Immunohistochemical Basigin Expression Level in Thyroid Cancer Tissues

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Research

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Abstract

**Background:** Thyroid cancer (TC) is the most common endocrine malignancy, Basigin (also known as BSG) plays a crucial role in tumor cell invasion, metastasis, and angiogenesis. This study was designed to identify the change of BSG expression in TC and its possible potential mechanism.

**Methods:** The BSG expression levels in TC were demonstrated using data collected from in-house immunohistochemical (IHC), RNA-sequencing (RNA-seq), microarrays and literatures. Integrated analysis was performed to determined BSG expression levels in TC comprehensively. The gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were performed with the integration of BSG co-expressed genes and differentially expressed genes (DEGs) in TC tissues to explore the potential mechanisms of BSG in TC.

**Results:** The protein expression level of BSG was significantly higher in TC cases based on the IHC experiments. In addition, the combined SMD for BSG expression was 0.39 (p<0.0001), the diagnostic odds ratio was 3.69, and the AUC of the sROC curve was 0.6986 using 1182 TC cases and 437 non-cancerous cases from 17 independent datasets. Furthermore, BSG co-expressed genes tended to be enriched in gene terms of the extracellular matrix (ECM), cell adhesion, and cell-cell interactions. The expression levels of nine hub BSG co-expressed genes were markedly up-regulated in TC cases.

**Conclusion:** BSG expression levels were closely correlated with the progression of TC and may affect the signals of the ECM, cell adhesion, and cell-cell interactions.

1. **Background**

Thyroid cancer (TC) is the most common endocrine malignancy, and its prevalence has increased dramatically in the last few years[1-3]. Histological types of TC include papillary carcinoma (PTC), follicular carcinoma (FTC), anaplastic carcinoma (ATC)/undifferentiated carcinoma, and medullary carcinoma (MTC)[4], and there have been different mutational profiles in these subtypes. Recent studies have confirmed a few molecular markers that have allowed better understanding of the molecular mechanisms of TC, for instance, BRAF and RAS point mutations, RET/PTC and PAX8/PPAR gene rearrangements, and so on[5-7]. In addition, the latest study found that Substance P/neurokinin-1 receptor(SP/NK-1R) system was over-expressed in TC than in normal thyroid tissues via immunohistochemical study, which could promoted the migration and invasion of cancer cells[8]. Targeting certain molecules associated with initiation and progression of TC will be a significant and promising research area. Therefore, it is critical to continue to study the molecular mechanisms involved in TC, which may provide new strategies for the diagnosis and treatment of TC patients in the future.

Basigin (also known as BSG, CD147, or Extracellular Matrix Metalloproteinase inducer [EMMPRIN]) is a single pass type 1 transmembrane protein that plays a crucial part in developmental processes, wound healing, nutrient transport, inflammation, arthritis, and microbial pathologies. BSG has also been verified as a potential stimulator of matrix metalloproteinases (MMPs) and is considered to be a prognostic
marker in cancer [9]. Accumulating studies have found that BSG is over-expressed in many different human tumor cell types, such as those caused by brain cancer, colon cancer, cervical cancer, and endometrial cancer. It also plays a crucial role in tumor cell invasion, metastasis, and angiogenesis [10-14]. The role of BSG in cancer suggests that it could be an effective therapeutic target. For instance, exciting clinical progress has been made in hepatocellular carcinoma (HCC) treatment by means of BSG-directed monoclonal antibodies [15].

As for TC, BSG also plays an important role in the tumorigenicity, invasion, metastasis, and degree of dedifferentiation [16, 17]. Recently, researchers have identified that BSG expression regulates tumor cell glycolysis, resulting in the progression of TC [17, 18]. In addition, researchers have also found that BSG gene silencing leads to growth inhibition of thyroid medullary carcinoma TT cells and alteration of the cell cycle [17]. Moreover, it was proved that BSG is involved in the invasiveness of FTC cells via regulation of MMPs [19]. For differentiated carcinoma (DTC), the expression of BSG and MMP-2 may be an important feature, and the expression of BSG may be useful to predict the prognosis of DTC patients [20, 21]. Furthermore, a study demonstrated that BSG inhibition may be a therapeutic target for TC patients [18].

In summary, accumulating evidence suggests that BSG exerts a variety of functions in TC progression. However, until now only one study has mentioned a possible molecular mechanism underlying TC: miR-125a-5p regulates BSG and is negatively correlated with its expression and function [18]. Within this context, little is known about the molecular mechanism of BSG in TC, and it is essential to further investigate its expression and function.

In this study, we analyzed cases gathered from immunohistochemistry (IHC), RNA-sequencing (RNA-seq), and gene microarray data to provide evidence to attest to the clinical value of BSG in TC. Co-expressed and differentially expressed genes (DEGs) of BSG in TC, along with the corresponding intersection genes and hub genes, were also obtained. In addition, gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were performed to intersect genes and explore the potential mechanisms of BSG in TC. We hope that our findings will broaden the horizons for future studies of TC and BSG.

2. Materials And Methods

2.1 BSG expression level in TC

2.1.1 Detecting protein expression levels of BSG by in-house immunohistochemistry

Two tissue microarrays (THC961 and THC1021) which contained 23 non-cancerous thyroid tissues and 125 TC (including PTC, FTC, MTC, and ATC) tissues were obtained from Fanpu Biotech, Inc. (Guilin, China). In addition, a number of 64 non-cancerous thyroid tissues and 46 TC tissues were collected from
the Department of Pathology, First Affiliated Hospital of Guangxi Medical University, from March 1 to December 1, 2018. The study was approved by the Ethical Committee of the First Affiliated Hospital of Guangxi Medical University. And all patients provided written informed consents for use of their samples in study. Subsequently, pathologists (Wei-jia Mo and Gang Chen) independently evaluated all slides using a semi-quantitative scoring system without knowing the clinical results in advance to classify the staining intensity and the percentage of positive cancer cells. The staining intensity score was divided into 0 (negative), 1 (weak), 2 (medium) or 3 (strong). And the proportion of staining score set as 0 (< 10%), 1 (11-25%), 2 (26-50%), 3 (51-75%) or 4 (76-100%). Finally, the final immune response score was determined by combining the intensity and proportion scores. In addition, a correlation analysis was constructed to determine the relationships between BSG expression levels, clinicopathological characteristics, and patient prognosis. Furthermore, we supplemented the immunohistochemical results of the BSG expression levels in thyroid cancer by searching the available literature and The Human Protein Atlas[22].

2.1.2 BSG mRNA expression in TC samples from TCGA RNA-seq data

Using The Cancer Genome Atlas (TCGA) database, we downloaded BSG expression data related to TC and para-cancerous tissues[23]. Then, the downloaded data were integrated and transferred into the quantile normalized log2 format to improve measurement accuracy. SPSS 25.0 (SPSS Inc. Chicago, IL, USA) was used to calculate the mean BSG expression and SD in 505 TC tissues and 59 non-cancer tissues, incorporating clinical information gathered from the TCGA database. Furthermore, a Kaplan-Meier survival curve combined with TC patients’ survival data was constructed using GraphPad Prism Version 7.0, in order to estimate the prognostic capacity of BSG in TC.

2.1.3 BSG expression in TC tissues from other microarray data

This study included The Gene Expression Database (GEO)[24], ArrayExpress[25], Oncomine[26], and the The Sequence Read Archive (SRA)[27] in order to search both Chinese and English medical literature for microarray data of BSG to analyze its clinical value in TC. Our search included data entered into the databases from the beginning to February 21, 2020. The retrieval formula of this study was as follows: (cancer OR carcinoma OR adenocarcinoma OR tumour OR tumor OR malignanc* OR neoplas*) AND (Thyroid OR Thyroidea OR Glandula thyroidea) AND (mRNA OR gene OR “messenger RNA”). Finally, only microchip data relevant to human samples that included BSG expression in TC tissues and non-cancerous tissues were selected.

2.1.4 Searching the literature for BSG expression

In order to supplement the new data on BSG expression in recent studies, we conducted a comprehensive literature search of electronic databases (including PubMed, Embase, Web of Science, Wiley Online Library, SpringerLink, Chinese National Knowledge Infrastructure, Chinese Biomedical Literature Database, Chinese VIP, and Wanfang database) to obtain eligible studies. The search retrieval formula for these databases was: (cancer OR carcinoma OR adenocarcinoma OR tumour OR tumor OR malignanc* OR neoplas*) AND (Thyroid OR Thyroidea OR Glandula thyroidea) AND (mRNA OR gene OR “messenger RNA”).
OR neoplas*) AND (Thyroid OR Thyroidea OR Glandula thyroidea) AND (“Basigin” OR “Ok blood group” OR “CD147” OR “EMMPRIN”). Only studies that included human BSG expression levels were selected.

2.1.5 Comprehensive analysis of BSG expression in TC

Data from four types of sources (IHC, RNA-seq, microarrays, and literature) were combined using Stata Version 12.0 in order to perform a comprehensive analysis to determine BSG expression levels in TC, and the standard mean difference (SMD) and 95% confidence interval (CI) were calculated. Furthermore, the summary receiver operating characteristic (sROC) curve was used to distinguish TC tissues from non-cancerous tissues, which were gathered from the above databases. Sensitivity and specificity, as well as the corresponding likelihood ratio (LR) and diagnostic odds ratio (DOR) were calculated using meta-disc Version 1.4.[28-30].

2.2 Validation of the expression of BSG using cell line data from CCLE

A search for the term “BSG” was performed on the Cancer Cell Line Encyclopedia (CCLE) database,[31] and a heat-map based on the expression of BSG in different TC cell lines was constructed using HemI (Heatmap Illustrator, version 1.0).

2.3 The potential mechanism of BSG expression in thyroid cancer

2.3.1 Identification of BSG co-expressed genes and differentially expressed genes (DEGs) in TC

To obtain the hub genes of BSG in TC, we first searched the cBioPortal for Cancer Genomics[32] and found the co-expressed genes of BSG. The DEGs from all gene microarray datasets and RNA-seq data were obtained using the RRA method. This method uses a probabilistic model for aggregation, which is resilient to noise, and can calculate the probability of significance of all elements and perform the final ranking[33-37]. The cutoff P value for the RRA was set as 0.05.

Eventually, the genes that intersected with the co-expressed genes and DEGs were selected for subsequent work.

2.3.2 GO and KEGG clustering analyses of BSG co-expressed genes and DEGs in TC

The BSG co-expressed genes and DEGs in TC were uploaded into a function annotation portal via The Database for Annotation, Visualization, and Integrated Discovery (DAVID)[38], an R studio software that provides a systematic and comprehensive analysis for gene lists.

2.3.3 Identification of BSG hub genes in TC

The protein interactions of BSG co-expressed genes and DEGs were obtained using the Search Tool for the Retrieval of Interacting Genes (STRING) network database, and we set the minimum required interaction score at 0.7. We then used CytoHubba, a plug-in of Cytoscape, which can identify hub objects and sub-networks from complex interactome to calculate gene nodes present in the interaction network.
We imported PPI results of STRING website into Cytoscape, selected the CytoHubba plug-in, and calculated the top ten genes through MCC algorithm which has a better performance on the precision of predicting essential proteins from the yeast PPI network[39]. And the network interface will show the connection of these hub nodes in the network. The darker the node color, the higher the score. Ultimately, the top 10 genes were selected as BSG hub genes in TC.

2.4 Statistical analysis

SPSS 25.0 software was chosen for data analysis, which included an independent student's t-test to determine the significance of BSG expression in two groups and a univariate analysis for three or more groups. A P value below 0.05 was considered statistically significant. Scatter diagrams whose data ranged between normal and THCY tissues were prepared by the GraphPad Prism 7.0 software. In addition, we established a ROC curve to evaluate the diagnostic role of BSG in TC and differentiated TC and non-cancerous tissue by area under the curve (AUC), which can be used as a diagnostic. Moreover, according to the AUC, the diagnostic efficacy can be divided into three levels: low (0.5-0.7), medium (0.7-0.9), and high (0.9-1.0).

3. Results

3.1 Immunohistochemical investigation for BSG protein expression levels

This study included 171 cases of TC tissues and 87 cases of non-cancerous tissues, which were collected from the First Affiliated Hospital of Guangxi Medical University (Figs. 1A-H). Based on these samples, the BSG protein expression in the 171 cases of TC tissues was notably higher than in the 87 cases of non-cancerous tissues (7.6±1.366 vs. 1.32±1.749, p<0.0001) (Fig. 1I). The AUC of BSG protein expression used to distinguish between TC cases and non-cancerous cases was 0.985 (95%CI: 0.971 to 0.999, p<0.0001). The sensitivity and specificity were 0.912 and 0.966, respectively (Fig. 1J). In addition, the relationship between BSG expression and clinical parameters is shown in supplementary Table 1.

3.2 BSG mRNA expression in TC samples

In total, we collected 505 TC samples and 59 non-cancerous samples for RNA-seq data analysis. Corresponding clinical information was also collected and is shown in Table 1. The BSG mRNA expression level in the TC samples was dramatically higher than in the non-cancerous samples (3.8±0.056 vs. 3.76±0.042, p<0.0001). Furthermore, the AUC of BSG mRNA expression was 0.7363 (95%CI: 0.6743 to 0.7983, p<0.0001), and the sensitivity and specificity were 0.602 and 0.814, respectively (Figs. 2A and 2B). Subsequently, the prognostic value of BSG expression was inspected. The Kaplan-Meier survival curve analysis revealed that TC patients with low BSG expression levels were more likely to have worse overall survival rates (Figs. 2C and 2D) than those with high BSG expression levels (log-rank p=0.5027). In addition, the HR value was 0.6988 (95%CI: 0.2564 to 1.905). More interestingly, TC
patients with high BSG expression levels tended to have worse rates of disease-free survival than those with low BSG expression levels (log-rank p=0.0947), and the HR value was 10.98 (95%CI: 0.6606 to 182.5).

3.3 BSG expression in TC tissues according to the microarray data analysis

After the retrieval using the GEO database, 15 microarrays of BSG expression datasets were selected for our study. These microarrays contained 506 TC cases and 291 non-cancerous cases (Fig. 3, Table 2). Of these 15 microarrays, 9 (GSE27155, GSE53072, GSE6004, GSE58545, GSE35570, GSE53157, GSE50901, GSE60542, and GSE58689) showed that the expression levels of BSG in TC cases were slightly higher than in non-cancerous cases. In order to further analyze the BSG expression levels in these microarrays, we merged the microarray data and performed a meta-analysis. The combined SMD for BSG was 0.11 (95%CI: -0.04 to 0.25) based on the fixed effect model (Supplementary Fig. 1A). The I-squared value and the P value were 0.0% and 0.570, respectively. A sensitivity analysis of BSG expression levels in TC tissues and non-cancerous tissues was performed to show the differences among the microarrays (Supplementary Fig. 1B). In addition, the publication bias was assessed by means of a funnel plot (Supplementary Figs. 1C and 1D). The publication bias was not significant for this meta-analysis of BSG expression according to Begg's test (p=0.805) and Egger's test (p=0.745). Furthermore, the funnel plot also displayed a symmetric shape.

The combined sensitivity of BSG was 0.47 (95%CI: 0.42 to 0.51, p<0.0001), and the specificity was 0.74 (95%CI: 0.69 to 0.79, p<0.0001) with a positive likelihood ratio of 1.53 (95%CI: 1.26 to 1.86, p=0.9913), a negative likelihood ratio of 0.79 (95%CI: 0.69 to 0.90, p=0.0367), and a diagnostic odds ratio of 2.38 (95%CI: 1.65 to 3.43, p=0.9277). Most importantly, the AUC of BSG reached 0.6539, showing that BSG has a good capacity to differentiate TC tissues from the non-cancerous tissues (Supplementary Fig. 2).

3.4 Integrated analysis of the in-house immunohistochemistry, RNA-seq, microarray and BSG expression data reported in the literature

In order to comprehensively and systematically analyze the BSG expression level in TC, we performed a meta-analysis combining all sources of data, which included 1182 TC cases and 437 non-cancerous cases (n=17). The combined SMD for BSG expression was 0.39 (95%CI: -0.19 to 0.97) according to a random effect model (I-squared value: 94.7%). In addition, the P value was <0.0001, confirming a significant difference in the sensitivity analysis (Figs. 4A and 4B). Moreover, the publication bias was not significant according to Begg’s test (p=0.323) and Egger’s test (p=0.508) (Figs. 4C and 4D). The combined sensitivity of BSG was 0.59 (95%CI: 0.56 to 0.62, p<0.0001), and the specificity was 0.80 (95%CI: 0.75 to 0.83, p<0.0001) with a positive likelihood ratio of 1.89 (95%CI: 1.30 to 2.74, p<0.0001), a negative likelihood ratio of 0.59 (95%CI: 0.45 to 0.79, p<0.0001), and a diagnostic odds ratio of 3.69 (95%CI: 1.99 to 6.87, p<0.0001). Ultimately, the AUC of the sROC curve, which estimated the capacity of BSG expression to distinguish TC cases from non-cancerous cases, was 0.6986 (Fig. 5).

3.5 Validation of BSG expression using CCLE cell coefficient data
Using the CCLE database, we found that the expression levels of BSG in thyroid cell lines were higher than in the majority of other types of cell lines (Fig. 6A). A heat-map of BSG expression in the TC cell lines was also constructed, and over half of the bands were red (Fig. 6B).

3.6 The potential functions and pathways of BSG in TC

We discovered 20092 BSG co-expressed genes using the cBioPortal for Cancer Genomics and 1447 DEGs using RRA methods. Finally, we discovered 1299 BSG co-expressed genes and DEGs by performing an intersection between the co-expressed gene data and the DEG data (Figs.7A).

Afterwards, GO and KEGG analyses were performed on these 1299 genes. The GO enrichment analysis unveiled enrichment of extracellular matrix (ECM) organization, cell adhesion, and inflammatory response in biological processes (BP). And we also discovered enrichment of Extracellular space, proteinaceous extracellular matrix, and extracellular region in cellular component (CC). Furthermore, enrichment of calcium ion binding, heparin binding and extracellular matrix structural constituent was also found in molecular function (MF) (Figs. 7B-D, Table 3). Most importantly, the result of the KEGG analysis revealed that co-expressed genes and DEGs of BSG tended to enrich in the following terms: ECM-receptor interactions, complement and coagulation cascades, staphylococcus aureus infection, p53 signaling pathway, and protein digestion and absorption (Fig. 7E, Table 3).

3.7 Hub genes of BSG in TC

Using PPI analysis and CytoHubba, we identified the top 10 genes as hub genes of BSG in TC (Fig. 8K): cyclin dependent kinase 1 (CDK1); kinesin family member 11 (KIF11); topoisomerase (DNA) II alpha (TOP2A); ribonucleotide reductase regulatory subunit M2 (RRM2); microtubule nucleation factor (TPX2); PDZ binding kinase (PBK); maternal embryonic leucine zipper kinase (MELK); DLG associated protein 5 (DLGAP5); kinetochore complex component (NDC80); and nucleolar and spindle associated protein 1 (NUSAP1).

The expression levels of 9 hub genes in TC tissues were markedly higher than in non-cancerous tissues except KIF11: CDK1 (6.7486±0.70712 vs. 6.4577±0.86347, p<0.0001); TOP2A (7.6083±1.19671 vs. 6.23±1.86183, p<0.0001); RRM2 (6.1999±1.25053 vs. 4.9689±2.35379); TPX2 (6.5675±1.05049 vs. 5.2402±1.88557, p<0.0001); PBK (4.1448±1.18686 vs. 2.9661±1.78316, p<0.0001); MELK (4.422±1.27704 vs. 2.8595±1.92385, p<0.0001); DLGAP5 (4.2636±1.36501 vs. 2.9592±2.27261, p<0.0001); NDC80 (4.988±1.02333 vs. 3.8061±1.70888, p<0.0001); NUSAP1 (6.4232±1.00652 vs. 5.4004±1.67015, p<0.0001) (Figs. 8A-J, Supplementary Table 2). Corresponding AUCs for these hub genes were also obtained, including CDK1 (AUC=0.6571); TOP2A (AUC=0.741); RRM2 (AUC=0.6911); TPX2 (AUC=0.7392); PBK (AUC=0.7275); MELK (AUC=0.753); DLGAP5 (AUC=0.7101); NDC80 (AUC=0.738); NUSAP1 (AUC=0.7234) (all p<0.0001) (Supplementary Fig. 3, Supplementary Table 2).

4. Discussion
This study had two main aims. One was to identify the change of Basigin (BSG) expression in thyroid cancer (TC) by collecting all possible sources of data, including data from IHC, RNA-seq, microarrays, and literature. The other was to create a preliminary study of the possible underlying mechanisms of BSG in TC using co-expressed genes and DEGs. We collected 1182 TC cases and 437 non-cancerous cases (n=17) from all possible sources, and the combined SMD for BSG was 0.39 (p<0.0001). The AUC, which was used to assess the capacity of BSG to distinguish TC from non-cancerous tissues, was 0.6986. Subsequently, we found that BSG levels were up-regulated in TC. We also performed GO and KEGG analyses for BSG co-expressed genes and DEGs, and the results showed that genes tended to enrich in terms which was corrected with the extracellular matrix (ECM). Therefore, we surmised that BSG plays a part in TC by means of infecting the ECM.

As we all know, TC is the most common endocrine malignancy. Moreover, present researchers who have studied BSG expression levels have shown that BSG plays an important part in tumor cell invasion, metastasis, and angiogenesis[40-44]. Previous studies tended to focus on the relationship between BSG and differentiation levels of TC[16, 17]. Nevertheless, few studies have contributed to understanding the relationship between BSG expression levels and TC. In this study, we constructed an analysis using a large sample gathered from all possible sources, including co-expressed genes and DEGs of BSG, which had never done before. We sincerely hope that the results of this study will assist with the development of TC therapy and provide a new direction for further study of BSG and TC.

Few studies have focused on the expression levels of the BSG protein and mRNA in TC, and previous studies were more likely to focus on how the differentiation level of TC is linked with the positive expression of BSG and MMP. In addition, the sample sizes of these studies were small, and the methods were limited to IHC. Aratake et al. [16, 45] indicated that BSG expression correlates significantly with the degree of dedifferentiation of TC based on immunocytochemical analysis. More interestingly, they also observed MMP-2 positive expression in tumor cells and/or the adjacent tissue in all anaplastic carcinoma (ATC) cases, and the cytological atypia of cells with BSG positive expression was greater than in cells with negative BSG expression. Tan et al. [20, 21] found that BSG and MMP-2 were expressed mainly in cancerous lesions by means of IHC staining of 156 differentiated carcinoma (DTC) cases. More importantly, they also observed that the positive expression of these two markers was more likely to be positively linked to extrathyroidal invasion, lymph node metastasis, depth of tumor invasion, and distant metastasis. This leads us to believe that the expression of BSG may be useful to predict the prognosis of DTC patients. Huang et al. [18, 46] evaluated the expression of BSG in 200 TC specimens and 20 nodular goiter specimens using IHC. They suggested that cytoplasmic BSG expression levels were significantly higher in TC tissues than in nodular goiter tissues, significantly higher across different pathological stages, and that they closely correlated with lymph node metastasis and depth of tumor invasion.

In the current study, we collected 171 cases of TC tissues and 87 cases of non-cancerous tissues from the First Affiliated Hospital of Guangxi Medical University and analyzed the BSG expression profiles. In doing so, we discovered that the protein expression levels of BSG were significantly higher in TC cases based on the IHC experiments. The AUC of BSG expression was 0.985, and the corresponding sensitivity
and specificity were 0.912 and 0.966, respectively. We also found that expression of the BSG protein tended to be statistically linked to cancer subtypes (p<0.0001) based on the analysis of clinical parameters. However, the number of undifferentiated carcinomas was only 2. Therefore, the more specific relationship between BSG expression levels and differentiation levels of TC needs further study and validation. More importantly, we also analyzed BSG expression profiles using 1182 TC cases and 437 non-cancerous cases (n=17) collected from all possible sources, including IHC, RNA-seq, and microarrays. The combined SMD for BSG expression was 0.39, the diagnostic odds ratio was 3.69, and the AUC of the sROC curve was 0.6986. Making use of 505 TC cases from RNA-seq based on the Kaplan-Meier survival curve analysis, we found that TC patients with high BSG expression levels tended to have slightly worse rates of disease-free survival. However, the number of cases with BSG mRNA expression level and disease-free time was inadequate and accounted for a small proportion. Therefore, the survival analysis had a large bias. And the prognostic ability of BSG to predict survival needs to be checked out by a large amount of cases and further validated.

Previous studies have shown that BSG plays an important role in cancers, but what mechanisms does it use to promote the occurrence and development of TC? A recent study[46] demonstrated that BSG was a cofactor of TNF receptor-associated factor 6 (TRAF6). Shi et al. [47] indicated that TRAF6 and BSG assisted with BSG-mediated MMP9 could form the TRAF6/CD147/MMP9 pathway and is associated with angiogenesis and metastasis in ATC. Omi et al. [19] suggested the involvement of BSG in the invasiveness of follicular carcinoma (FTC) cells via regulation of MMPs based on immunoblot and IHC techniques. In addition, they indicated that up-regulated expression of BSG in FTC was driven by epidermal growth factor (EGF) via the PI3K, ERK, and JNK pathways. Furthermore, a previous study [48] also found that the up-regulated expression of BSG directly contributed to tumor angiogenesis by stimulating vascular endothelial growth factor (VEGF) production via the PI3K/AKT pathway. Li et al. [17] revealed that silencing of BSG in the TT cells in medullary carcinoma (MTC) could drive the inhibited proliferation of TT cells and the alteration of the cell cycle. Huang et al.[49] first confirmed that BSG took part in glycolysis and the transmembrane transport of lactate assisting with monocarboxylate transporters 1 (MCT1), a facilitator of lactate transport. This resulted in the decrease of extracellular pH and tumor progression. Fanelli et al.[50] revealed that Thyroid Stimulating Hormone (TSH) further up-regulated the MCT/BSG complex, which is necessary for BSG expression, and assisted BSG translocation to the cytoplasmic membrane. In addition, Huang et al.[18] indicated that miR-125a-5p functions as a tumor suppressor in TC by directly targeting and repressing BSG expression, thereby inhibiting aerobic glycolysis and subsequently suppressing cancer cell viability, migration, and invasion.

In the present study, we constructed GO and KEGG clustering analyses of 1299 candidate BSG co-expressed genes and DEGs in TC. And these BSG co-expressed genes and DEGs tended to enrich in pathways which were associated with ECM, cell adhesion, cell-cell interactions and angiogenesis. In the KEGG pathway analysis, genes were more likely enriched in the following terms: ECM-receptor interactions, complement and coagulation cascades, Staphylococcus aureus infections, the p53 signaling pathway, and in protein digestion and absorption. At the same time, the thyroid hormone synthesis pathways, pathways in cancers, and the PI3K-Akt signaling pathway were also evident. Due to
these results, BSG, a member of the immunoglobulin superfamily, was determined to be a potential stimulator of MMP. It stimulated MMP-mediated ECM degradation, cell adhesion, and cell-cell interactions and played a crucial part in tumor cell invasion\cite{9, 51-54}. BSG could also promote tumor angiogenesis via regulating MMPs and VEGF. Additionally, many previous researchers have studied the relationship between BSG and human cancers and further confirmed the role of BSG in the development of human tumors. For example, Li et al.\cite{55} indicated that BSG promoted cell proliferation, metastasis, and invasion in breast cancer via accommodating the expression of MMP-9 and VEGF. Qiao et al.\cite{43} revealed that BSG expression was significantly up-regulated in malignant bone cancer. Moreover, the expression of TRACP, MMP-2, MMP-9, and c-Src (osteoclast specific genes) was analyzed by RT-PCR. In a word, BSG promoted the development of TC via MMPs, infecting the ECM and promoting cancer cell invasion. This was also true of the PI3K-Akt signaling pathway.

The important role played by BSG in tumor cell invasion makes this gene an excellent target for cancer treatment. At present, there are options for targeted therapy of TC. Based on the immune response to specific antigenic peptides present on the tumor surface or cytoplasm, several immunotherapeutic strategies have been developed. For instance, chimeric antigen receptor (CAR)-T cell therapy targeting intercellular adhesion molecule (ICAM)-1 was preclinically validated in TCs, especially in PTC and ATC\cite{56}. Capdevila et al. demonstrated the effectiveness of blocking checkpoints with programmed death-1 (PD-1)/PD ligand 1 (PD-L1) axis inhibitors in ATC\cite{57, 58}. Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) could also be utilized in TC therapy. Furthermore, chemokine receptor targeted immunotherapy for thyroid cancer is also worthy of attention, since previous studies have found that chemokines and their receptors such as CXCR1, CXCR2, CXCR3, CXCR4, CXCR7, DARC, CCR3, CCR6, CCR7 and CXCL5-CXCR2 axis play an important role in determining the cellular immunophenotype of thyroid tumor microenvironment\cite{59}. Moreover, some novel drugs were utilized to TC therapy. For example, apatinib could induce apoptosis and autophagy of PTC cells through PI3K/Akt/mTOR signaling pathway\cite{60}. Previous studies have indicated that emodin inhibited the angiogenesis and metastasis of ATC by regulating TRAF6 mediated pathways, including the TRAF6/CD147/MMP9 pathway\cite{47}. Some studies have provided evidence that down-regulation of BSG via lentivirus vector-based RNAi decreased cell proliferation, matrigel invasion, and tumor formation in breast cancer, especially in MCF-7 breast cancer cells\cite{61, 62}. Zhang et al.\cite{63} provided evidence that chimeric antigen receptor T cells induced by doxycycline targeting BSG can be used in the treatment of liver cancer. Another study also found that doxycycline inhibited the proliferation of gallbladder cancer cells by down-regulating the expression levels of BSG and induced an early apoptosis response in cancer cells\cite{64}. In addition, Wang et al.\cite{65} found that Metuzumab could inhibit metastasis of esophageal cancer via blocking the function of BSG. Furthermore, BSG inhibitors are now being developed and tested \cite{66-68}, and we hope our study can provide some evidence for BSG targeted treatment of TC.

There were still some limitations in this study. First, only 11 TC cell lines were collected from the CCLE database. Second, the insufficiency of RT-qPCR analysis was also evident. Besides, the number of cases with BSG mRNA expression levels and disease-free time was limited making the survival analysis had a
large bias. The current results of survival analysis need to be checked out. Most importantly, the specific mechanisms underlying BSG in TC need further validation in vitro and in vivo.

5. Conclusion

In this study, we demonstrated the up-regulation of BSG expression in TC using meta-analysis and statistical analysis on the data collected from all possible sources, including IHC experiments, RNA-seq data, and microarray data. More importantly, we showed that BSG levels were closely correlated with the progression of TC may by affecting the ECM, cell adhesion, and cell-cell interactions. We hope further study can be performed to discover the specific molecular machinery of BSG that promotes the biological aggressiveness of TC.

Abbreviations

ATC: anaplastic carcinoma; AUC: Area under the curve; CAR: chimeric antigen receptor; CCLE: Cancer Cell Line Encyclopedia; CI: Confidential interval; CTLA-4: Cytotoxic T-lymphocyte-associated protein 4; DAVID: Database for Annotation, Visualization, and Integrated Discovery; DEGs: differentially expressed genes; DOR: diagnostic odds ratio; DTC: differentiated carcinoma; ECM: extracellular matrix; FTC: follicular carcinoma; GEO: Gene expression omnibus; GO: Gene ontology; GO-BP: GO biological process; GO-CC: GO cellular component; GO-MF: GO molecular function; HCC: hepatocellular carcinoma; IHC: immunohistochemistry; KEGG: Kyoto Encyclopedia of Genes and Genomes; MMPs: matrix metalloproteinases; MTC: medullary carcinoma; kinetochore complex component; PD-1: programmed death-1; PD-L1: PD ligand 1; PPI: Protein-protein interaction; PTC: papillary carcinoma; RNA-seq: RNA-sequencing; RT-qPCR: Real-time quantitative polymerase chain reaction; SMD: Standard mean difference; SRA: Sequence Read Archive; sROC: Summary receiver operating characteristic; SP/NK-1R: Substance P/neurokinin-1 receptor; STRING: Search Tool for the Retrieval of Interacting Genes; TCGA: The Cancer Genome Atlas; TC: thyroid cancer; TSH: thyroid stimulating hormone; UTC: undifferentiated carcinoma; VEGF: vascular endothelial growth factor.

Declarations

Ethics approval and consent to participate

Approval for the study was obtained from the Ethics Committee of the First Affiliated Hospital of Guangxi Medical University [Fanpu (No. 23 in 2018)]. Informed consent was obtained from all the patients whose tissues were used in this study.

Consent for publication

Not applicable.
Availability of data and materials

The datasets generated and/or analysed during the current study are available in the TCGA repository, https://cancergenome.nih.gov/, and the GEO repository, https://www.ncbi.nlm.nih.gov/geo/. The IHC data that support the findings of this study are available from Fanpu Biotech, Inc. (Guilin, China) but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of Fanpu Biotech, Inc. (Guilin, China).

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

G Chen, YY Pang, ZG Huang, XZ Tang, ZX Wei and W Ma designed the study. WP Guo, QQ Lai and JY Gan collected and analyzed the data. WP Guo, XL Huang and XF Liu wrote the manuscript. G Chen, ZG Huang, XJ Li, D Tang and W Ma revised the paper. All authors read and approved the final manuscript.

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Tables

Table 1 Clinical pathological parameters and BSG expression in TC data from TCGA database
| Characteristic          | expression of BSG\(\log_2(x)\) | n     | mean±SD   | t/F value | p value |
|------------------------|----------------------------------|-------|-----------|-----------|---------|
| Tissue                 | TC                               | 505   | 3.8±0.056 | 6.647     | <0.0001 |
|                        | non-cancerous                    | 59    | 3.76±0.042|           |         |
| Gender                 | Female                           | 369   | 3.7946±0.05313 | 1.175 | 0.081   |
|                        | Male                             | 136   | 3.8044±0.06241 |       |         |
| Age                    | >=60                             | 120   | 3.8031±0.05706 | -1.33 | 0.184   |
|                        | <60                              | 385   | 3.7954±0.05546 |       |         |
| Pathologic T           | T1                               | 143   | 3.7887±0.05514 | 1.5   | 0.201   |
|                        | T2                               | 166   | 3.7991±0.05678 |       |         |
|                        | T3                               | 171   | 3.8006±0.05538 |       |         |
|                        | T4                               | 23    | 3.8128±0.04993 |       |         |
|                        | Tx                               | 2     | 3.7882±0.12413 |       |         |
| T                      | T1-T2                            | 309   | 3.7943±0.05618 | 1.17  | 0.311   |
|                        | T3-T4                            | 194   | 3.802±0.05479 |       |         |
|                        | Tx                               | 2     | 3.7882±0.12413 |       |         |
| Pathologic N           | N0                               | 230   | 3.8047±0.06035 | 4.387 | 0.013   |
|                        | N1                               | 225   | 3.7893±0.04527 |       |         |
|                        | Nx                               | 50    | 3.7985±0.07198 |       |         |
| Pathologic M           | M0                               | 283   | 3.794±0.05303 | 1.922 | 0.147   |
|                        | M1                               | 9     | 3.8248±0.05971 |       |         |
|                        | Mx                               | 213   | 3.8004±0.05911 |       |         |
| Stage                  | I                                | 285   | 3.7925±0.05688 | 4.011 | 0.008   |
|                        | II                               | 52    | 3.8195±0.06654 |       |         |
|                        | III                              | 113   | 3.7948±0.04918 |       |         |
|                        | IV                               | 55    | 3.8057±0.04748 |       |         |
| Stage                  | I-II                             | 337   | 3.7967±0.05918 | -0.323 | 0.747   |
|                        | III-IV                           | 168   | 3.7984±0.04876 |       |         |
| Subtype                | Other, specify                   | 9     | 3.8297±0.06582 | 14.593 | <0.0001 |
|                        | Thyroid Papillary Carcinoma      | 358   | 3.7883±0.04898 |       |         |
| Class/usual                                      |   |          |
|-------------------------------------------------|---|----------|
| Thyroid Papillary Carcinoma - Follicular        | 102| 3.8266±0.06996 |
| Thyroid Papillary Carcinoma - Tall Cell         | 36 | 3.7948±0.0421  |

TC: thyroid cancer.

Table 2 Expression levels of BSG in the microarrays
| ID       | year | n     | Mean±SD       | t value | P value |
|----------|------|-------|---------------|---------|---------|
| GSE27155 | 2011 | TC    | 78 3.6650±0.2420 | 1.302   | 0.196   |
|          |      | non-cancerous | 21 3.5886±0.2249 |         |         |
| GSE53072 | 2013 | TC    | 5 3.8166±0.0956  | 0.389   | 0.715   |
|          |      | non-cancerous | 4 3.7995±0.0188 |         |         |
| GSE65144 | 2015 | TC    | 12 9.5326±0.4483 | -0.722  | 0.478   |
|          |      | non-cancerous | 13 9.6457±0.3306 |         |         |
| GSE29315 | 2012 | TC    | 31 9.1377±0.7861 | -0.166  | 0.869   |
|          |      | non-cancerous | 40 9.1715±0.9021 |         |         |
| GSE3678  | 2006 | TC    | 7 6.9376±0.251   | -0.693  | 0.501   |
|          |      | non-cancerous | 7 7.1255±0.4929 |         |         |
| GSE6004  | 2006 | TC    | 14 7.9174±0.5241 | 0.027   | 0.979   |
|          |      | non-cancerous | 4 7.9096±0.4714 |         |         |
| GSE29265 | 2012 | TC    | 29 6.9111±0.5993 | -0.151  | 0.880   |
|          |      | non-cancerous | 20 6.9352±0.4666 |         |         |
| GSE58545 | 2015 | TC    | 27 8.3943±0.7030 | 0.868   | 0.390   |
|          |      | non-cancerous | 18 8.1597±1.1135 |         |         |
| GSE58689 | 2015 | TC    | 27 8.3943±0.7030 | 0.868   | 0.390   |
|          |      | non-cancerous | 18 8.1597±1.1135 |         |         |
| GSE35570 | 2015 | TC    | 65 9.8489±0.5833 | 1.315   | 0.191   |
|          |      | non-cancerous | 51 9.7066±0.5724 |         |         |
| GSE33630 | 2012 | TC    | 59 9.6007±0.4657 | -1.286  | 0.201   |
|          |      | non-cancerous | 45 9.7057±0.3300 |         |         |
| GSE53157 | 2013 | TC    | 24 10.8278±0.5792 | 1.218   | 0.235   |
|          |      | non-cancerous | 3 10.3846±0.7451 |         |         |
| GSE50901 | 2014 | TC    | 61 1.3010±0.8936 | 0.444   | 0.659   |
| Experiment | Year | Tissue | Non-cancerous Cases | Mean ± SD | Z-Score | P-Value |
|------------|------|--------|---------------------|-----------|---------|---------|
| GSE3467    | 2005 | TC     | 9                   | 8.7789 ± 0.4506 | -0.829  | 0.419   |
|            |      |        | 9                   | 8.9390 ± 0.3642 |         |         |
| GSE60542   | 2015 | TC     | 58                  | 8.9624 ± 0.5898 | 1.910   | 0.059   |
|            |      |        | 34                  | 8.7006 ± 0.5161 |         |         |

Table 3 BSG-related bioinformation analysis in thyroid cancer tissue. The top twenty significant pathways in GO and KEGG pathway.
| Category | Term                                   | Count | %   | P-Value    |
|----------|----------------------------------------|-------|-----|------------|
| GO_BP    | extracellular matrix organization      | 52    | 4   | 5.9E-17    |
| GO_BP    | cell adhesion                          | 81    | 6.2 | 1.4E-14    |
| GO_BP    | inflammatory response                  | 62    | 4.8 | 6E-10      |
| GO_BP    | signal transduction                    | 128   | 9.8 | 1.5E-07    |
| GO_BP    | collagen catabolic process             | 19    | 1.5 | 2.2E-07    |
| GO_BP    | endothelial cell migration             | 12    | 0.9 | 1.9E-06    |
| GO_BP    | positive regulation of synapse assembly| 17    | 1.3 | 3.5E-06    |
| GO_BP    | cellular response to tumor necrosis factor| 23  | 1.8 | 5.8E-06    |
| GO_BP    | response to drug                       | 44    | 3.4 | 6.9E-06    |
| GO_BP    | leukocyte migration                    | 24    | 1.8 | 0.00001    |
| GO_BP    | positive regulation of apoptotic process| 43  | 3.3 | 0.000011   |
| GO_BP    | BMP signaling pathway                  | 18    | 1.4 | 0.000014   |
| GO_BP    | vasculogenesis                         | 15    | 1.2 | 0.00021    |
| GO_BP    | skeletal system development            | 25    | 1.9 | 0.00024    |
| GO_BP    | angiogenesis                           | 34    | 2.6 | 0.00031    |
| GO_BP    | response to mechanical stimulus        | 15    | 1.2 | 0.00004    |
| GO_BP    | response to axon injury                | 10    | 0.8 | 0.00055    |
| GO_BP    | positive regulation of gene expression | 37    | 2.8 | 0.00007    |
| GO_BP    | SMAD protein signal transduction       | 15    | 1.2 | 0.000072   |
| GO_BP    | cell chemotaxis                        | 15    | 1.2 | 0.00015    |
| GO_CC    | extracellular space                    | 199   | 15.3| 1.9E-26    |
| GO_CC    | proteinaceous extracellular matrix     | 70    | 5.4 | 1.3E-22    |
| GO_CC    | extracellular region                   | 208   | 16  | 2.4E-20    |
| GO_CC    | extracellular exosome                  | 311   | 23.9| 6E-20      |
| GO_CC    | extracellular matrix                   | 62    | 4.8 | 4.5E-15    |
| GO_CC    | cell surface                           | 80    | 6.2 | 1E-10      |
| GO_CC    | collagen trimer                        | 26    | 2   | 1.5E-09    |
| GO_CC    | integral component of plasma membrane  | 151   | 11.6| 2.5E-08    |
| GO_CC    | plasma membrane                        | 361   | 27.8| 4.4E-08    |
| GO_CC    | basement membrane                      | 20    | 1.5 | 1.1E-06    |
| GO_CC    | receptor complex                       | 24    | 1.8 | 0.00015    |
| GO_CC    | perinuclear region of cytoplasm        | 71    | 5.5 | 0.000023   |
| GO_CC    | endoplasmic reticulum lumen            | 29    | 2.2 | 0.00011    |
| GO_CC    | dendritic spine                        | 19    | 1.5 | 0.00013    |
| GO_CC    | Z disc                                 | 21    | 1.6 | 0.00014    |
| GO_CC    | integral component of membrane         | 409   | 31.5| 0.0002     |
| GO_CC    | anchored component of membrane         | 20    | 1.5 | 0.00022    |
| GO_CC       |                      |   |       |         |
|-------------|----------------------|---|-------|---------|
| focal adhesion |                     | 46| 3.5   | 0.00041 |
| endoplasmic reticulum |                 | 82| 6.3   | 0.00064 |
| cell         |                      | 17| 1.3   | 0.0012  |
| calcium ion binding |                | 99| 7.6   | 1.2E-11 |
| heparin binding |                    | 37| 2.8   | 1E-10   |
| extracellular matrix structural constituent |           | 20| 1.5   | 5.3E-08 |
| identical protein binding |               | 89| 6.8   | 2.1E-07 |
| integrin binding |                   | 23| 1.8   | 1.6E-06 |
| protein homodimerization activity |            | 79| 6.1   | 0.00035 |
| glycoprotein binding |                | 15| 1.2   | 0.00089 |
| SMAD binding |                     | 12| 0.9   | 0.00098 |
| extracellular matrix binding |            | 9 | 0.7   | 0.0022  |
| transforming growth factor beta binding |          | 7 | 0.5   | 0.00042 |
| calcium-dependent phospholipid binding |         | 13| 1     | 0.00042 |
| collagen binding |                   | 13| 1     | 0.00058 |
| aldehyde dehydrogenase (NAD) activity |            | 7 | 0.5   | 0.00061 |
| receptor activity |                  | 29| 2.2   | 0.00073 |
| low-density lipoprotein receptor activity |          | 6 | 0.5   | 0.0011  |
| chemokine activity |                 | 11| 0.8   | 0.0014  |
| actin filament binding |              | 20| 1.5   | 0.0014  |
| calcium-dependent protein binding |            | 12| 0.9   | 0.0015  |
| structural constituent of muscle |            | 10| 0.8   | 0.0017  |
| protein binding |                   | 642| 49.4 | 0.0018  |
| KEGG_PATHWAY  | ECM-receptor interaction | 25| 1.9   | 1.3E-08 |
| KEGG_PATHWAY  | Complement and coagulation cascades | 20| 1.5   | 4.5E-07 |
| KEGG_PATHWAY  | Staphylococcus aureus infection | 17| 1.3   | 1.2E-06 |
| KEGG_PATHWAY  | p53 signaling pathway | 18| 1.4   | 6.2E-06 |
| KEGG_PATHWAY  | Protein digestion and absorption | 20| 1.5   | 0.00022 |
| KEGG_PATHWAY  | Malaria | 14| 1.1   | 0.00046 |
| KEGG_PATHWAY  | Amoebiasis | 21| 1.6   | 0.0001  |
| KEGG_PATHWAY  | Transcriptional misregulation in cancer | 28| 2.2   | 0.00012 |
| KEGG_PATHWAY  | Phagosome | 26| 2     | 0.00013 |
| KEGG_PATHWAY  | Focal adhesion | 32| 2.5   | 0.00015 |
| KEGG_PATHWAY  | Pathways in cancer | 49| 3.8   | 0.0005  |
| KEGG_PATHWAY  | Pertussis | 15| 1.2   | 0.0012  |
| KEGG_PATHWAY  | Cytokine-cytokine receptor interaction | 33| 2.5   | 0.0013  |
| KEGG_PATHWAY  | Thyroid hormone synthesis | 14| 1.1   | 0.0019  |
| KEGG_PATHWAY  | Leukocyte transendothelial migration | 19| 1.5   | 0.0023  |
| KEGG_PATHWAY               |               |     |     |     |
|---------------------------|---------------|-----|-----|-----|
| Cell adhesion molecules (CAMs) | 21            | 1.6 | 0.0047 |
| Mineral absorption        | 10            | 0.8 | 0.0048 |
| Leishmaniasis             | 13            | 1   | 0.0063 |
| PI3K-Akt signaling pathway | 40            | 3.1 | 0.0065 |
| TGF-beta signaling pathway | 14            | 1.1 | 0.0097 |

**Figures**
The results of BSG immunohistochemical staining. A. BSG staining in non-cancer tissues (×200); B. BSG staining in non-cancer tissues (×200); C. BSG staining in non-cancer tissues (×400); D. BSG staining in non-cancer tissues (×400). E. BSG staining in TC tissues (×200); F. BSG staining in TC tissues (×200). G. BSG staining in TC tissues (×400); H. BSG staining in TC tissues (×400). I. The expression of BSG in 171 TC and 87 non-cancerous thyroid tissues. J. The ROC curve was generated to assess the diagnostic ability of BSG in 171 TC and 87 non-cancerous thyroid tissues. The AUC was 0.985(CI: 0.971 to 0.999, p<0.0001), and the corresponding sensitivity and specificity was 0.912 and 0.966, respectively.
Figure 2

Clinical role of upregulation of BSG mRNA level in thyroid cancer (TC) based on RNA-sequencing data. A, B: Expression level of BSG mRNA between TC and non-cancerous thyroid controls. C: Overall survival, Log-rank p= 0.5027, Hazard Ratio (logrank)= 0.6988 (95%CI: 0.2564 to 1.905). D: Disease free survival, Log-rank p= 0.0947, Hazard Ratio (Mantel-Haenszel)= 10.98 (95%CI: 0.6606 to 182.5).
A. $P = 0.501$

B. $P = 0.979$

C. $P = 0.196$

D. $P = 0.880$

E. $P = 0.201$

F. $P = 0.191$

G. $P = 0.659$

H. $p = 0.715$

I. $p = 0.235$

J. $P = 0.390$

K. $P = 0.390$

L. $P = 0.478$

M. $P = 0.419$

N. $p = 0.869$

O. $P = 0.059$
Figure 3

Scatter plots of BSG mRNA expression in thyroid cancer (TC) based on 15 GEO gene microarrays. A: Microarray GSE3678. B: Microarray GSE6004. C: Microarray GSE27155. D: Microarray GSE29265. E: Microarray GSE33630. F: Microarray GSE35570. G: Microarray GSE50901. F: Microarray GSE53072. I: Microarray GSE53751. J: Microarray GSE58545. K: Microarray GSE58689. L: Microarray GSE65144. M: Microarray GSE3467. N: Microarray GSE29315. O: Microarray GSE60542.

Figure 4

Integrated assessment of the CDK1 mRNA level in thyroid cancer (TC) based on all available data. A: Forest plot; B: Sensitivity; C: Funnel plot (Begg's test); D: Funnel plot (Egger's test)
Figure 5

Expression level of BSG confirmed using sROC curves including all available data. A: Sensitivity; B: Specificity; C: Positive likelihood ratios; D: Negative likelihood ratios; E: Diagnostic odds ratio; F: AUC of sROC.
Figure 6

Expression of BSG mRNA in thyroid cancer (TC) cell lines from the Cancer Cell Line Encyclopedia (CCLE). A: Expression of CDK1 mRNA in all cell types; B: Expression of CDK1 mRNA in all TC cell lines.
Figure 7
The potential mechanism of BSG in thyroid cancer (TC) assessed by GO annotation and KEGG enrichment analysis. A: Interaction of BSG co-expressed genes and differentially expressed genes (DEGs) in TC; B, C, D: GO analyses; E: KEGG enrichment analysis.
Figure 8

Hub genes of BSG in TC. A-J: Scatter plots of top 10 hub genes in thyroid cancer (TC) based on RNA-seq data: A: Cyclin dependent kinase 1 (CDK1); B: kinesin family member 11 (KIF11); C: topoisomerase (DNA) II alpha (TOP2A); D: ribonucleotide reductase regulatory subunit M2 (RRM2); E: microtubule nucleation factor (TPX2); F: PDZ binding kinase (PBK); G: maternal embryonic leucine zipper kinase (MELK); H: DLG...
associated protein 5 (DLGAP5); I: kinetochore complex component (NDC80); J: and nucleolar and spindle associated protein 1 (NUSAP1). K: Top 10 hub genes calculated by String and CytoHubba of Cytoscape based on the intersect genes.

**Supplementary Files**

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