AXL/GAS6 Inhibition Induces Apoptosis and Suppresses Cell Proliferation and Migration by Blocking the PI3K/AKT Signaling Pathways in Thyroid Cancer Cells in Vivo

Zhenhua Zhang (zhangzh1944@163.com)
Henan Provincial People's Hospital
Zijie Su
Henan Provincial People's Hospital
Ji Zhang
Henan Provincial People's Hospital

Research Article

Keywords: GAS6, AXL, thyroid cancer, CAL62, PI3K/AKT pathway, AX inhibitor, apoptosis, proliferation, migration

Posted Date: January 3rd, 2022

DOI: https://doi.org/10.21203/rs.3.rs-966855/v1

Abstract

Background: The Gas6/Axl-PI3K/Akt pathway is known as one of the most critical molecular signaling pathways involved in the regulation of key cellular processes. GAS6 has emerged as the perfect target for many malignancy treatments, but its role in thyroid cancer remains less described. This study aimed to evaluate both knockdown and overexpression effects of GAS6 on thyroid cancer cell proliferation, migration, and viability.

Methods: Both RT-PCR and western blot analysis were performed to evaluate mRNA and protein expressions of GAS6; cell viability was assessed by MTT assay; then, TUNEL apoptosis, Transwell and migration assays were monitored to determine the effects of GAS6 knockdown or GAS6 overexpression on 850-5C and CAL62 thyroid cancer cells.

Results: The mRNAs and protein expressions of GAS6 were the highest in CAL62 cancer cells. AXL/GAS6 knockdown through the application of siGAS6 and XL184, an AXL inhibitor, strongly diminished the proliferation and migration levels of CAL62 by inducing cell apoptosis. Meanwhile, overexpression of GAS6 produced the inversed effects, and the protein levels of PI3K, AKT, and p-AKT were significantly up and downregulated, accordingly.

Conclusion: GAS6 inhibition promotes apoptosis and represses the proliferation of thyroid cancer cells by activating PI3K/AKT pathway; thus, provides a novel target for thyroid cancer therapy.

Introduction

Thyroid cancer is a typical endocrinal malignant tumor with regularly increased prevalence from the last decade (1, 2). Thyroid carcinoma includes anaplastic thyroid cancer (ATC), follicular thyroid cancer (FTC), papillary thyroid cancer (PTC), and poorly differentiated thyroid cancer (PDTC); all originated from thyroid follicular cells. Among them, PDTC and ATC represent the main forms of distinguished thyroid cancer; they belong to relatively rare tumors connected with aggressive human malignancies (3). In 2014, the median survival rate of patients diagnosed with ATC was about 3-5 months after diagnosis, and the advanced PDTC patients were likely to reach metastatic levels (4, 5). Deeper investigations are required to identify the molecular mechanisms that govern the development of thyroid cancer cells.

Previous studies have described the PI3K/Akt signaling pathway as regularly involved in key regulating mechanisms, especially those related to kinase flow in tumor cell biology, including cell, proliferation, autophagy, and apoptosis (6, 7). Because of its pivotal role, many studies have suggested the PI3K/Akt signaling pathway as a valuable target for cancer therapy (8). Furthermore, the PI3k/Akt pathway was reported to be an essential modulator of the DNA damage repair pathway as well as cholesterol ester generation in prostate cancer cells (9).

GAS6 (growth arrest-specific gene 6) belongs to the family of vitamin K-dependent proteins (10), which are connected with various receptors of tyrosine kinases (RTKs) like Sky, Axl, and Mer (11–13). Several

studies have demonstrated the implication of GAS6 in many carcinomas, including hepatocellular carcinoma (14), gastric cancer (15), oral squamous cell carcinoma (16), prostate cancer (17). It is well known that Gas6/Axl signaling promotes tumor cell development and immune evasion, which makes the Axl a promising target for various cancer therapies. Deeper investigations are warranted to carry out the exact mechanism by which Gas6/Axl signaling intervenes in various cancer physiologies.

Therefore, this study aimed to evaluate both the knockdown of GAS6 and GAS6 overexpression effects on thyroid cancer cell key processes. Thus, the expression patterns of GAS6 at mRNA and protein levels in the CAL62 thyroid cancer cells were first evaluated through RT-PCT and western blot assessments; Transwell and wound healing assays were applied to examine the proliferation and migration of thyroid cancer cells transfected with empty vector, siGAS6, and GAS6 overexpression vector. Then, flow cytometry and TUNEL assays were performed to examine cell apoptosis while MTT, Transwell and wound-healing assays were performed to evaluate cell viability, proliferation and migration. Finally, the protein expression levels of the PI3K, AKT, and p-AKT were measured via western blotting. Our results demonstrated that inhibition of GAS6 can be considered as a new and effective therapeutic target for thyroid cancer.

Methods

Cell lines and chemicals

Human thyroid cancer cell line (CAL62, 850-5C, NIM, OCUT-1) and human normal thyroid cell line (Nthy-ori 3-1) were purchased from the ATCC, Manassas, VA, USA. MEM medium (11095072), fetal bovine serum FBS (16000-044), trypsin, and double antibodies were purchased from Gibco; MTT cell proliferation and cytotoxicity detection kit were purchased from Biyuntian Company (C0009); Lipofectamine 2000 transfection reagent and DMSO were purchased from Invitrogen Company (Cat. No. 15596-018); GAS6 siRNA (Cat. No. 106266) and AXL siRNA (Cat. No. 1218) were purchased from ThermoFisher.

Co-culture of thyroid cancer cell lines

CAL62 and Nthy-ori 3-1 cell lines were cultured in the Dulbecco's modified Eagle medium (DMEM, Invitrogen-Gibco, 11095072) supplemented with 10% Fetal Bovine Serum (FBS, Invitrogen-Gibco, 16000-044). XL184 was dissolved in dimethyl sulfoxide (DMSO) at concentrations of 7.0 nM (IC50 of XL184), and the vehicle was used as a control (18). Then, CAL62 cells were assessed with three different concentrations of XL184 for 96 h and harvested for further assay.

Plasmids, siRNAs assessments, and cell transfection

The pcDNA3.1 empty vector and plasmid constructs containing the GAS6 (pcDNA3.1-GAS6) were purchased from Obio Technology (Shanghai, China). CAL62 cells were transfected with pcDNA3.1 empty vector or pcDNA3.1-GAS6 to form the control system or the GAS6 overexpression system, respectively. GAS6-siRNAs (siGAS6-1, siGAS6-2, and siGAS6-3) were obtained from GenePharma (Shanghai, China). when cell confluence was up to 85% following the manufacturer's protocol, CAL62 cells were transfected

with GAS6-siRNAs. 6h after transfection, cells were further cultivated with a new culture medium and harvested after 48 h.

MTT assay

CAL62 cells were seeded into the 96-well plates and their proliferation ability of cells transfected with siGAS6 or pcDNA3.1-GAS6 were evaluated 48 h post-transfection by the MTT assay (3-(4,5-dimethylthiazol)-2,5- diphenyltetrazolium bromide) from Sigma-Aldrich, Shanghai, China, according to the manufactory's recommendations Finally, the absorbance of each well was measured at 595 nm by a microplate reader (ELx808 Bio-Tek Instruments, City, ST, USA).

Cell migration

Cell migration was assessed using Transwell and wound-healing assays with the ACL62 cell line. For the Transwell assay, 5×105 cells in the 200L serum-free medium were added into the upper chamber and incubated for 24h at the atmosphere of 5% CO2 and 37 ℃ when the lower chamber was containing the appropriate medium. After that, cells were rinsed with PBS when the medium was discarded and then neutralized with methanol. Then, cells were splashed with 0.1% crystal violet and reckoned, captured under 100-fold magnification through at least three fields randomly. For the wound-healing assay, straight lines were drawn at the lower part of the 6-well plates outside with disinfected rulers and markers. 5×105 cells plated in the 6-well plates were refined for 24 h and opposite lines were drawn with the tip of the pipettor. Cells were refined in the medium for another 24h and the scratch wound was caught with a magnifying microscope under 40-overlap amplification.

TUNEL assay

Cell fixation was performed for 15 min for each group (control or siGAS6 or pcDNA3.1-GAS6) using 4% paraformaldehyde at room temperature, dewaxed and hydrolyzed in xylene three times for 5 min, two-times 10 min in 100% ethanol, two-times 10 min 95% ethanol, and two-times 5 min ddH2O, respectively. Then, a fresh-made TUNEL solution was used to perform the incubation step in a humidified chamber (37°C for 1 h), and the slides were transferred on the DAPI (Sigma), five times cleaned with PBS, fixed with the Dako fluorescence mounting medium, and observed on Nikon Eclipse Ti2 microscope or Olympus BX51.

RNA extraction and reverse transcription-polymerase chain reaction

Total RNA was reversely transcribed into cDNA using TRIzol Total RNA Extraction Reagent Shanghai (BiomedWorld Shanghai) following the manufacturer's instructions. The relative expressions of GAS6 and AXL genes were determined by RT-PCR performed with SYBR Premix Ex Taq (Takara, Beijing, China) on ABI 7500 fast real-time PCR system (Applied Biosystems). The relative levels of GAS6 and AXL were normalized to the GAPDH in each sample.

Western blotting

Immunoblotting was conducted to evaluate the protein expression of GAS6, AXL, AKT, and p-AKT. Proteins extraction from cells lysed in RIPA buffer (Beyotime, Beijing, China) containing the protease inhibitor cocktail (Sigma) was performed by SDS-PAGE and transfected to a PVDF membrane (Millipore). After blocking in fat-free milk, the film was analyzed with the essential antibodies at 4°C overnight followed by secondary antibody incubation. CLARITYTM Western ECL substrate (Bio-Rad) was used to visualize the signals. The protein level was quantified using Image J software normalized with β-actin.

Statistical analysis

All data were obtained from three independent experiments least, and each experiment was performed following the experimental requirements. All data were shown as mean ± SD and statistical analyses were performed using SPSS 17.0 software (SPSS, Chicago, IL, USA). The student t-test was used for comparisons between groups. $p < 0.05$ was deemed as statistically significant.

Results

GAS6 is highly expressed in the thyroid carcinoma cell lines.

GAS6 was differentially expressed in the thyroid carcinoma cell lines, and GAS6 was highly expressed in cancer tissues. In comparison to other tumors, GAS6 expression was significantly higher in papillary thyroid Carcinoma (Figure.1A and 1B). In a recent study, the survival curve drawn based on the TCGA data predicted that the up-regulation of GAS6 was closely associated with the unproductive diagnosis in the thyroid tumor (19). Herein, the survival rate was predicted as lower in cancer cells with the lower expression of GAS6 according to the TCGA data (Figure.1C). RT-qPCR and western blot results confirmed the differential expression of GAS6 in the four thyroid cancer cell lines including CAL62, 850-5C, NIM, and OCUT-1 at mRNA and protein levels, respectively. GAS6 exhibited the highest expression in CAL62 cell lines, up to two folds higher than that of 850-5C, which exhibited the lowest expression (Figure.1D). These TCGA database results confirmed that GAS6 could play potential roles in thyroid carcinoma evolution.

Overexpression of GAS6 enhanced proliferation of CAL62 cells.

First of all, 850-C5 (which initially displayed the lowest expression GAS6) and CAL62 (which initially had the highest expression GAS6) thyroid were transfected with a GAS6 over-expressive vector (pcDNA3.1-GAS6) and GAS6 inhibitor (siGAS6), respectively. Then, the expression characterization of GAS6 in the thyroid cancer cells was carried out using both qPCR and western blotting. The results showed that compared to normal cell lines Nthy-ori3-1 (control), the expression of GAS6 was almost four folds higher in cells under pcDNA3.1-GAS6 treatment and less than three folds downregulated in siGAS6 cells (Figure.2A). Moreover, the MTT assay revealed that overexpression of GAS6 lightly promoted thyroid cancer cell viabilities while its inhibition suddenly dropped down the cell viability rate at more than two folds smaller than that of the control groups (Figure.2B).

GAS6 expression regulates PI3K and p-Akt protein expression in 850-05 and CAL62 cancer cells.

The PI3K/Akt pathway is well known for its dominant role in thyroid carcinomas (20). Given the evidence for GAS6 implication in cell growth and development, we evaluated the effect of GAS6 on the PI3K/Akt pathway using RT-qPCR and western blot analysis in different groups. We found that GAS6 mRNA expression significantly diminished in all siGAS6 groups when compared to control cells while treatment with GAS6 overexpression vectors improved the protein mRNA of GAS6 850-C5 cells. Moreover, western blot assessments showed that the protein expression of GAS6 was suddenly lowered in CAL62 cells under siGAS6 treatment but upregulated in 850-C5-GAS6 overexpression cells. On the other hand, the expression levels of PI3K/Akt pathway biomarkers (PI3K AKT and p-Akt), were well improved in 850-C5-GAS6 overexpression cells when compared to normal cells, but greatly reduced when GAS6 was inhibited (Figure 3A and 3B). This section of findings demonstrated that modification of GAS6 expressions directly affects the PI3K/Akt signaling pathway in thyroid cancer cells.

XL184 inhibits the protein expressions of PI3K and p-Akt in 850-05 and CAL62 cancer cells.

Previously described as a potent inhibitor of VEGFR2 (IC50=0.035 nmol/L), MET (IC50=1.3 nmol/L), and RET (IC50=5.2 nmol/L), XL184 can also inhibit both GAS6-AXL and HGF-MET pathways in cancer cells (21). Given the evidence for the co-expression of GAS6 and AXL in thyroid cancer cells, we performed a second Western bolt assay to characterize the effects of XL184 treatments on the PI3K/AKT signaling pathway. We found that the protein expressions of PI3K, AKT, and p-Akt in CAL62 cancer cells were also downregulated (Figure 4A). Moreover, the MTT assay showed that the viability rate of Cal62 cells was markedly also reduced in cells treated with XL184 (Figure 4B). These results were almost similar to those gotten from the siGAS6 groups, suggesting that GAS6 might play essential roles in key processes of thyroid cancer growth. Thus, to confirm the interaction between GAS6 with AXL in thyroid cancer cells, cells were co-transfected FLAG-tagged AXL with or without HA-tagged GAS6. Co-IP assay with HA flag antibodies showed that GAS6-HA could interact with AXL protein (Figure 4C)

GAS6 mediated the apoptosis of CAL62 cells

flow cytometry and TUNEL assays were monitored to examine apoptotic events in transfected cells. The flow cytometry assay was carried out in order to evaluate the differential levels of transfected cells apoptosis. According to the flow cytometry results, compared with human papillary thyroid cancer cells transfected with empty vector, CAL62 cells under siGAS6 and XL184 treatments had stronger apoptotic abilities, while GAS6 overexpression suppressed apoptosis of CAL62 cells when compared with the control groups (Figure.5A). In addition, we TUNEL assay was used to visualize apoptotic cells and the results also confirmed that the apoptotic rates of GAS6 overexpression cells were strongly reduced in comparison with those of the control groups and significantly enhanced in siGAS6 and XL184 groups (Figure. 5B). According to these results, GAS6 inhibition promotes the apoptosis of thyroid cancer cells by activating the PI3K/Akt pathway.

GAS6 mediates the proliferation and migration of CAL62 cells through the PI3K/Akt pathway

Both Transwell and wound healing experiments were used to determine the cell migration abilities of CAL62 cells treated with a GAS6 over-expressive vector (pcDNA3.1-GAS6), GAS6 inhibitor (siGAS6), and

XL184, respectively. The Transwell assay results showed that compared with CAL62 cells transfected with empty vector, GAS6 overexpression (pcDNA3.1-GAS6) promotes the cells migration, while inhibition of GAS6 expression (si-GAS6 group) and XL184 treatment slows down the migration of human papillary thyroid cancer cells (Figure.6A). In addition, the wound healing results showed that compared with the control group, the migration abilities of both siGAS6 and XL184 groups were significantly reduced. Then, the protein level of PI3K, AKT, and the phosphorylation of AKT (p-AKT) was greatly decreased in the CAL62 cells treated with siGAS6. On contrary, GAS6 overexpression cells (pcDNA3.1-GAS6 groups) exhibited accelerated rates of migration when compared with the control group (Figure.6B).

Discussion

Many studies have targeted the roles of GAS6 in cancer development. It has been reported that the GAS6 gene exhibited high expressions in many human cancers including colorectal, thyroid, lung carcinomas, breast, ovarian cancer, etc (2, 22, 23). According to the TCGA data, the expression of GAS6 was confirmed in the thyroid cancer cell lines and the expression level of GAS6 mRNAs was the highest CAL62 cell lines. At the same time, Western blot analysis showed a similar trend of results for GAS6 protein expression in CAL62 in comparison with three other thyroid cancer cells.

The PI3K/Akt signaling pathway is an essential metabolic route in thyroid cancers; it plays a pivotal role in the control of cell apoptosis, proliferation, migration, and survival. Cabozantinib (XL184) is a huge inhibitor of GAS6/AXL and HGF-MET pathways in cancer cells. A recent study demonstrated that oral administration of cabozantinib induced rapid and robust inhibition of tumor growth in various xenograft models, causing prolonged survival (24). Also, GAS6 overexpression resulted in cell relocation through a bash intensification in JNK- and ERK1/2-dependent mechanisms (19). More interesting, GAS6/AXL flagging was recently reported as fundamental for postponing reactions of cell senescence by controlling the PI3K/Akt/FoxO signaling pathway; subsequently, GAS6 activated the PI3K/AKT pathway the following binding to its receptor AXL (25). A much recent study reported that GAS6/AXL axis also plays important role in drug resistance and metastasis occurrences in breast cancer by activating the Akt/GSK-3β/β-catenin signaling, and proposed the latter as a novel therapeutic quarry for researches on drugs resistance and metastasis evolutions in cancer (26).

Assuming that the overexpression of GAS6 in thyroid cancer resulted in elevated cell metabolisms like proliferation, migration, and invasion and that GAS6 coo-expresses with AXL, we speculated that GAS6/AXL pathway could make a promising target for researches on thyroid cancer therapy. Inhibition of the GAS6/AXL pathway can be practically achieved by using AXL tyrosine kinases inhibitors (TKIs) or blocking antibodies against GAS6 and AXL (27). XL184, known as Cabozantinib, is an inhibitor of various kinase including AXL used is in phases 2 and 3 of clinical trials against several cancers, including medullary thyroid, brain, and NSCLC cancers (28).

The present study aimed to investigate the effects of the GAS6-overexpression (pcDNA3.1-GAS6) system in 850-5C cells and the GAS6-knockdown system (siGAS6) in CAL62 cells. The results in vitro revealed

that overexpression of GAS6 enhanced cell proliferation, migration, invasion, and vice versa. Moreover, the up-regulation or down-regulation of GAS6 efficiently activated or shut down the PI3K/AKT pathway. All the thyroid cancer cells treated with XL184 or siGAS6 exhibited the diminished ability of cell proliferation, migration, and invasion; interestingly, the PI3K/AKT pathway was strongly inhibited, causing the increase of apoptosis rate of tumor cells, accordingly. In contrast, pcDNA3.1-GAS6 overexpression induced inverse effects like inhibition of apoptosis and acceleration of cell migration. These results suggested that blocking GAS6/AXL axis could be an effective target in thyroid cancer in vitro. However, deeper experiments on GAS6/AXL pathway in vivo are still required.

In summary, GAS6 was confirmed to be significantly expressed in both the thyroid cancer cells, inhibition of GAS6 promoted CAL62 cell apoptosis, reduced their proliferation, migration, and invasion by inhibited the PI3K/AKT signaling pathway while the GAS6 overexpression induced the opposite reactions. From these results, our study demonstrated that GAS6 activated the PI3K/AKT pathway by connecting to AXL in thyroid cancer, and proposed that GAS6/AXL pathway would be an effective target for thyroid cancer therapy.

Abbreviations

ATC: anaplastic thyroid cancer; FTC: follicular thyroid cancer; PTC: papillary thyroid cancer; PDTC: poorly differentiated thyroid cancer

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

Not applicable.

Authors' contributions

ZZ conceived the project and was a major contributor in writing the manuscript. ZZ and ZS conducted the research. JZ was responsible for data analysis. All authors read and approved the final manuscript.

Acknowledgements

Not applicable.

References

1. Vecchia CL, Malvezzi M, Bosetti C, Garavello W, Bertuccio P, Levi F, et al. Thyroid cancer mortality and incidence: A global overview. International Journal of Cancer Journal International Du Cancer. 2015;136(9):2187-95.
2. Corm Ac K OM, Chung WY, Fitzpatrick P, Cooke F, Flynn B, Harrison M, et al. Growth arrest-specific gene 6 expression in human breast cancer. British Journal of Cancer.
3. Fahiminiya S, Kock LD, Foulkes WD. Biologic and clinical perspectives on thyroid cancer: To the editor. New England Journal of Medicine. 2016;375(23):2306-7.
4. Vathaire FD, Schlumberger M, Delisle MJ, Francese C, Challeton C, Genardiére E, et al. Leukaemias and cancers following iodine-131 administration for thyroid cancer. British Journal of Cancer.
5. Ibrahimpasic, Ghossein, L D, Carlson, Nixon, L F, et al. Outcomes in patients with poorly differentiated thyroid carcinoma. The Journal of clinical endocrinology and metabolism. 2014.
6. Roy B, Pattanaik AK, Das J, Bhutia SK, Behera B, Singh P, et al. Role of PI3K/Akt/mTOR and MEK/ERK pathway in Concanavalin A induced autophagy in HeLa cells. Chemico-Biological Interactions. 2014;210:96-102.
7. Ersahin T, Tuncbag N, Cetin-Atalay R. The PI3K/AKT/mTOR interactive pathway. Molecular BioSystems. 2015;11(7):1946-54.
8. Polivka J, Janku F. Molecular targets for cancer therapy in the PI3K/AKT/mTOR pathway. Pharmacology & Therapeutics. 2014;142(2):164-75.
9. Wu S, Wu F, Jiang Z. Effect of HOXA6 on the proliferation, apoptosis, migration and invasion of colorectal cancer cells. International Journal of Oncology. 2018;52.
10. Manfioletti G, Brancolini C, Avanzi G, Schneider C. The protein encoded by a growth arrest-specific gene (gas6) is a new member of the vitamin K-dependent proteins related to protein S, a negative coregulator in the blood coagulation cascade. Molecular & Cellular Biology. 1993;13(8):4976-85.
11. Varnum BC, Young C. Axl receptor tyrosine kinase stimulated by the vitamin K-dependent protein encoded by growth. Nature: International weekly journal of science. 1995;373(6515):págs. 623-6.
12. Chen, Jian, Carey, Kendall, Godowski, Paul J. Identification of Gas6 as a ligand for Mer, a neural cell adhesion molecule related receptor tyrosine kinase implicated in cellular transformation. Oncogene. 1997.

13. Lemke, G. Biology of the TAM Receptors. Cold Spring Harb Perspect Biol. 2013;5(11):a009076.
14. Lee HJ, Jeng YM, Chen YL, Chung L, Yuan RH. Gas6/Axl pathway promotes tumor invasion through the transcriptional activation of Slug in hepatocellular carcinoma. Carcinogenesis. 2014;35(4):769-75.
15. Sawabu T, Seno H, Kawashima T, Fukuda A, Uenoyama Y, Kawada M, et al. Growth arrest-specific gene 6 and Axl signaling enhances gastric cancer cell survival via Akt pathway. Molecular Carcinogenesis. 2010;46(2):155-64.
16. Tao J, Liu G, Wang L, Liu H, Wei QY. Elevated Serum Gas6 Is a Novel Prognostic Biomarker in Patients with Oral Squamous Cell Carcinoma. Plos One. 2015;10(7):e0133940.
17. Gas6 induces proliferation in prostate carcinoma cell lines expressing the Axl receptor. Journal of Cellular Physiology. 2005;204(1):36-44.
18. Sameni M, Tovar EA, Essenburg C, Chalasani A, Linklater ES, Borgman A, et al. Cabozantinib (XL184) Inhibits Growth and Invasion of Preclinical TNBC Models. Clinical Cancer Research. 2015;22(4).
19. None. Integrated Genomic Characterization of Papillary Thyroid Carcinoma. Cell. 2014;159(3):676-90.
20. Nozhat Z, Hedayati M. PI3K/AKT Pathway and Its Mediators in Thyroid Carcinomas. Molecular Diagnosis & Therapy. 2016;20(1):13-26.
21. Bentzien F, Zuzow M, Heald N, Gibson A, Yakes M. In vitro and in vivo activity of cabozantinib (XL184), an inhibitor of RET, MET, and VEGFR2, in a model of medullary thyroid cancer. Thyroid Official Journal of the American Thyroid Association. 2013;23(12).
22. Ito M, Nakashima M, Nakayama T, Ohtsuru A, Nagayama Y, Takamura N, et al. Expression of receptor-type tyrosine kinase, Axl, and its ligand, Gas6, in pediatric thyroid carcinomas around chernobyl. Thyroid. 2002;12(11):971-5.
23. Ito M, Nakashima M, Nakayama T, Ohtsuru A, Nagayama Y, Takamura N, et al. Expression of receptor-type tyrosine kinase, Axl, and its ligand, Gas6, in pediatric thyroid carcinomas around chernobyl. Thyroid. 2002;12(11):971-5.
24. Sameni M, Tovar EA, Essenburg C, Chalasani A, Linklater ES, Borgman A, et al. Cabozantinib (XL184) Inhibits Growth and Invasion of Preclinical TNBC Models. Clinical Cancer Research. 2015;22(4).
25. Meric F, Lee WP, Sahin A, Zhang H, Hung MC. Expression Profile of Tyrosine Kinases in Breast Cancer. Clinical Cancer Research. 2002;8(2):361-7.
26. Wenshu, Sun, Jiro, Fujimoto, Teruhiko, Tamaya. Coexpression of Gas6/Axl in human ovarian cancers. Oncology. 2004.
27. Wimmel A, Glitz D, Kraus A, Roeder J, Schuerma Nn M. Wimmel A, Glitz D, Kraus A, Roeder J, Schuermann M.. Axl receptor tyrosine kinase expression in human lung cancer cell lines correlates with cellular adhesion. Eur J Cancer 37: 2264-2274. European Journal of Cancer. 2001;37(17):2264-74.

28. Lee Y, Lee M, Kim S. Gas6 induces cancer cell migration and epithelial–mesenchymal transition through upregulation of MAPK and Slug. Biochem Biophys Res Commun. 2013;434(1):8-14.

Figures

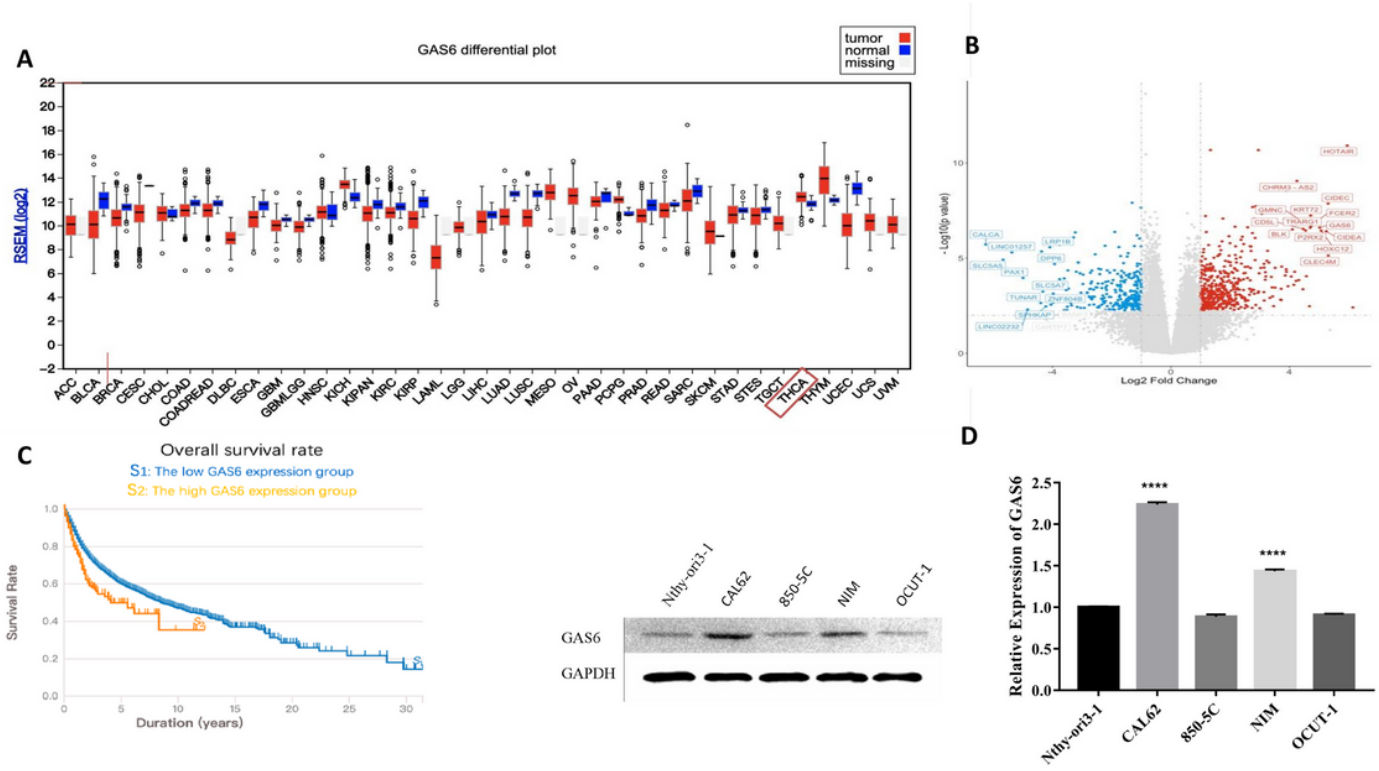

Figure 1

GAS6 was abnormally expressed in the thyroid carcinoma cell lines. Based on the TCGA database, GAS6 was overexpressed in the thyroid cancer tumor tissues (**A** and **B**). And, the survival curve was drawn according to the TCGA data (**C**). The mRNA expression of GAS6 in the thyroid cancer cell lines, including CAL62, 850-5C, NIM and OCUT-1, was confirmed by RT-qPCR (**D**). The protein levels of these cell lines mentioned above were evaluated by western blot analysis (**E** and **F**) (*$p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$ *vs* Nthy-ori3-1).

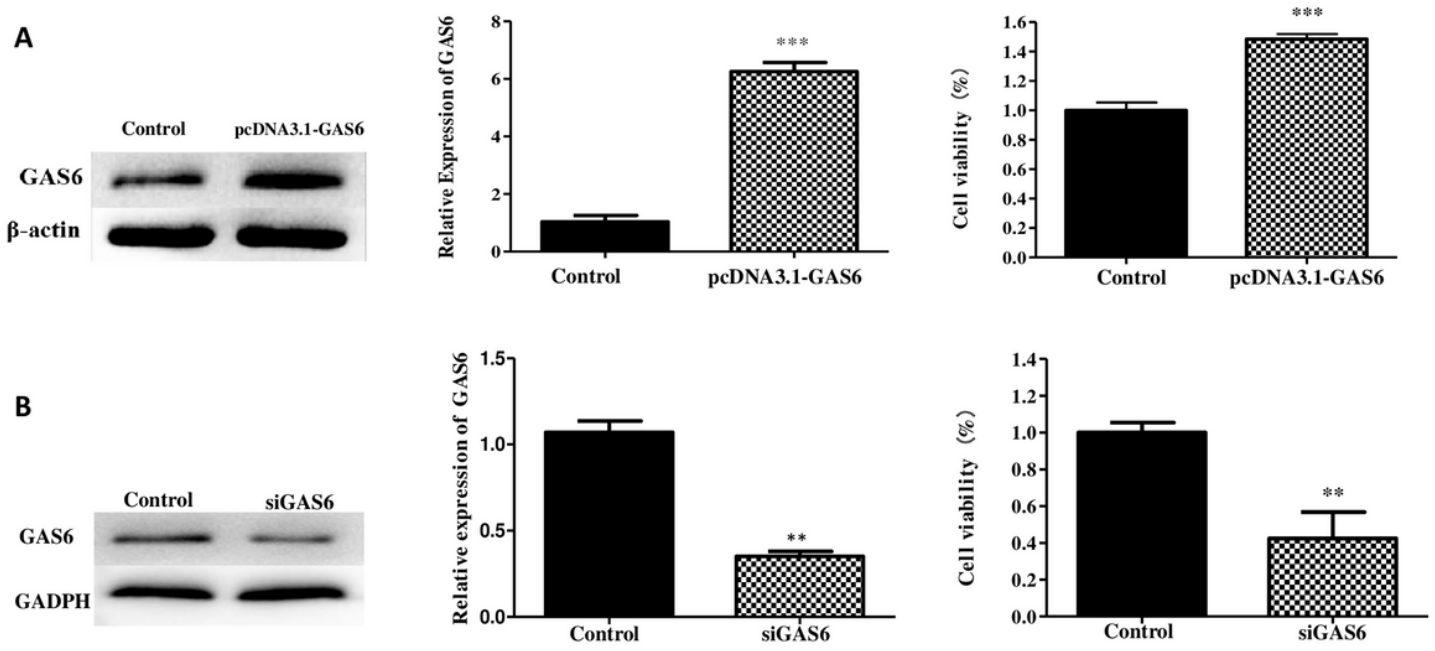

Figure 2

Overexpression of GAS6 enhanced cell proliferation, migration, and invasion, and activated PI3K/AKT signaling pathway in 850-5C cells. The mRNA expression of GAS6 in the GAS6 overexpression system was verified by RT-qPCR (**A**). Then, cell proliferation (**B**), migration (**C**), and invasion (**D**) ability were measured in the GAS6 overexpression system. The related proteins of PI3K/Akt pathways were determined by western blot analysis in the GAS6 overexpression system (**E**) (*** $p < 0.001$, **** $p < 0.0001$ *vs* control).

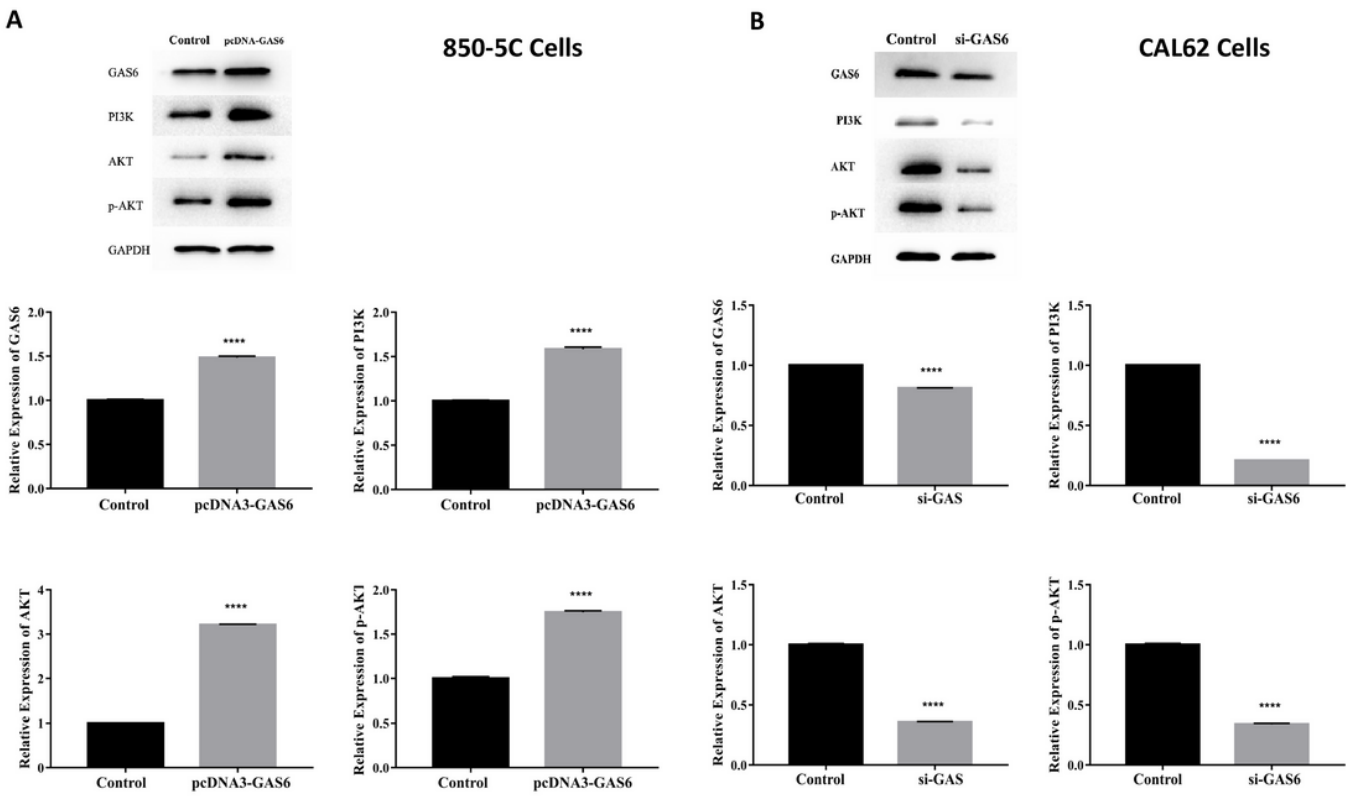

Figure 3

Knockdown of GAS6 impaired cell proliferation, migration, and invasion, and inhibited PI3K/AKT signaling pathway in CAL62 cells. The mRNA expression of GAS6 in the GAS6 knockdown system was verified by RT-qPCR (**A**). Then, cell proliferation (**B**), migration (**C**), and invasion (**D**) ability were measured in the GAS6 knockdown system. The related proteins of PI3K/Akt pathways were determined by western blot analysis in the GAS6 knockdown system (**E**) (** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ *vs* control).

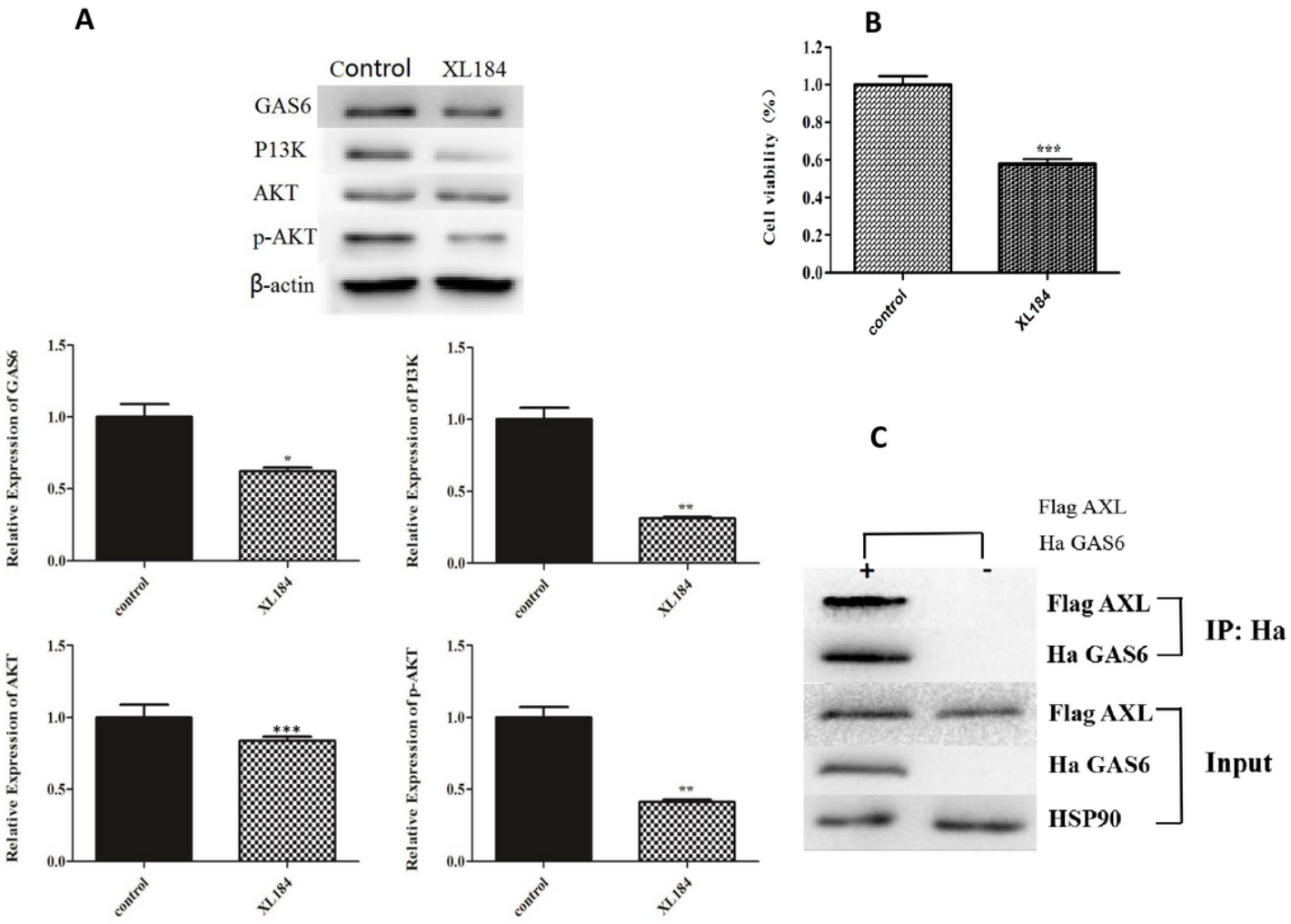

Figure 4

GAS6 interacted with AXL. Western Blot showing expression of key proteins in downstream pathways (**A**); MTT assay showing the viability rate of cells treated with XL184 (**B**); Co-IP assay was performed to determine whether GAS6 interact with AXL (**C**).

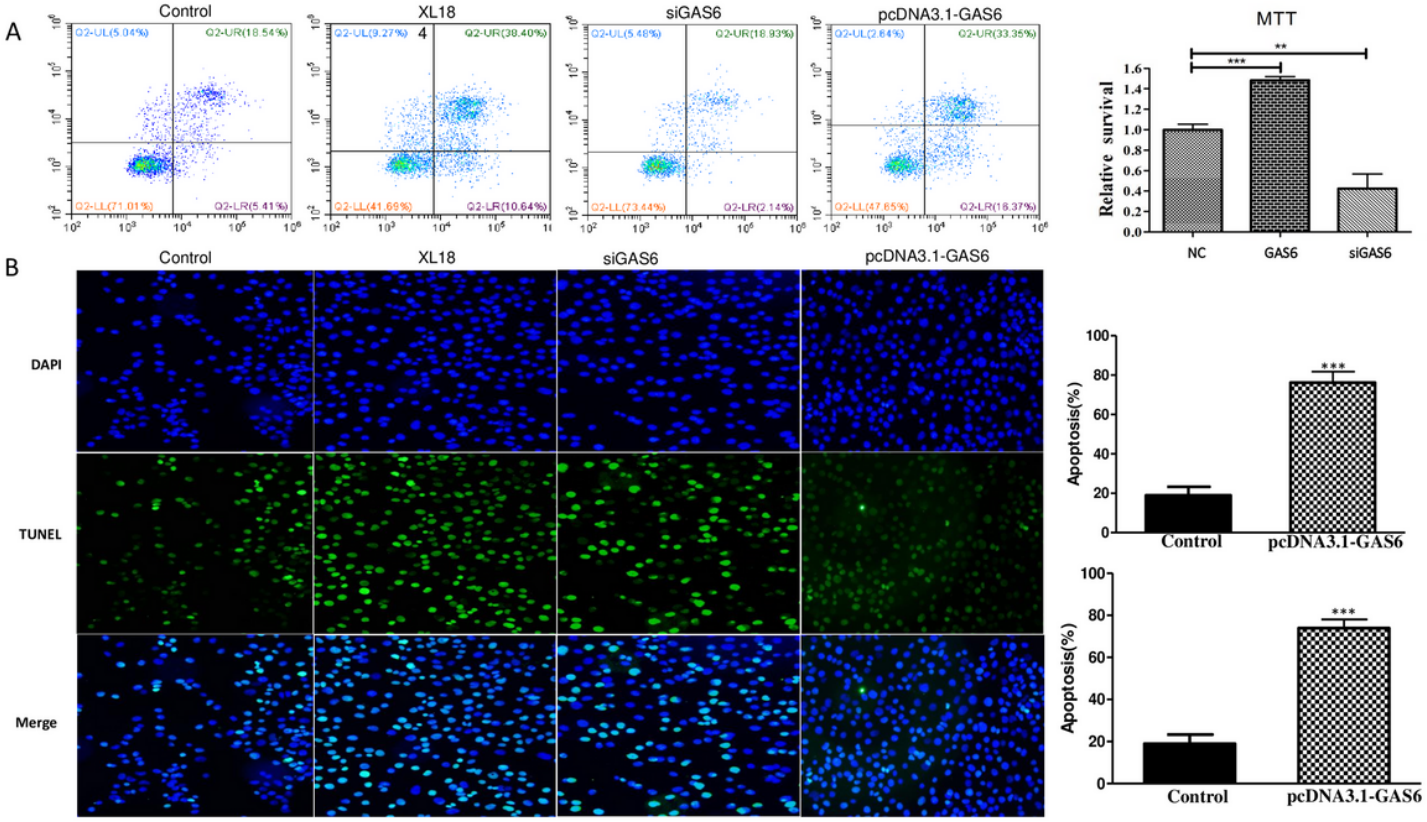

Figure 5

Cell apoptosis was evaluated via flow cytometry assays and TUNEL assays. Cell apoptosis was characterized by flow cytometry. *P<0.05 *vs*. NC, **P<0.01 *vs*. vector (**A**) Cells stained with TUNEL and DAPI were considered as apoptotic (**B**), *P<0.05 vs. vector or NC (magnification, ×200). (C). TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling; pcDNA3.1-GAS6, overexpression vector ofGAS6; vector-only control; NC, negative control; siGAS6, GAS6 siRNA inhibitor. PI, propidium iodide; FITC (7 nM) (*$p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ *vs* control).

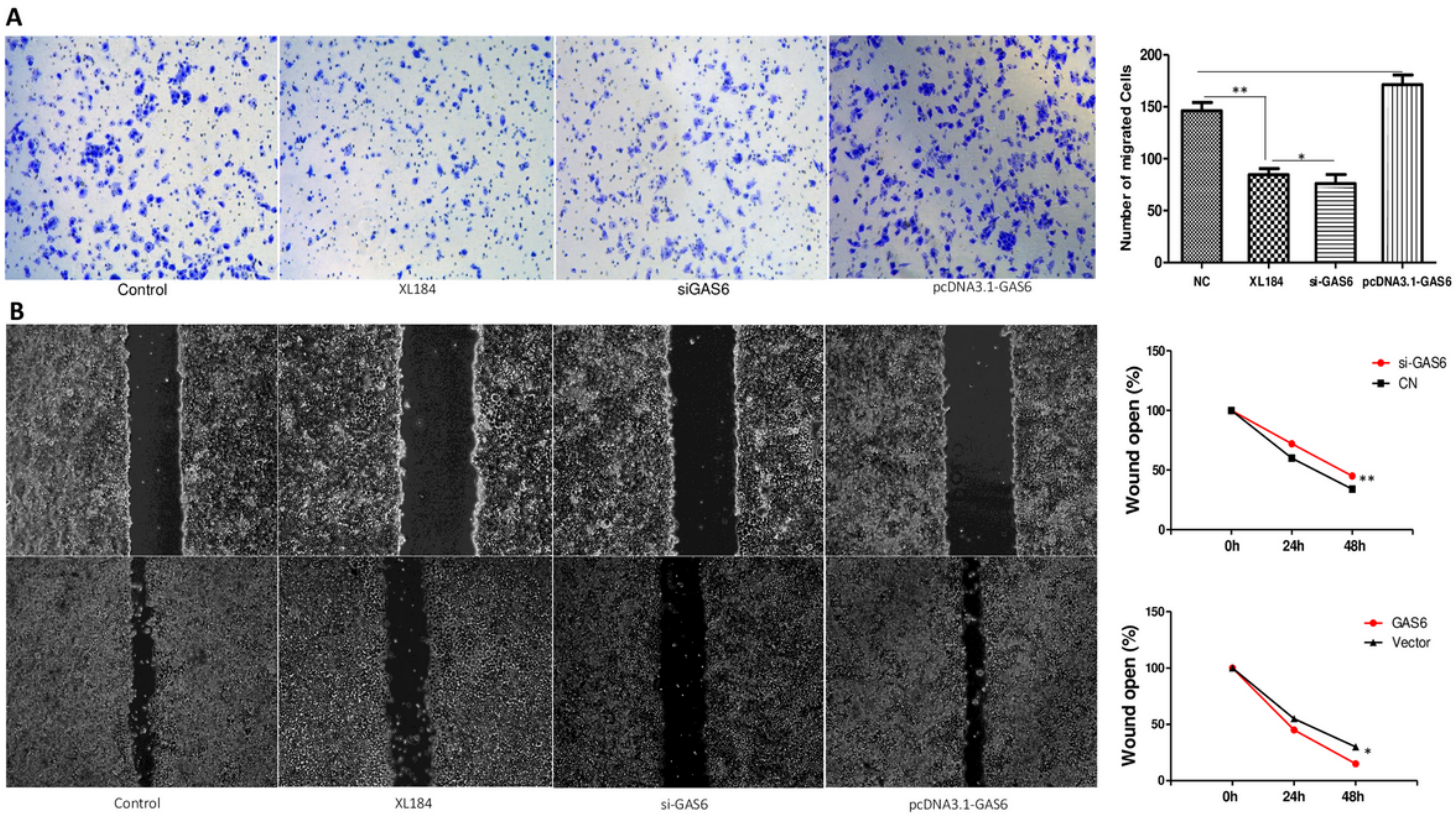

Figure 6

Cell migration evaluated by Transwell and wound-healing analysis. Transwell assay was monitored to examine the migration of thyroid cancer cells (**A**); **P<0.01 vs. control. Wound-healing assay was carried out to investigate the migration of thyroid cancer cells (**B**); (*$p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ *vs* control). pcDNA3.1-GAS6, overexpression vector ofGAS6; vector-only control; NC, negative control; siGAS6, GAS6 siRNA inhibitor.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- editableWB1.pptx