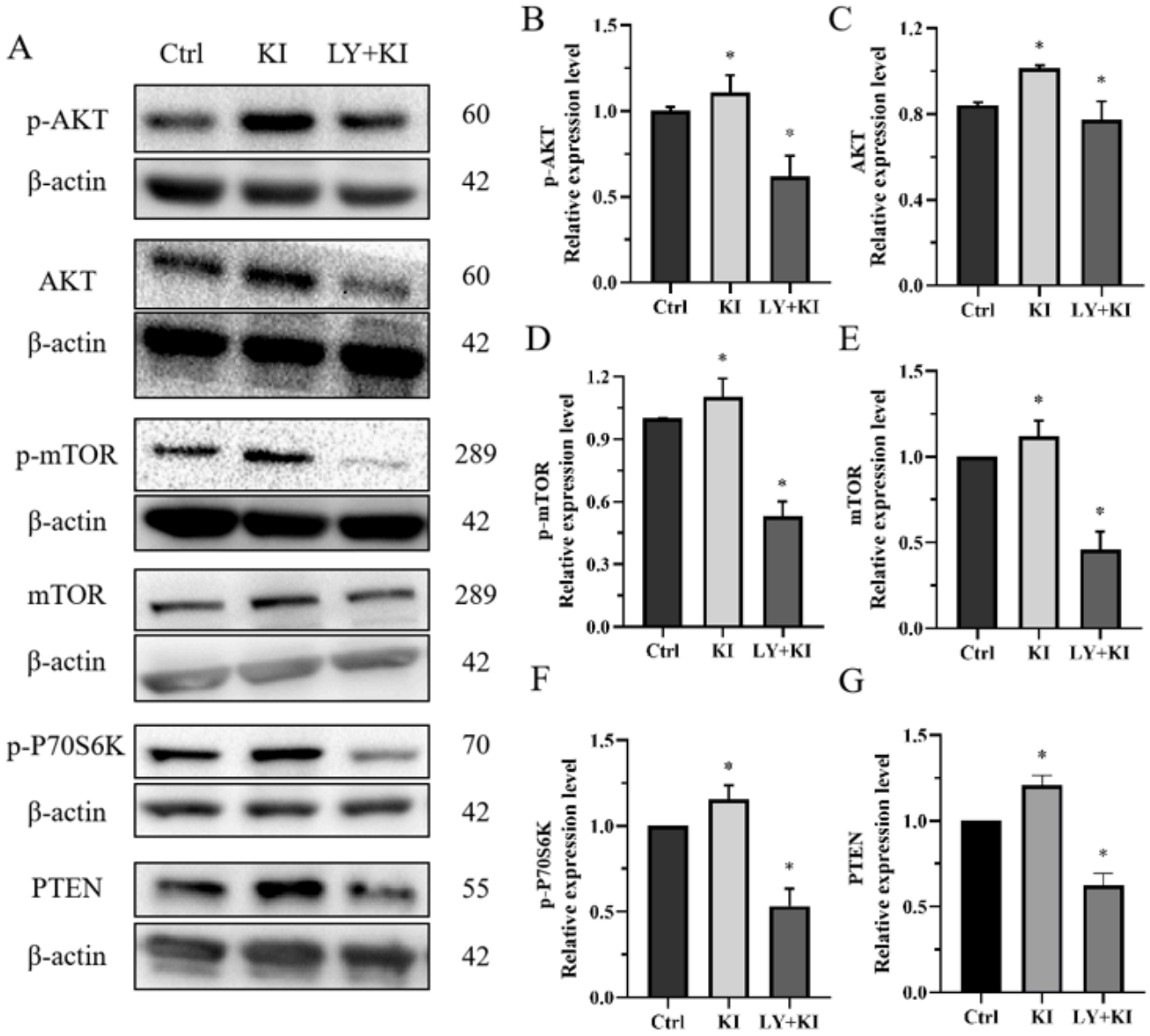

Figure 3

Effect of high iodine on the expression of AKT/mTOR pathway in papillary thyroid cancer cells. (A) Western Blot detection of intracellular AKT/mTOR signaling pathway-related protein expression in TPC-1 cells co-treated with or without 10 μmmol/L LY294002 and 10^{-3} mmom/L KI for 48 h. The control was a DMSO control group, and the 10^{-3} mmom/L KI-treated group was added with the same proportion of DMSO and the concentrations were all <1%. (B) Changes in p-AKT protein expression after treatment. (C) Changes in AKT protein expression after treatment. (D) Changes in p-mTOR protein expression after treatment. (E) Changes in mTOR protein expression after treatment. (F) Changes in p-P70S6K protein expression after treatment. (G) Changes in PTEN protein expression after treatment. $^{*}P<0.05$.

Figure 4.

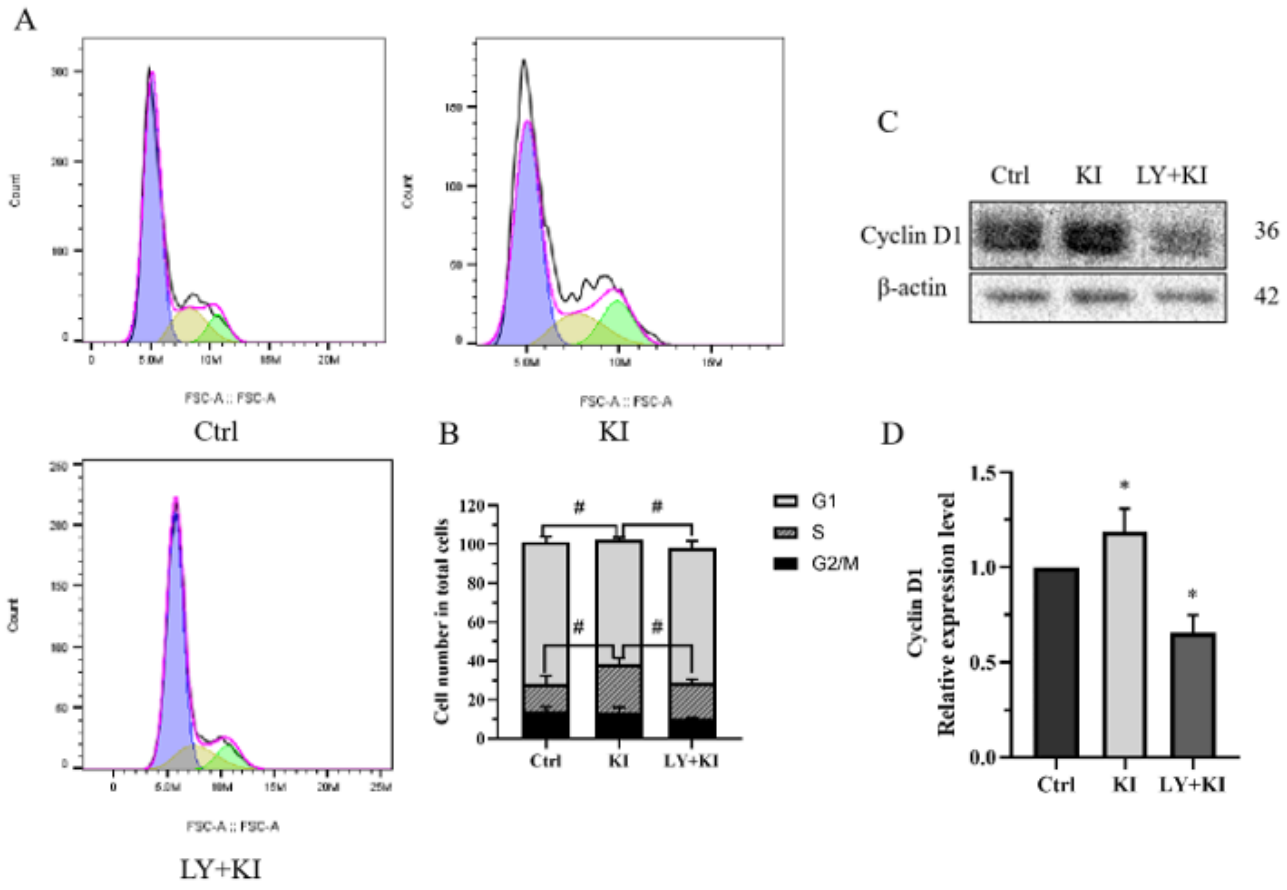

Figure 4

Effect of high iodine on the cell cycle of papillary thyroid carcinoma. (A) Flow cytometry was performed to detect cell cycle changes in TPC-1 cells treated with or without 10 μmmol/L LY294002 and 10^{-3} mmol/L KI for 48 h. The control group was the DMSO control group, and the 10^{-3} mmol/L KI treatment group was also treated with the same proportion of DMSO, and the concentration of DMSO was <1% in both groups. (B) Changes in the proportion of cell volume in each cell cycle in different treatment groups. (C) Western Blot detection of cell cycle-related protein Cyclin D1 expression in TPC-1 cells co-treated with or without 10 μmmol/L LY294002 and 10^{-3} mmom/L KI for 48 h. The control was the DMSO control group, and the same proportion of DMSO was also added to the 10^{-3} mmom/L KI treatment group, and the concentrations were all <1%. (D) Changes in Cyclin D1 protein expression after treatment. $^{*}P<0.05$.