S100A6 Promotes the Development and Apoptosis of Thyroid Cancer Through the PI3K/AKT/Mtor Pathway

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Primary research

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Abstract

Background

Thyroid cancer (TC) is the most common endocrine malignant tumor. High levels of S100A6 have been associated with progression in some type of human cancers, but its role in the molecular pathogenesis of these cancers is largely unknown. This study investigated the expression and functional roles of S100A6 in human thyroid cancer.

Methods

TCGA database and qRT-PCR were used to examine the expression of S100A6 in thyroid cancer. CCK-8 assay, colony formation assay, Transwell assay, invasion assay and western blot assay were used to identify the biological function of S100A6 in thyroid cancer cells. Flow cytometry is used to assess apoptosis.

Results

Compared with normal thyroid tissues, S100A6 is overexpressed in thyroid cancer tissues and cell lines. Silencing S100A6 can inhibit the proliferation, migration and invasion of TC cells, and induce their apoptosis.

Conclusions

S100A6 promotes the progression of thyroid cancer through PI3K/AKT/mTOR pathway and can be used as a target in the future treatment of thyroid cancer.

Background

Thyroid cancer (TC) is the most common endocrine tumor, and in the last decade its incidence has rapidly and sharply increased worldwide. Thyroid cancer ranks fifth among all cancers in females.[1] Most patients with thyroid cancer have a good prognosis after surgery, with a 10-year survival rate of > 90%. However, some patients still experience recurrence and metastasis after treatment. The overall survival rate of patients with local recurrence is approximately 70–85%, while the long-term survival rate of patients with distant metastasis is 30%-60%.[2–4] Therefore, the mechanism of TC needs to be further studied.

S100A6 mainly exists in the cytoplasm, cell membrane, and nuclear envelope, including the inner surface of the nuclear membrane, and is a member of the S100A calcium-binding protein family. Several studies have shown that S100A6 is related to the occurrence and development of various tumors, including colon cancer, hepatocellular carcinoma, pancreatic cancer, and gastric cancer.[5–9] Therefore, we speculate that the abnormal expression of S100A6 is closely related to the proliferation and differentiation of tumor cells.
Moreover, S100A6 mostly accumulates at the edge of the tumor, which may also be related to tumor invasion and migration. However, its role in thyroid cancer remains unclear.

The PI3K/AKT/mTOR pathway is an important pathway in cancer development. It regulates multiple processes, including cell survival, cell cycle progression, DNA repair, protein synthesis, glucose metabolism, angiogenesis, and cell migration. In this study, we verified, using real-time quantitative PCR, that S100A6 was highly expressed in tumor tissues; we then explored its function in thyroid cells by gene silencing and western blot analysis. The results indicated that S100A6 may be an important tumor-promoting gene in thyroid cancer through the activation of the PI3K/AKT/mTOR pathway.

**Methods**

**Patients and thyroid tissue sample**

Thyroid cancer tissues (42 pairs) and corresponding normal tissues were collected from patients in the thyroid surgery department of the First Affiliated Hospital of Wenzhou Medical University, China, and stored in liquid nitrogen. Two pathologists performed the histological examinations of all specimens to confirm the histological diagnosis. All patients signed a letter of consent, and the patient's information was approved by the Ethics Committee of the First Affiliated Hospital of Wenzhou Medical University (approval no. 2012-57).

**Bioinformatics**

Information of 502 thyroid cancer patients with complete clinical case characteristics (age, sex, LNM, tumor size, clinical stage, etc.) was downloaded from the TCGA database for analysis. The public gene chip dataset was downloaded from the Gene Expression Omnibus (https://www.ncbi.nlm.nih.gov/geo/). The GSEA software (GSEA v3.0, http://www.broadinstitute.org/gsea) was used to perform a single GSEA between the low S100A6 and high S100A6 expression groups in TCGA.

**RNA isolation and real-time reverse transcription/polymerase chain reaction (qPCR)**

Total RNA of TC and normal tissues was extracted using TRIzol reagent (Thermo Fisher Scientific, Waltham, USA) according to the manufacturer's instructions. cDNA synthesis was performed using the ReverTra Ace qPCR RT kit (Toyobo, Osaka, Japan). The cDNA was stored at -20°C for further processing. To detect the expression level of S100A6, qRT-PCR was performed using The Applied Biosystems 7500 Real-Time PCR system (Applied Biosystems; Thermo Fisher Scientific, Inc.). The primer sequences used for quantitative PCR were as follows: GAPDH forward 5'-GTCTCCTCTGACTTCAACAGCG-3'/ reverse 5'-ACCACCCGTGGCTGTTAGCCA-3'; S100A6 forward 5'-GAAATTGCAAGGCTGATGGAAG-3'/ reverse 5'-GGCTTCATTGTAGATCAAAGCC-3'.

**Cell culture**
Human TC cell lines (FTC, KTC-1, and BCPAP) were provided by Professor Mingzhao Xing of Johns Hopkins University School of Medicine (Baltimore, Maryland, USA). The Htorti-3 cell line was purchased from the Stem Cell Bank, Chinese Academy of Sciences. TPC-1 and BCPAP cell lines were cultured in RPMI-1640 containing 10% fetal bovine serum (FBS) (Gibco, Invitrogen, Carlsbad, CA, USA); FTC was cultured in DMEM medium (Gibco, Invitrogen, Carlsbad, CA, USA). Three cell lines were incubated at 37°C with 5% CO2 in a standard cell culture incubator (Thermo Fisher Scientific).

Cell transfection

Thyroid cells were seeded in a six-well plate and transfected for 24 h. S100A6 was silenced by siRNA using Lipofectamine RNAiMAX transfection reagent (Invitrogen, Grand Island, NY, USA). Both Si-S100A6 and Si-NC were purchased from GenePharma (Shanghai, China). 48 hours after transfection, cells were subjected to the next experiment. The sequences were as follows: Si-S100A6, forward 5’-AAGCTGCCAGGATGCTGAAATT-3’; Si-NC, forward 5’-UUCUCCGAACGUGUCAGUTT-3’ and reverse 5’-ACGUGACACGUUCGGAGATT-3’.

Cell proliferation and colony formation assay

Cell Counting Kit-8 (Beyotime Biotechnology, Shanghai, China) and clone formation experiments were used to evaluate the proliferative ability of the cells. Transfected cells were spread (1000 cells per well) in a 96-well plate. Approximately 100 µL of cck8 were added to each well, and the cells were incubated at 37°C for 3 h. The absorbance of each well at 450 nm was measured for four consecutive days.

Clone formation experiment

The transfected TC cells were seeded into a 6-well plate and incubated under the same conditions for 5-8 days. Cells were then fixed with 4% paraformaldehyde at room temperature for 15 min, and stained with crystal violet. Pictures were taken under a strong light by a digital camera. Each experiment was repeated three times.

Cell migration and invasion assays

Transwell assays were performed to evaluate the migration and invasion capabilities of the cells. Transfected and control cells were collected using trypsin. The Transwell chamber (#3422, Corning, NY, USA) and Matrigel Invasion Chamber (#354480; Corning Biocat, New York, USA) was used in the assays. First, 600 µL of medium containing 10% FBS were added to the lower chamber and the cells (35000 cells per well) were transferred to the upper chamber and incubated at 37°C for 20 h. Cells migrating from the upper chamber to the lower chamber were fixed and stained with crystal violet for 15 min. Three fields of view were randomly selected, and images were taken using a microscope at ×5 magnification. Each experiment was repeated 3 times.

Apoptosis analysis
Annexin V-FITC apoptosis kit (#556,547; Becton, Dickinson and Company, Franklin Lakes, NJ, USA) was used to detect cell apoptosis. Cells were collected, and stained with added Annexin V-FITC and PI in the dark for 15 min. The cells were analyzed using a flow cytometer (BD Biosciences, Franklin Lakes, USA). The apoptosis rate was equal to the percentage of late apoptosis (Q2) and the percentage of early apoptosis (Q3).

**Western blot analysis**

Total protein was extracted 48 h after transfection, and protein concentrations were determined using bicinchoninic acid assay (BCA). Protein lysates were separated by 10% SDS-PAGE electrophoresis and transferred to polyvinylidene fluoride (PVDF) membranes (EMD Millipore, Billerica, MA, USA). The PVDF membranes were blocked with skim milk for 2 h at room temperature and then washed three times with TBST. Cells were incubated with primary antibodies overnight at 4°C. The primary antibodies are as follows: BCL-2 (Abcam, Cambridge, Massachusetts, USA); S100A6, p-AKT, p-PI3K, p-MTOR, BAX and β-Actin (Proteintech, Wuhan, China). Membranes were rewashed with TBST three times and incubated with secondary antibodies (anti-mouse IgG or anti-rabbit IgG) for 1 h at room temperature. A chemiluminescence kit (Thermo Scientific, USA) was used to expose the films. Image Lab software was used to quantitatively analyze the bands.

**Statistical analysis**

Statistical analysis was performed using SPSS 23.0, and GraphPad Prism was used to create charts. Student’s t-test or one-way ANOVA was used to assess differences between groups. Data in the figure are the mean ± standard deviation. P<0.05, represents statistical significance.

**Results**

**S100A6 is highly expressed in thyroid cancer**

We downloaded transcriptome sequencing data from The Cancer Genome Atlas (TCGA) and GSE3678 to evaluate the expression of the S100A protein family in TC. Two data sets showed that s100a6 expression was significantly higher in tumor tissue than in normal thyroid tissue (P<0.0001, Figure 1A). In addition, we used the Tumor Immunity Estimation Resource (TIMER) database to perform a whole-cancer analysis of the expression of S100A6. Results showed that S100A6 was highly expressed in a variety of cancers (Figure 1B). In the TCGA database, compared with normal thyroid tissue, S100A6 is highly expressed in 502 pairs of thyroid cancer tissues (P<0.0001, Figure 2A), and the same was observed in the GSE3678 dataset (P<0.05, Figure 2B). In addition, we performed RNA sequencing on 70 pairs of thyroid cancer tissues and their paired adjacent tissues, and found the same phenomenon (P<0.0001, Figure 2C). To verify this phenomenon, we used qRT-PCR to analyze 42 pairs of thyroid cancer tissues and corresponding adjacent tissues. As expected, S100A6 was overexpressed in the thyroid cancer tissues (P<0.0001, Figure 2D).
S100A6 expression level is related to the clinicopathological characteristics of thyroid cancer

Next, we assessed the relationship between the expression level of S100A6 and clinical features in the TCGA database. According to the expression level of S100A6, patients were divided into two groups: a high-expression and low-expression group. In the TCGA cohort, higher S100A6 expression was associated with a higher T stage (p<0.001), a greater incidence of lymph node metastasis (LNM) (p<0.0001), a higher disease stage (p<0.001), and a higher frequency of the classical type (p<0.0001) (Table 1). In addition, the expression of S100A6 was related to tumor size, LNM, and disease stage in the validated cohort (Table 2). We also found that most patients with higher disease stages had higher expression of S100A6 (Figure 2E-2G). Therefore, we believe that the clinicopathological characteristics of patients with thyroid cancer are related to the expression levels of S100A6.

High expression of S100A6 increases the risk of lymph node metastasis in thyroid cancer

Furthermore, we speculated that S100A6 may be the main risk factor for LNM in TC. Univariate logistic regression analysis showed that the expression levels of S100A6, age, disease stage, and histological type were related to the risk of LNM. Multivariate logistic regression analysis showed that these factors were also associated with LNM risk (Table 3).

The significance of S100A6 expression in the diagnosis of patients with thyroid cancer

To clarify the diagnostic value of S100A6, we performed an ROC curve analysis. In the TCGA database, the AUC was 84% (P<0.0001). Similarly, in the verification cohort and the GSE3678 database, the AUCs were 82.48% and 91.84%, respectively (Figure 2H). In addition, S100A6 also had diagnostic significance for T stage (Figure 2I). Therefore, we believe that the expression of S100A6 may be useful for the diagnosis of thyroid cancer.

S100A6 promotes cell proliferation and clone formation

We assessed the expression levels of S100A6 in normal cells and various thyroid cells by RT-qPCR and found that S100A6 was highly expressed in KTC-1, BCPAP, and FTC cell lines; therefore, these cell lines were selected for in vitro experiments (Figure 3A). First, we used siRNA to knockdown S100A6 in TC cell lines. RT-qPCR and western blotting revealed a reduction in the expression level of S100A6 in the three cell lines (Figure 3B,C). Then, we evaluated the effect of S100A6 on the proliferation of PTC cells. CCK-8 suggested that S100A6 knockdown reduced the proliferation of TC cells (Figure 3D, p<0.001). Clone formation experiments also proved this hypothesis (Figure 3E). Therefore, down-regulation of S100A6 inhibited the proliferation of PTC cell lines.

Knockdown of S100A6 reduces the migration and invasion of thyroid cancer cells

As mentioned above, since the expression of S100A6 is closely related to the LNM of TC, we used the Transwell assay and Matrigel invasion chamber assays to verify the effect of S100A6 on cell motility. Compared with the control group, the migration and invasion abilities of cells transfected with Si-
S100A6 were significantly decreased (P<0.001, Figure 3F,G). These results indicated that S100A6 promotes the migration and invasion of TC cells.

**S100A6 inhibits apoptosis and promotes the proliferation, migration and invasion of thyroid cancer cells by activating the PI3K/AKT/mTOR pathway**

Single gene set enrichment analysis (GSEA) was used to identify genes in TC that may be regulated by S100A6. The TC patients from TCGA were divided into two groups, S100A6 high expression group and S100A6 low expression group. The results showed that genes involved in apoptosis and related genes that activate the AKT pathway may be related to the expression of S100A6 (Figure 4A-B). We used flow cytometry to detect cell apoptosis after transfection. After silencing S100A6, the apoptosis of KTC-1, BCPAP, and FTC cell lines increased, especially in early apoptotic cells (Figure 4C). Next, we quantified the protein levels of several representative markers of the AKT pathway and apoptosis. Activation of the PI3K/AKT/mTOR pathway is widely involved in the occurrence and development of thyroid cancer.[16-18] We found that knockdown of S100A6 significantly reduced the expression of p-mTOR, p-PI3K, and p-AKT (Figure 4D). In addition, pro-apoptotic genes (Bax) and anti-apoptotic (Bcl-2) are representative markers of the apoptosis pathway. Down-regulation of S100A6 enhanced the expression of Bax and decreased the expression of Bcl-2 (Figure 4E).

**AKT activator SC79 can reverse the knockdown effect of Si-RNA**

To further confirm that S100A6 affects the occurrence and development of thyroid cancer through the PI3K/AKT/mTOR pathway, we conducted rescue experiments. SC79 is an AKT activator that can directly bind to AKT to activate AKT[19]. Using SC79 to treat transfected cells, and to perform functional tests, CCK-8 and colony formation experiments proved that transfected cells treated with SC79 had a stronger proliferation ability than simply transfected cells (Figure 5A,C). Transwell and Matrigel invasion chamber assays also showed that activated AKT could enhance the migration and proliferation of transfected cells (Figure 5D,E). Flow cytometry analysis showed that compared with Si-S100A6 group, SC79 inhibited cell apoptosis (Figure 5B).

**Discussion**

The incidence of thyroid cancer ranks fifth among women[1]. Although the prognosis is good, patients who relapse or have distant metastases still have a higher risk of death. For this reason, exploring the mechanism of the occurrence and development of thyroid cancer and identifying biomarkers is the aim of our research.

S100A6 is a calcium cellular peripheral protein that regulates cytoskeletal protein dynamics, cell proliferation, differentiation, calcium metabolism, ubiquitination, and acetylation. Previous studies have reported the high expression of [20–22]. S100A6 in several cancers. However, no studies have reported the mechanism of S100A6 in thyroid cancer.
First, we consulted multiple public databases and found that the expression level of S100A6 was upregulated in cancer tissues. For further verification, we examined our validated cohort (42 pairs of thyroid cancer tissues and matched adjacent tissues). We found that the expression level of S100A6 was related to clinicopathological characteristics. In addition, the ROC curve also showed that S100A6 has diagnostic value. Through functional studies, we found that S100A6 promotes the proliferation, migration, and invasion of thyroid cells, and reduces their apoptosis. Western blotting assays proved that S100A6 promotes the occurrence of thyroid cancer through the PI3K/AKT/mTOR pathway and affects the expression of apoptotic gene Bcl-2 and Bax. We verified this conclusion by using rescue experiments.

This study had several limitations. First, the results obtained were not verified through in vitro experiments. In addition, further studies are required to elucidate the mechanism by which S100A6 regulates the PI3K/AKT/mTOR pathway. In summary, we discovered that S100A6 is an effective oncogene, and can be a potential target for the diagnosis and treatment of thyroid cancer.

**Conclusion**

S100A6 promotes the proliferation, migration and invasion of TC cells, and inhibits their apoptosis. S100A6 promotes the progression of thyroid cancer through PI3K/AKT/mTOR pathway and can be used as a target in the future treatment of thyroid cancer.

**Abbreviations**

TC: Thyroid cancer; TCGA: The Cancer Genome Atlas; qRT-PCR: Quantitative Real-time PCR; CCK-8: Cell Counting Kit-8; GSEA: Gene Set Enrichment Analysis; TIMER: Tumor Immunity Estimation Resource; LNM: lymph node metastasis.

**Declarations**

**Ethics approval and participation consent**

All patients signed a letter of consent, and the patient's information was approved by the Ethics Committee of the First Affiliated Hospital of Wenzhou Medical University (approval no. 2012-57).

**Consent for publication**

Not applicable.

**Availability of data and materials**

The sources of public datasets supporting the conclusions of this study are shown in this article. Other raw data are available on the main electronic data storage system of the First Affiliated Hospital of Wenzhou Medical University.
Competing interests

The authors declare that they have no conflicts of interest.

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Author's contribution

BRC is the first author and contributed to the research design. CHS and DNZ conducted a literature search. YHS got the data. CLH and HHL wrote this article. KFT and WZ revised the article and finally approved the version to be submitted. The final manuscript read and approved by all authors.

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**Tables**

Table 1. The association between S100A6 expression and clinicopathological features in the TCGA cohort
| Factors          | Patients | High expression | Low expression | p      |
|------------------|----------|-----------------|----------------|--------|
| Gender           |          |                 |                |        |
| Female           | 367      | 178             | 189            | 0.314  |
| Male             | 135      | 73              | 62             |        |
| Age (years)      |          |                 |                |        |
| <60              | 389      | 199             | 190            | 0.393  |
| ≥60              | 113      | 52              | 61             |        |
| T stage          |          |                 |                |        |
| I+II             | 308      | 131             | 177            | <0.001*|
| III+IV           | 193      | 120             | 73             |        |
| N stage          |          |                 |                |        |
| N0               | 280      | 107             | 173            | <0.001*|
| Nx               | 222      | 144             | 78             |        |
| M stage          |          |                 |                |        |
| M0               | 283      | 147             | 186            | 0.504  |
| Mx               | 9        | 6               | 3              |        |
| Disease stage    |          |                 |                |        |
| I+II             | 333      | 147             | 186            | <0.001*|
| III+IV           | 167      | 103             | 64             |        |
| Neoplasm focus type |      |                 |                |        |
| Unifocal         | 276      | 143             | 133            | 0.419  |
| Multifocal       | 226      | 108             | 118            |        |
| Histological     |          |                 |                |        |
| Classical        | 356      | 205             | 151            | <0.001*|
| Others           | 146      | 46              | 100            |        |
| New event        |          |                 |                |        |
| No               | 458      | 224             | 234            | 0.155  |
| Yes              | 44       | 27              | 17             |        |

Abbreviations: S100A6, S100 calcium-binding protein 1; TCGA, The Cancer Genome Atlas;
Table 2. The correlation between S100A6 expression and clinicopathological factors in the validated cohort

| Factors                      | Patients | High expression (%) | Low expression (%) | p     |
|------------------------------|----------|---------------------|--------------------|-------|
| Age (years)                  |          |                     |                    |       |
| ≥55                          | 15       | 9                   | 6                  | 0.334 |
| <55                          | 27       | 12                  | 15                 |       |
| Gender (mm)                  |          |                     |                    |       |
| female                       | 23       | 10                  | 13                 | 0.800 |
| male                         | 19       | 9                   | 10                 |       |
| Lymph node metastasis        |          |                     |                    |       |
| No                           | 19       | 4                   | 15                 | 0.004*|
| Yes                          | 23       | 15                  | 8                  |       |
| Tumor size                   |          |                     |                    |       |
| ≥10                          | 25       | 18                  | 7                  | 0.002*|
| <10                          | 17       | 4                   | 13                 |       |
| Neoplasm focus type          |          |                     |                    |       |
| Unifocal                     | 34       | 17                  | 17                 | 0.524 |
| Multifocal                   | 8        | 3                   | 5                  |       |
| Disease stage (AJCC7)        |          |                     |                    |       |
| I+II                         | 24       | 10                  | 14                 | 0.027*|
| III+IV                       | 14       | 11                  | 3                  |       |

Table 3. Univariate and multivariate COX regression analysis for overall survival in the TCGA cohort
| Parameters                        | Univariate analysis |                      |                     | Multivariate analysis |                      |                     |
|----------------------------------|---------------------|----------------------|---------------------|-----------------------|----------------------|---------------------|
|                                  | HR                  | 95% CI               | p                   | HR                    | 95% CI               | p                   |
| S100A6 expression (high vs. low) | 2.126               | 1.410-3.207          | <0.001*             | 0.468                 | 0.314-0.697          | <0.001*             |
| Gender                           | 0.695               | 0.438-1.104          | 0.123               |                       |                      |                     |
| Age (years) (≥60 vs. <60)        | 0.288               | 0.159-0.523          | <0.001*             | 0.31                  | 0.178-0.538          | <0.001*             |
| T stage (T1+T2 vs T3+T4)         | 1.476               | 0.919-2.371          | 0.107               |                       |                      |                     |
| Disease Stage (I+II vs. III+IV)   | 4.459               | 2.590-7.676          | <0.001*             | 5.631                 | 3.465-9.153          | <0.001              |
| Histological type (classical vs. others) | 3.014               | 1.828-4.970          | <0.001*             | 2.75                  | 1.732-4.512          | <0.001              |
| Neoplasm focus type (Unifocal vs. Multifocal) | 1.491               | 0.982-2.64           | 0.061               |                       |                      |                     |
| Disease Stage (I+II vs. III+IV)   | 4.459               | 2.590-7.676          | <0.001*             | 5.631                 | 3.465-9.153          | <0.001              |

Abbreviations: TCGA, The Cancer Genome Atlas; S100A6, S100 calcium-binding protein 1; HR: Hazard ratio; CI: Confidence interval.

**Figures**
Figure 1

S100A6 is upregulated in thyroid cancer (A) Analysis of the expression level of the S100 family in the TCGA database and GSE 3678. (B) S100A6 is overexpressed in a variety of cancers in TCGA. The Wilcoxon test was used to assess the statistical significance of the differential expression.

*p<0.05, **p<0.01, ***p<0.001.
Figure 2

S100A6 is highly expressed in TC (A-B) In the TCGA and GSE3678 database, the expression level of S100A6 in TC tissues was higher than that in normal tissues. (C) S100A6 was highly expressed in tumor tissues among 70 pairs of RNA sequencing tissues. (D) S100A6 was overexpressed in tumor tissues in the validation cohort using qRT-PCR. (E-G) Different T, N, and disease stage show that the expression level of S100A6 is different. Higher S100A6 expression is often associated with poorer clinicopathological
characteristics (H) In the TCGA and GSE3678 database, and verification cohort, the ROC curve shows that S100A6 has diagnostic value. (I) In TCGA, the diagnostic significance of S100A6 expression levels at different T stages. Statistical analysis: (A) Mann-Whitney test (B) Paired t-test (C-D) Wilcoxon test (E-G) Kruskal-Wallis test *p<0.05,**p<0.01,***p<0.001, ****p<0.0001.

Figure 3
S100A6 is highly expressed in thyroid cell lines and promotes its proliferation, migration and invasion. (A) Compared with the normal thyroid cell line HTORI-3, S100A6 was relatively highly expressed in the TC cell line. (B) In the three cell lines, the expression level of S100A6 in the Si-S100A6 group was lower than that in the Si-NC group. (C) In the Si-S100A6 group, the S100A6 protein expression level was lower than that of the Si-NC group. (D-E) CKK-8 analysis and clone formation experiments in three TC lines. (F-G) Migration and invasion experiments were performed on the Si-NC and Si-S100A6 groups; 3 fields of view were randomly selected for analysis and measurement under the microscope. Statistical analysis: (D) Two-way analysis of variance (A, B, C, E, F, G) Student's t-test. *p<0.05, **p<0.01, ***p<0.001
****p<0.0001.
Figure 4

S100A6 promotes the development of thyroid cancer through the PI3K/AKT/mTOR pathway while inhibiting its apoptosis. (A-B) A single GSEA based on TCGA data indicates that S100A6 is associated with apoptosis and the AKT pathway. (C) Silencing S100A6 promotes the apoptosis of TC cells. At protein level, down-regulation of S100A6 increased BAX expression and decreased the expression of Bcl-2. (D) After silencing S100A6, p-mTOR, p-PI3K and p-AKT were down-regulated at the protein level. (E)
Western blot analysis showed that at the protein level, down-regulation of S100A6 can increase the expression of Bax and decrease the expression of Bcl-2. Statistical analysis: (C) Student's t-test. *p<0.05, **p<0.01.

**Figure 5**

AKT activator SC79 can reverse the knockdown effect of Si-RNA (A,C) CCK-8 and clone formation experiments were performed on the three groups Si-NC+DMSO, Si-S100A6+DMSO, and Si-S100A6+SC79.
(B) Compared with the Si-S100A6+DMSO group, SC79 promotes cell apoptosis. (D-E) Transwell migration and Matrigel invasion assays were performed on three groups, namely: Si-NC+DMSO, Si-S100A6+DMSO, and Si-S100A6+SC79. Statistical analysis: Two-way analysis of multiple comparisons (A, B, C, D, E). *p<0.05,**p<0.01,***p<0.001,****p<0.0001.