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*Original Article*

# Integrative analysis of BSG expression in NPC through immunohistochemistry and public high-throughput gene expression data

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**Abstract:** Background: Though basigin (BSG) was reported to be overexpressed in nasopharyngeal carcinoma (NPC) and correlate with the development of NPC, the molecular basis of BSG in NPC remained elusive. The aim of the research was to investigate BSG expression in NPC and the potential molecular mechanism underlying it. Materials and methods: BSG expression in NPC tissues was detected with immunohistochemistry. Chi-square test, Kruskal-Wallis test and Spearman correlation test were performed to examine the relationship between BSG expression and the clinico-pathological features as well as EGFR and P-53 expression in NPC. In addition, data from the Human Protein Atlas (HPA) database and oncomine were collected to validate BSG expression in NPC. Meta-analysis was conducted to investigate the association between BSG expression and the clinico-pathological variables of NPC. The prognostic value and the alteration of BSG gene status were also analyzed with data from The Cancer Genome Atlas (TCGA). Results: BSG presented notably higher expression in NPC tissues than in non-cancer tissues. Moreover, IHC results showed that BSG expression was significantly correlated with tumor progression. A positive correlation was also found between BSG expression and EGFR, P53 expression. Meta-analysis confirmed that BSG was indicative of lymph node metastasis and TNM stage in NPC. Additionally, data from cBioPortal indicated that alteration of BSG gene existed in 5% of NPC cases and BSG correlative genes were obtained from the Co-expression Analysis in TCGA. Conclusion: BSG was overexpressed in NPC and might have an oncogenic effect on the tumorigenesis and progression of NPC.

**Keywords:** BSG, nasopharyngeal carcinoma, immunohistochemistry, clinical significance, molecular mechanism

## Introduction

Nasopharyngeal carcinoma (NPC) is a malignancy arising from nasopharynx which has an unbalanced distribution of morbidity in different regions of the world. NPC highly prevailed in Southern China, Southeast Asia and North Africa with an incidence rate of approximately 30 per 100,000, while NPC is rarely found in white populations [1-17]. Currently, radiotherapy and concurrent chemoradiotherapy (CCRT) are the preferential treatment for NPC. Despite great effort in enhancing diagnostic and therapeutic strategies, the 5-year overall survival for locally-advanced NPC patients remained poor [18]. Therefore, a novel target is urgently needed for an efficient treatment option for NPC.

Basigin (BSG), also known as extracellular matrix metalloproteinase inducer (EMMPRIN), belongs to the immunoglobulin (Ig) superfamily, exhibiting a high level of expression in various types of cancers [19-27]. Abundant researches indicated that BSG, as a transmembrane glyprotein, plays a vital part in tumor growth, metastasis, angiogenesis and apoptosis [28], which can be attributed to its capacity of inducing the release of VEGF and hyaluronan as well as stimulating the fibroblasts adjacent to tumors to promote the secretion of matrix metalloproteinases (MMP) that degrade the extra-cellular matrix [29]. The role of BSG in the tumorigenesis and development of cancer implies that BSG has potential diagnostic and therapeutic value in NPC.

**Table 1.** Relationship between BSG expression and clinico-pathological variables

| Variables | Total (n) | BSG expression n (%) | | $X^2$ | P |
|---|---|---|---|---|---|
| | | Negative | Positive | | |
| Tissue | | | | 77.543 | P<0.001 |
| Non-cancer tissues | 54 | 41 (75.9%) | 13 (24.1%) | | |
| NPC | 393 | 77 (19.6%) | 316 (80.4%) | | |
| Histological type | | | | 0.985 | P=0.321 |
| Adenocarcinoma | 4 | 0 (0.0%) | 4 (100.0%) | | |
| Squamous carcinoma | 389 | 77 (19.8%) | 312 (80.2%) | | |
| Squamous differentiation | | | | 1.999 | P=0.157 |
| Differentiated | 251 | 55 (21.9%) | 196 (78.1%) | | |
| Undifferentiated | 138 | 22 (15.9%) | 116 (84.1%) | | |
| Gender | | | | 0.006 | P=0.936 |
| Female | 121 | 24 (19.8%) | 97 (80.2%) | | |
| Male | 272 | 53 (19.5%) | 219 (80.5%) | | |
| Age | | | | 0.101 | P=0.751 |
| ≤50 | 208 | 42 (20.2%) | 166 (79.8%) | | |
| >50 | 185 | 35 (18.9%) | 150 (81.1%) | | |
| T category | | | | 20.794[a] | P<0.001 |
| T1 | 30 | 14 (46.7%) | 16 (53.3%) | | |
| T2 | 32 | 7 (21.9%) | 25 (78.1%) | | |
| T3 | 27 | 2 (7.4%) | 25 (92.6%) | | |
| T4 | 22 | 0 (0.0%) | 22 (100.0%) | | |
| N category | | | | 16.620[a] | P=0.001 |
| N0 | 6 | 2 (33.3%) | 4 (66.7%) | | |
| N1 | 22 | 11 (50.0%) | 11 (50.0%) | | |
| N2 | 62 | 9 (14.5%) | 53 (85.5%) | | |
| N3 | 21 | 1 (4.8%) | 20 (95.2%) | | |
| TNM stage | | | | 18.803[a] | P<0.001 |
| I | 6 | 2 (33.3%) | 4 (66.7%) | | |
| II | 22 | 11 (50.0%) | 11 (50.0%) | | |
| III | 48 | 9 (18.8%) | 39 (81.3%) | | |
| IV | 35 | 1 (2.9%) | 34 (97.1%) | | |

a: Kruskal-Wallis H test was performed. The rest of the scores were from Chi-square test.

BSG was observed to present high expression in NPC tissues and play a crucial role in the malignant progression of NPC [30, 31]. Although the overexpression of BSG has been reported by several studies, most of these studies investigated the clinico-pathological significance of BSG in NPC simply by immunohistochemistry. Only Du et al. used in vitro experiment as a supplement for IHC to illustrate the effect of BSG on the migration of NPC cells [32]. Above all, the molecular mechanism of BSG expression in NPC has not been fully elucidated yet. Therefore, the present study was designed to comprehensively assess the clinico-pathological significance of BSG expression in NPC with a combined method of immunohistochemistry (IHC), meta-analysis and data-mining in public databases, we also attempt to unveil the molecular mechanism underlying BSG expression in NPC.

## Materials and methods

### *Tissue samples*

We collected a total of 393 NPC tissues diagnosed as NPC from January 2011 to December 2013 for the study. The original site of tumor along with metastatic nodes was involved in selecting retrievable biopsy samples. Besides, a collection of 54 cases of control nasopharyngeal tissues comprised of chronic nasopharyngitis, rhinopolyp or fresh autopsy specimen were also included in our study. A total of 142 females and 305 males participated in our study. The age of patients ranged from 18 to 85 years old with an average of 59.6. Moreover, the clinical stages of 111 NPC patients without distant metastasis were carefully classified according to the seventh edition of UICC Staging System for NPC. Based on the pathological examination of primary tumor sites and adjacent lymph nodes, there were 6, 22, 48 and 35 patients belonging to the category of stage I, II, III and IV, respectively. All the paraffin-embedded tissues constituted the subjects of tissue microarrays (TMAs). As shown in **Table 1**, the clinico-pathological characters of the samples were available from archived pathological files. Approval of the study protocol was given by The Ethical Committee of First Affiliated Hospital of Guangxi Medical University. All the patients

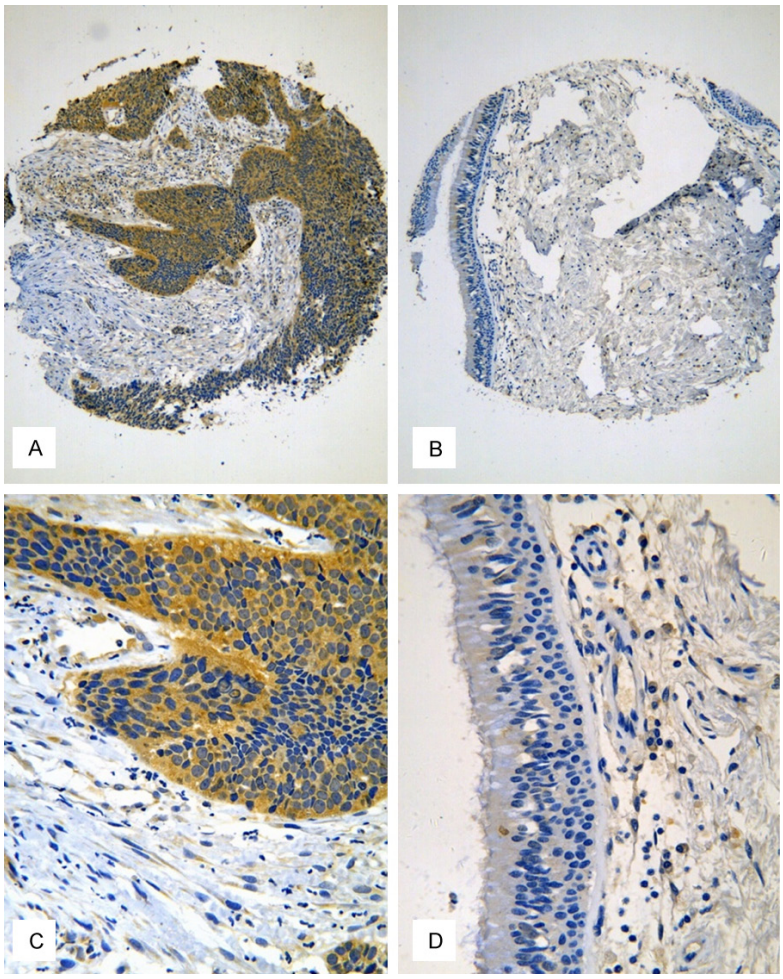


**Figure 1.** The representative staining pattern of BSG in NPC tissues and non-cancer tissues. BSG expression was obviously observed in the cytoplasm of NPC tissues (A and C). Non-cancer nasopharynx tissues exhibited weak or no BSG expression (B and D). The magnification of (A and B) was 100× and the magnification of (C and D) was 400×.

signed the written informed consents before the entry of the study.

*IHC*

All the tissue samples were sliced into 4 μm serial sections after fixation in formalin and paraffin embedding. Immunohistochemical staining was conducted on these sections to detect the expression of BSG. All the tissue sections were soaked in xylene solutions for the purpose of deparaffinization before staining. Alcohols with a series of concentration gradients were used for the rehydration process. Primary antibodies adopted in the study for immune-staining was rabbit monoclonal anti-BSG antibody (at 1/1000 dilution) (Abcam's RabMAb technology). We performed a standard avidin-biotin immunoperoxidase complexes detection system in accordance with the manufacturer's constitution. To ensure the accuracy of the experiment, the evaluation of immunostaining for each sample was conducted by two pathologists (Gang Chen and Yi-wu Dang), and divergence of views was resolved through discussion. The intensity of BSG expression in cytoplasm determined the immunereactivities of BSG expression in tissue samples. Criteria was established that tissue with BSG expression in more than 25% of tumor cells would be considered as BSG-positive. The immunostaining of EGFR and P53 expression was assessed according to the proportion of cells with positive expression in total cells. At least 10 typical fields in high magnification (40×40) from necrotic areas were randomly selected for counting. Results of the immunostaining were divided into four groups: (-) for negative staining, (+) for weak staining, (++) for moderate staining and (+++) for strong staining.

*Statistical analysis for IHC*

All the statistical analysis for IHC was performed by SPSS 22.0. Frequency and percentage were calculated for the classified variables. We used Chi-square test to evaluate the difference of BSG expression between two opposite groups of clinico-pathological factors. Kruskal-Wallis H test was carried out to compare the difference of BSG expression in more than two groups of varied clinico-pathological features. Furthermore, the correlations between BSG expression and clinical variables, EGFR or P53 were assessed by Spearman correlation test.

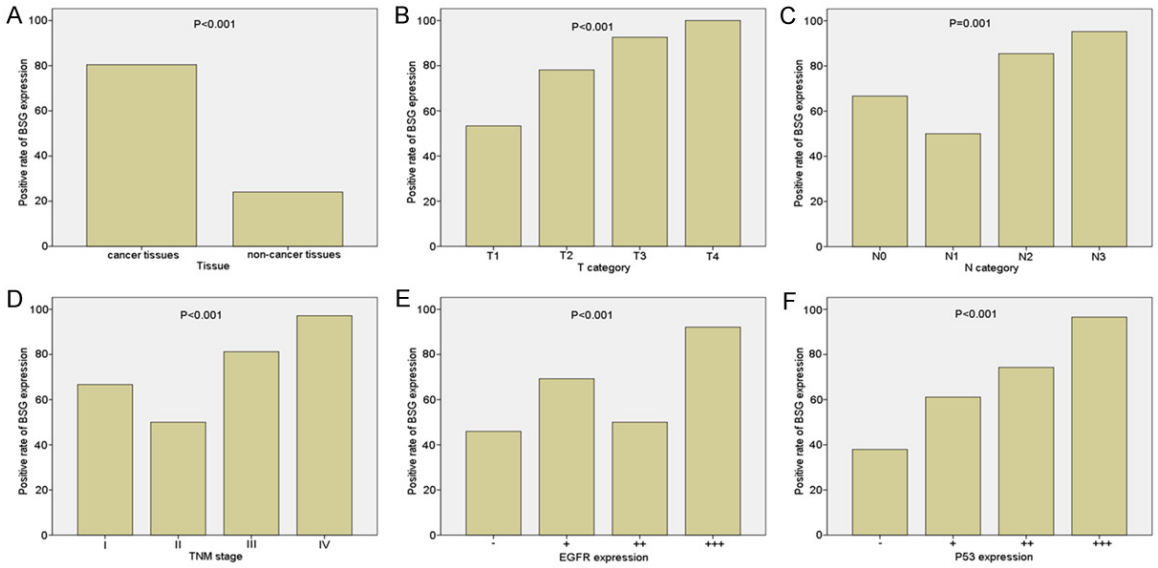


**Figure 2.** The relationship between BSG expression and the clinico-pathological variables of NPC. A. The relationship between BSG expression and Tissue. B. The relationship between BSG expression and T category. C. The relationship between BSG expression and N category. D. The relationship between BSG expression and TNM stage. E. The relationship between BSG expression and EGFR expression. F. The expression between BSG expression and P53 expression. BSG expression varied significantly in different subgroups of tissue types (P<0.001), T stage (P<0.001), N stage (P=0.001), TNM stage (P<0.001), EGFR expression (P<0.001) and P53 expression (P<0.001).

All the *P*-values in the statistical analysis were two-tailed, and P<0.05 was considered as statistically significant.

*BSG expression in head and neck cancer from the HPA database*

Apart from IHC, we also compared BSG expression in normal human tissues and head and neck cancer tissues from the HPA database. The HPA database provided abundant data of transcriptome and proteomes in specific human tissues through RNA-sequencing analysis and immunohistochemistry analysis. Additionally, functional analyses of proteomes in secretion, membrane, cancer and drug were also included in HPA, which proved that HPA served as an advantageous tool in protein expression analysis and medical diagnostics [33, 34]. Since NPC belongs to head and neck cancer, BSG expression in head and neck cancer tissues might indirectly reflect BSG expression level in NPC tissues.

*BSG expression in head and neck cancer from oncomine*

Oncomine is a cancer microarray database that contained 65 gene expression datasets covering a wide type of human cancers. Thus, we took advantage of this high-throughput database to mine BSG expression in NSP tissues and normal tissues. However, only BSG expression in head and neck cancer tissues was available in oncomine and we compared BSG expression in NPC and normal tissues using data of BSG expression in head and neck cancer tissues for NPC falls to the category of head and neck cancer.

*Investigation of the clinic-pathological significance of BSG in NPC through a combined meta-analysis of in-house IHC and literature studies*

*Literature research and study selection:* Eleven databases including Pubmed, Embase, Science Direct, Wiley online library, Chochrane library, Web of science, Springerlink, VIP, Sinomed, WanFang and CNKI were searched. The following combination of terminologies: (nasopharyngeal carcinoma) AND (BSG OR CD147 OR BSG OR basigin OR HaB18G) was used for searching eligible studies. We also searched references lists in relevant articles and review articles. Further selection for eligible studies was based on the inclusion and exclusion criteria. Studies were included if they met the following criteria:

**Table 2.** The relationship between BSG expression and two molecular markers

| Molecular markers | Total (n) | BSG expression n (%) | | H | P |
|---|---|---|---|---|---|
| | | Negative | Positive | | |
| EGFR | | | | 20.726 | P<0.001 |
| - | 37 | 20 (54.1%) | 17 (45.9%) | | |
| + | 52 | 16 (30.8%) | 36 (69.2%) | | |
| ++ | 20 | 10 (50.0%) | 10 (50.0%) | | |
| +++ | 38 | 3 (7.9%) | 35 (92.1%) | | |
| P53 | | | | 23.931 | P<0.001 |
| - | 29 | 18 (62.1%) | 11 (37.9%) | | |
| + | 54 | 21 (38.9%) | 33 (61.1%) | | |
| ++ | 35 | 9 (25.7%) | 26 (74.3%) | | |
| +++ | 29 | 1 (3.4%) | 28 (96.6%) | | |

Kruskal-Wallis H test was performed.

**Table 3.** The correlation between BSG expression and clinico-pathological variables

| Variables | r | P |
|---|---|---|
| Tumor infiltration (T) | 0.426 | P<0.001 |
| Lymph node metastasis (N) | 0.348 | P<0.001 |
| TNM stage | 0.386 | P<0.001 |
| Squamous differentiation | -0.072 | P=0.158 |
| EGFR | 0.301 | P<0.001 |
| P53 | 0.399 | P<0.001 |

(1) Studies that compared BSG expression in NPC tissues and non-cancer tissues. (2) Studies that were published in English or Chinese. (3) Studies that provided any of the data: expression value of BSG in NPC patients, the clinico-pathological features of NPC patients and the prognostic or diagnostic data of BSG in NPC. Studies were excluded according to the following criteria: (1) studies that contained no non-cancer control of NPC tissues. (2) No sufficient data can be extracted from the study to analyze the clinico-pathological significance of BSG expression in NPC. (3) Studies that belong to the types of review, meta-analysis, case report or conference reports.

*Data extraction*

The following information were extracted from the included studies: first author, publication year, country, number of NPC tissues (M/F), number of Pharyngitis (M/F), age (median), antibody, staining for BSG positivity, blinded reading, differentiation grade (low/Undifferentiated), lymph node metastasis (no/yes), WHO classification (II/III), T stage (T1-T2/T3-T4), TNM stage (I-II/III-IV), recurrence and metastasis (No/Yes).

*Quality assessment*

The Newcastle-Ottawa Scale (NOS) was applied to evaluate the quality of the included studies [35]. Each study was assessed by the scale in three aspects including selection of participants, study comparability, and the ascertainment of outcomes of interest. A maximum of 9 points can be awarded to a study. Studies were considered to be of good quality with a score ≥7.

*Statistical analysis for meta-analysis*

Odds ratios (OR) with 95% confidential interval (95% CI) were employed to pool the overall effects of all the literature studies and in-house IHC data. Cochran Q and $I^2$ statistics were utilized to evaluate the heterogeneity between all the studies. A fixed-effect model was applied to calculate the pooling effects of all the literature studies and in-house IHC data when no significant heterogeneity was detected, ($I^2 \leq 50\%$, $P>0.05$); conversely, a random-effect model was used when significant heterogeneity existed between studies ($I^2>50\%$, $P<0.05$) [36]. Then, sensitivity analysis and subgroup analysis were performed to identify the sources of heterogeneity. Finally, Begg's test with funnel plot and Egger's test were performed to evaluate the publication bias [37].

*TCGA data extraction*

*Kaplan-Meier survival analysis of BSG expression in head and neck cancer from Oncolnc:* To assess the prognostic significance of BSG expression in NPC patients, we searched BSG expression in head and neck squamous cell carcinoma (HNSCC) as well as the corresponding prognostic data of HNSCC patients from Oncolnc. The primitive expression values of BSG were log-2 transformed and grouped according to the average value of all the expres-

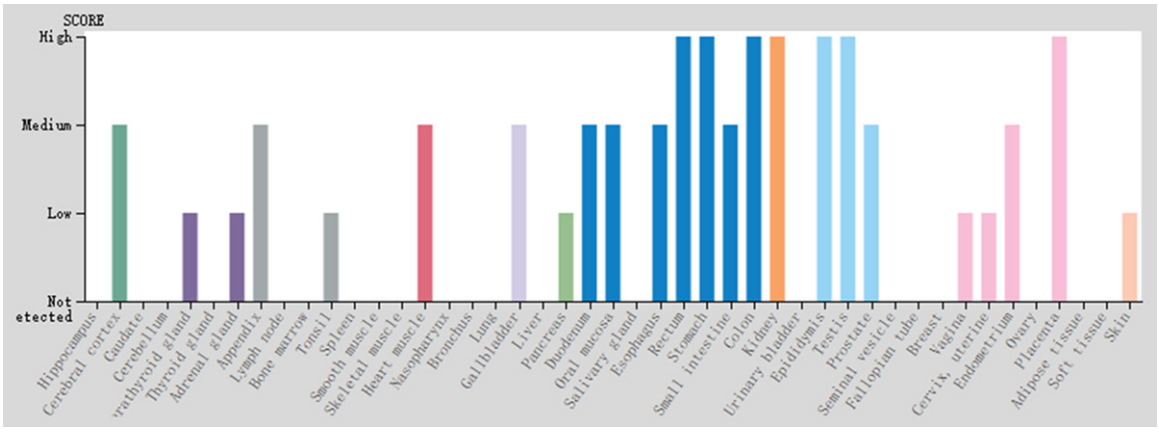


**Figure 3.** BSG expression in different normal human tissues. BSG expression profile in different normal human tissues were downloaded from HPA. No expression of BSG was detected in normal nasopharynx tissues.

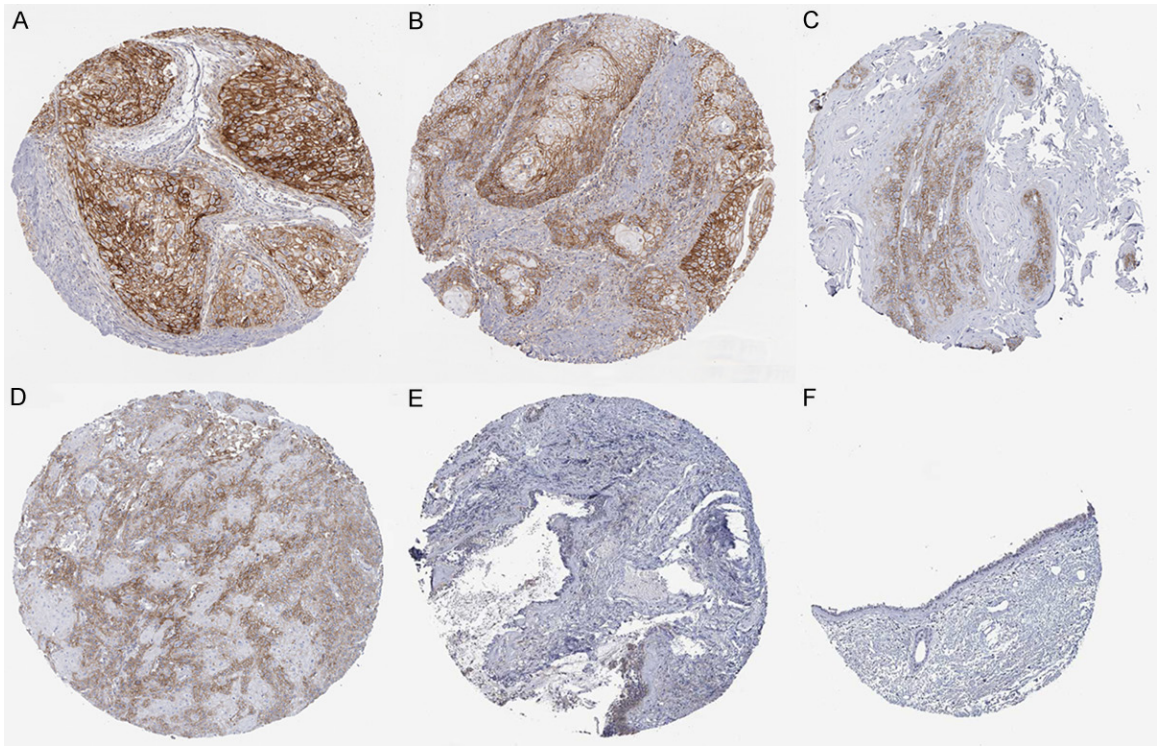


**Figure 4.** Pattern of BSG expression in head and neck cancer and normal tissues. (A) High staining of BSG in the cytoplasm and membrane of head and neck cancer cells. (B-D) Medium staining of BSG in the cytoplasm and membrane of head and neck cancer cells. (E and F) No BSG expression was detected in normal nasopharynx tissues. All the immunostaining pictures were downloaded from HPA database. Antibody HPA036048 (A and B; E and F) and CAB002427 (C and D) were used for staining.

sion data. Then, Kaplan Meier survival analysis was carried out by SPSS v.22.0 to assess the influence of BSG on the survival of NPC patients.

*Gene alteration of BSG in NPC from cBioPortal*

Alteration of BSG gene status in NPC patients was obtained from cBioPortal (http://www.cBioPortal.org/index.do) for the analysis of BSG gene status in the progression of NPC. Moreover, we also constructed a co-expression network with genes related to BSG from cBioPortal OncoPrint, which might facilitate understanding the interaction between genes associated with BSG. The impact of the alteration of BSG gene status on the prognosis of NPC

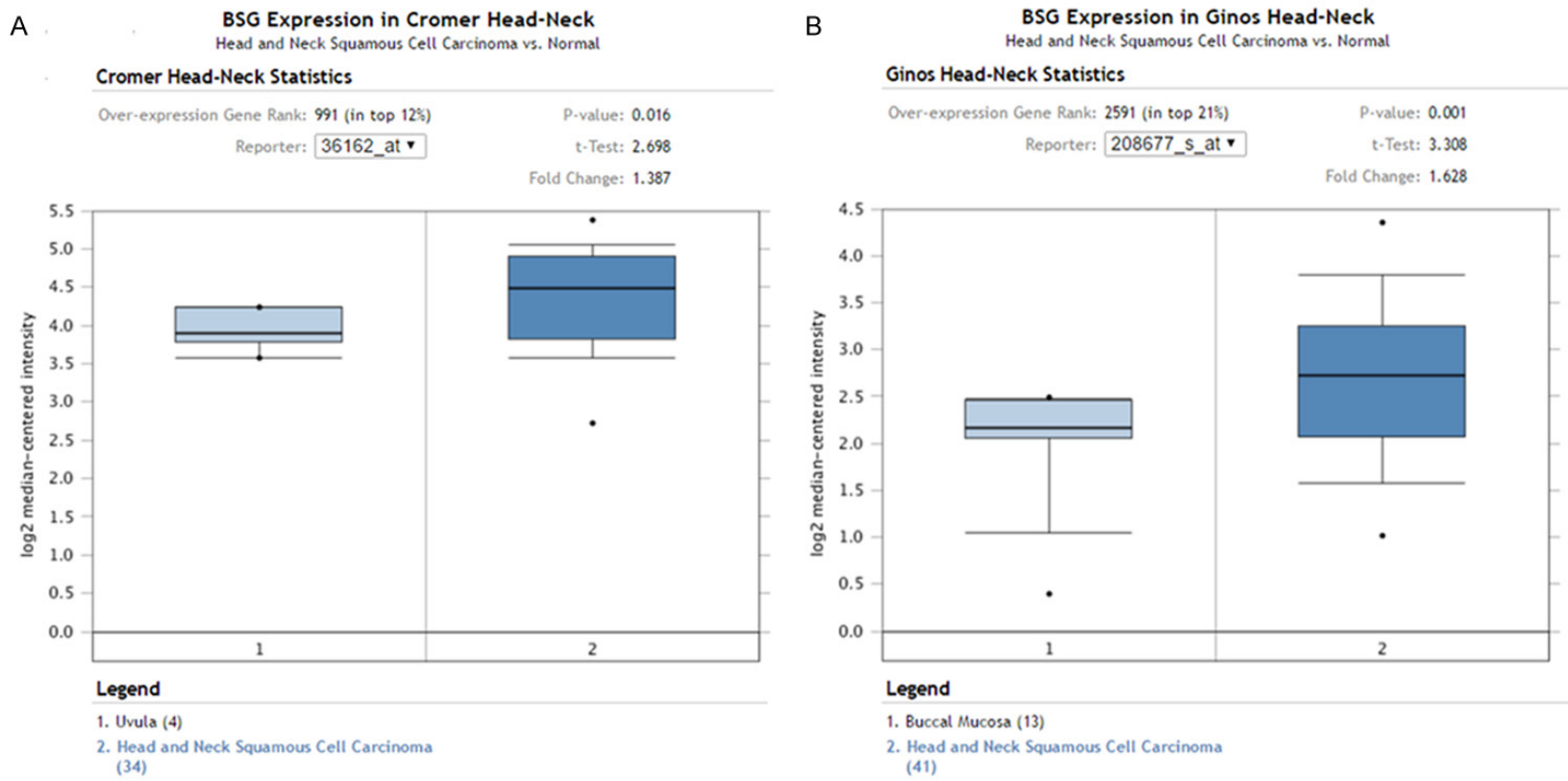


**Figure 5.** The difference of BSG expression in head and neck cancer and non-cancer tissues. A. BSG expression in 4 non-cancer tissues and 34 HNSCC tissues from Cromer Head-Neck Statistics (P=0.016). B. BSG expression in 13 non-cancer tissues and 41 HNSCC tissues from Ginos Head-Neck Statistics (P=0.001). BSG expression was significantly higher in HNSCC tissues than in normal tissues (Both P<0.05).

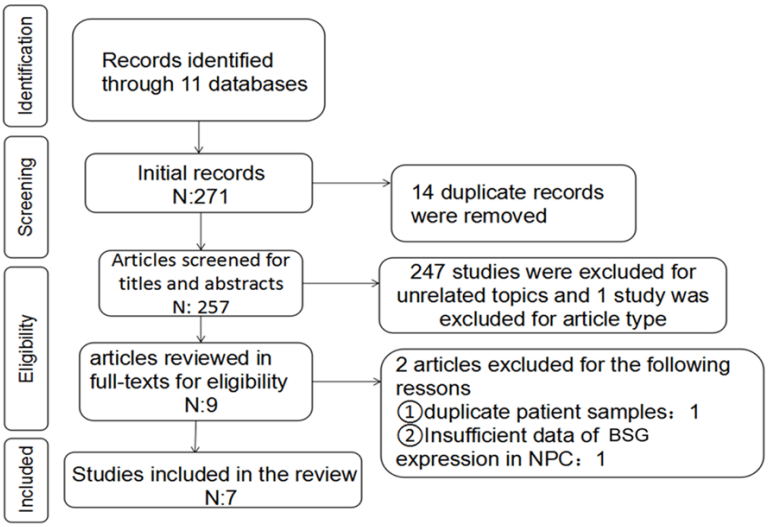


**Figure 6.** The flowchart of meta-analysis. A total of 271 studies appeared as the initial records. After 14 duplicate records were removed, 247 studies were excluded by screening titles and abstracts. Then, two studies were excluded in full-text reviewing. Finally, seven studies were enrolled for meta-analysis.

patients was evaluated by Kaplan Meier Survival Curves.

Genes co-amplified with BSG in HNSCC was acquired from the Co-expression Analysis in TCGA. Top 20 genes that significantly correlated with BSG in HNSCC were selected and visualized by heatmap.

## Results

*BSG expression in NPC tissues*

As shown in **Figure 1**, four representative images of immunostaining illustrated BSG expression pattern in NPC tissues (n=393) and non-cancer tissues (n=54). A vast majority of NPC tissues presented positive BSG immunostaining in the cytoplasm of the tumor cells. In contrast, a weak staining or no staining was observed in non-cancer tissues. Four cases of adenocarcinoma tissues and 389 cases of squamous carcinoma tissues constituted the whole NPC samples. BSG expression in the two sub-types of NPC was of no statistical significance (P=0.321). Subgroups analysis with respect of adenocarcinoma was excluded from our study due to limited samples of adenocarcinoma tissues. Results from statistical analysis demonstrated that BSG expression was obviously higher in NPC tissues with statistical significance (80.4%) compared with that in non-cancer tissues (24.1%) (P< 0.001) (**Figure 2A**).

*Relationship between BSG expression and clinico-pathological features*

A panel of clinico-pathological parameters was adopted to investigate the clinical significance of BSG expression in NPC. As shown in **Table 1**, no significant relationship was established between BSG expression and histological types of cancer, squamous differentiation, gender and age. BSG expression showed considerable difference between NPC tissues (80.4%) and non-cancer tissues (24.1%) ($X^2$=77.543, P< 0.001). In regard with the malignant progression of NPC, BSG expression was observed significantly higher in advanced T category (T3 and T4) (96.0%) than in early T category (T1 and T2) (66.1%) (H=20.794) (P< 0.001). BSG expression also bore significant difference in the course of lymph node metastasis (N), which can be proved by the higher expression of BSG in advanced N category (N2 and N3) (88.0%) compared with that in early N category (N0 and N1) (53.6%) (H=16.620, P=0.001). With respect to the overall clinical stage, BSG expression was remarkably up-regulated in patients with advanced TNM stage (III and IV) (88.0%) than in patients with early TNM stage (I and II) (53.6%) (H=18.803, P<0.001). The expression patterns of BSG in different groups of T category, N category, and TNM stage were illustrated in **Figure 2B-D**.

*Relationship between BSG expression and two common molecular markers for NPC*

As shown in **Table 2**, the variation of BSG expression was further analyzed in four sequential subgroups of EGFR and P53 expression. As a consequence, BSG reached a positive rate of expression in groups with positive EGFR expression (73.6%) in comparison with that in negative EGFR expression group (45.9%) (H=20.726, P<0.001), showing a statistical significance. Similarly, BSG expression experienced a remarkable up-regulation in patients with posi-

**Table 4.** The basic information of the included studies

| | Characteristic of all the selected studies | | | | | | |
|---|---|---|---|---|---|---|---|
| First author | Aihua Luo | Dan Liu | Juan Zhang | Tian Huang | Ziming Du | Shuzhen Han | Bo Huang |
| Year | 2012 | 2012 | 2014 | 2013 | 2009 | 2013 | 2004 |
| Country | China | China | China | China | China | China | China |
| N of NPC tissues (M/F) | (22/8) | (37/18) | (47/34) | (41/12) | (174/58) | (37/13) | 71 |
| N of Pharyngitis (M/F) | (17/8) | 37 | 43 | 15 | | (21/9) | (17/13) |
| Age (median) | 49 | 45 | 47.11±10.20 | 51.9 | 46 | 43 | NR |
| Antibody | Mouse anti human BSG polyclonal antibody | Rabbit anti human BSG polyclonal antibody | Rabbit anti human BSG polyclonal antibody | Rabbit anti-BSG | Primary antibody against BSG | Mouse anti human BSG polyclonal antibody | mouse anti human BSG monoclonal antibody M 0703 |
| Staining for BSG positivity | ≥2 | ≥2 | ≥2 | ≥5% | ≥1 | ≥2 | ≥5% |
| Blinded reading | Yes | Yes | Yes | Yes | Yes | Yes | NR |
| Differentiation grade (low/undeffirentiated) | | | (52/29) | | | | |
| Lymph node metastasis (no/yes) | | | 32/49 | (20/33) | (52/145) | | |
| WHO classification (II/III) | | | | | 50/147 | | |
| T stage (T1-T2/T3-T4) | | | | | (80/117) | | |
| TNM stage (I-II/III-IV) | | | 33/48 | | (48/149) | (6/44) | |
| Recurrence | | | | | (50/147) | | |
| Metastasis (No/Yes) | | | | | (171/26) | (9/41) | |
| Quality assessment | 6 | 6 | 7 | 7 | | 7 | 7 |

NPC: nasopharyngeal carcinoma; M: male; F: female.

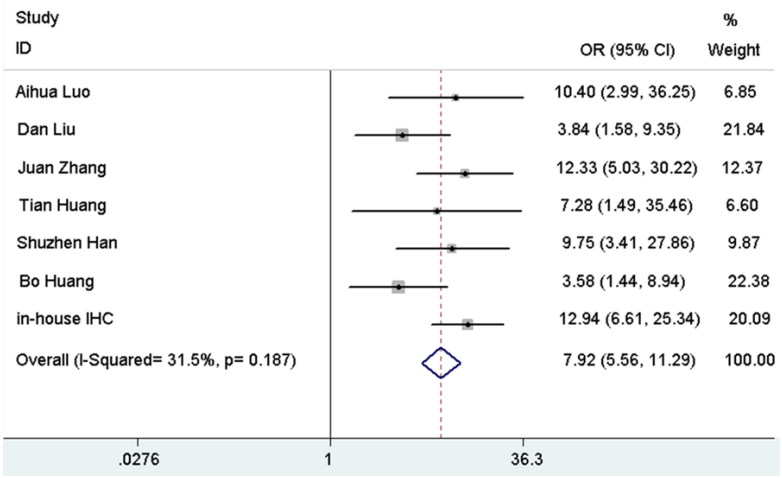


**Figure 7.** The forest plot of BSG expression in NPC. Higher BSG expression was closely associated with the occurrence of NPC (OR=7.92, 95% CI=5.56-11.29, $I^2$=31.5%, p=0.187).

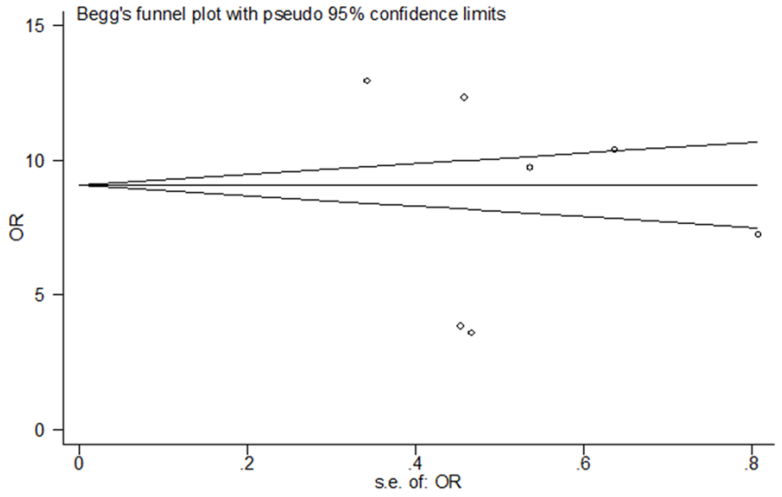


**Figure 8.** Publication bias test. The results from Begg's test and Egger's test as well as the symmetrical funnel plot indicated that no publication bias was found (P=0.548).

tive expression of P53 (73.7%), compared with that in patients with negative P53 expression (37.9%) (H=23.931, P<0.001). The difference of BSG expression in four sequential subgroups of EGFR and P53 expression were displayed in **Figure 2E** and **2F**.

*Spearman's correlation test*

As illustrated in **Table 3**, examination from spearman's correlation test demonstrated that BSG expression was closely associated with tumor infiltration (r=0.426, P<0.001), lymph node metastasis (r=0.348, P< 0.001), TNM stage (r=0.386, P<0.001), EGFR expression (r=0.301, P<0.001) and P53 expression (r=0.399, P< 0.001). Additionally, a significant relationship was confirmed between BSG expression and EGFR (r=0.301, P<0.001) or P53 expression (r=0.399, P<0.001).

*BSG expression in head and neck cancer from HPA database*

As shown in the expression profile of BSG from HPA (http://www.proteinatlas.org/ENSG00000172270-BSG/tissue), no expression of BSG was detected in normal nasopharynx tissues (**Figure 3**). Immunohistochemical results in **Figure 4** also revealed that BSG presented negative expression in normal nasopharynx tissues while moderate or even strong BSG expression was recorded in the cytoplasm or membrane of head and neck cancer cells.

*BSG expression in head and neck cancer from oncomine*

In oncomine database, the distribution of BSG expression in normal tissues and head and neck cancer tissues were available from two microarray chips including Human Genome U95A-Av2 Array and Human Genome U133A Array, which contained 38 samples (4 normal tissues and 34 head and neck squamous cell carcinoma tissues) (**Figure 5A**) and 54 samples (13 normal tissues and 41 head and neck squamous cell carcinoma tissues) (**Figure 5B**), respectively. From the result, BSG expression was obviously higher in head and neck squamous cell carcinoma tissues than in normal tissues (both *P* values from t-Test <0.05) (**Figure 5**).

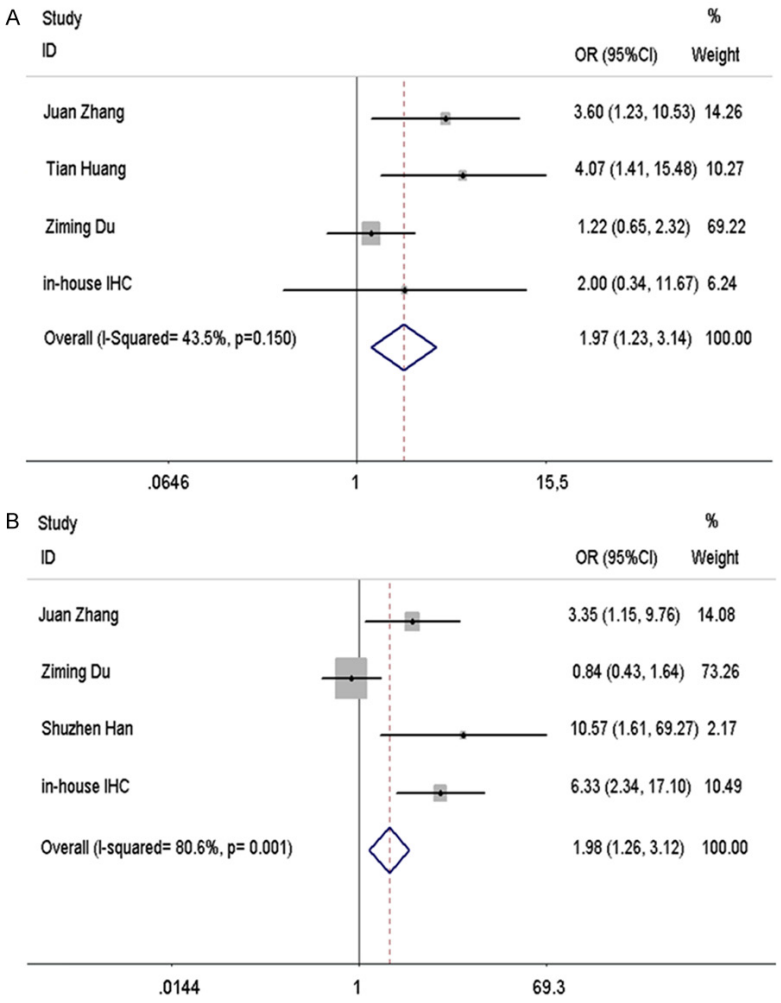


**Figure 9.** Forest plot of BSG expression in the lymph node metastasis of and TNM stage of NPC. A. Higher BSG expression predicted lymph node metastasis (OR=1.97, 95% CI=1.23-3.14, $I^2$=43.5%, P=0.150). B. The forest plot revealed that higher BSG might be indicative of advanced TNM stage (OR=1.98, 95% CI=1.26-3.12) with non-negligible heterogeneity ($I^2$=80.6%, P=0.001).

*Investigation of the clinico-pathological significance of BSG in NPC through a combined meta-analysis of IHC and literature*

As described in the flowchart (**Figure 6**), there were a total of 271 articles in the initial records. After removing 14 duplicate records, 248 studies were excluded by screening the abstracts of the articles. Two studies were excluded due to the duplicate samples and insufficient data of BSG expression in NPC, respectively. Eventually, seven studies with 572 NPC tissues and 180 nasopharyngitis tissues were included for our meta-analysis. The basic information of the included studies was listed in **Table 4**. All the studies were from China. Since the study from Du et al. was not a case control study [32], NOS evaluation was conducted in other six studies and they all scored no less than six points.

After the selection of qualified literature studies, in-house IHC data were integrated into the meta-analysis to comprehensively assess the clinico-pathological significance of BSG in NPC. As shown in **Figure 7**, higher BSG expression was closely associated with the occurrence of NPC (OR=7.92, 95% CI=5.56-11.29). Since no significant heterogeneity was detected ($I^2$=31.5%, p=0.187), fixed effect model was used to aggregate BSG expression in NPC and nasopharyngitis tissues. As for the publication bias, the symmetrical funnel plot indicated that no publication bias was found (P=0.548) (**Figure 8**). We also evaluated the correlation between BSG expression and the malignant development of NPC such as lymph node metastasis and TNM stage. The forest plot in **Figure 9A** suggested that higher BSG expression predicted lymph node metastasis (OR=1.97, 95% CI=1.23-3.14, $I^2$=43.5%, P=0.150). An OR value of 1.98 from **Figure 9B** (95% CI=1.26-3.12) indicated that higher BSG expression might be also related to the advanced TNM stage; however, the result bore significant heterogeneity ($I^2$=80.6%, P=0.001). Considering the characteristic of our data, sensitivity analysis was performed and the study of Du et al. was found to contribute to the heterogeneity significantly (**Figure 10A**), which was supported by the result that when the study of Du et al. was removed, the remaining studies yielded an OR value of 5.10 (95% CI=2.59-10.07) with a remarkably decreased heterogeneity ($I^2$=0.0%, P=0.508) (**Figure 10B**).

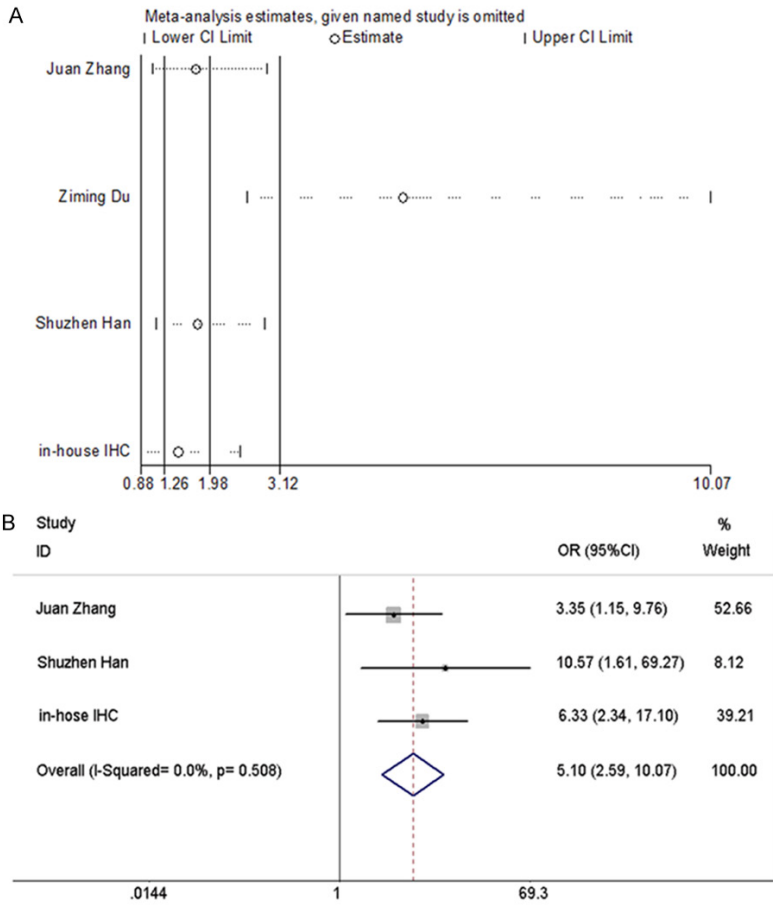


**Figure 10.** Sensitivity analysis and the corrected forest plot of BSG expression in TNM stage of NPC. A. The sensitivity analysis identified the study of Du et al. as the major contributor of the significant heterogeneity between studies. B. The corrected forest plot was created by excluding the study of Du et al. to re-assess the relationship between BSG expression and TNM stage of NPC. The result indicated higher correlation between BSG expression and TNM stage of NPC with significantly reduced heterogeneity between studies (OR=5.10, 95% CI=2.59-10.07, $I^2$=0.0%, P=0.508).

*TCGA data extraction*

The expression and prognostic data of BSG in HNSCC tissues downloaded from Oncolnc were attached in **Figure 11**. According to the Kaplan Meier Survival analysis from 496 HNSCC specimens in Oncolnc (**Figure 12**), no significant difference was observed between the survival of HNSCC patients with high BSG expression and those with low BSG expression (P=0.292).

From BSG gene alteration data of 528 NPC patients (530 samples) in cBioPortal, 28 cases exhibited BSG gene alteration, which consisted of 4 cases of deep deletion, 22 cases of mRNA up-regulation and 2 cases of mRNA down-regulation (**Figure 13A**). The survival analysis for BSG gene alteration in the overall survival and disease-free survival of NPC patients were displayed in the Kaplan Meier survival curves. Nevertheless, the results bore no significance (Both P>0.05) (**Figure 13B** and **13C**). Gene co-expression network illustrated that BSG was closely associated with some genes including PPIB, L1CAM, SLC7AB, PPIL2, SLC7A7 and ATP1B3 (**Figure 14**). These genes were also actively involved in the regulation network.

According to the Co-expression analysis in TCGA, 18823 genes were measured for expression values in 628 samples comprised of 338 samples of no value and 290 HNSCC samples. The correlation between BSG and genes co-amplified with BSG were calculated. The results revealed that genes including HCN2, GZMM, RNF126, FGF22, POLRMT, PRSSL1, CDC34, AZU1, PRTN3, ELANE, CFD, SBNO2, STK11, C19orf20, MADCAM1, FSTL3, EFNA2, C19orf24, TMPR559 and UQCR11 were the top 20 genes that significantly correlated with BSG (all correlation value ≥0.887) (**Figure 15**).

## Discussion

Currently, mounting studies reported that BSG plays a critical role in the multi-step process of various cancers. The study conducted by Huang et al. indicated that BSG accelerate the progression of thyroid carcinoma through the interaction with MTC1, which affect the glycolysis of tumor cells [38]. Xu et al. reported that BSG was involved in the regulation of MAPK/ERK pathway to facilitate 5-FU resistance, cell invasion ability and EMT in colorectal cancer [39]. The clinico-pathological values of BSG overex-

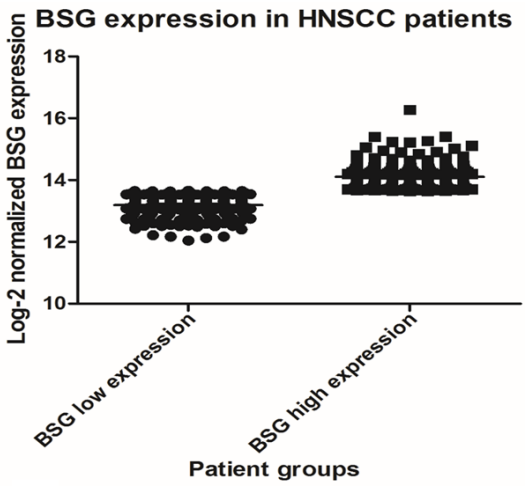


**Figure 11.** Expression data of BSG in HNSCC from OncoInc. There were 244 and 252 HNSCC patients in groups of BSG low expression and BSG high expression, respectively. All the primitive expression data were log-2 normalized and then visualized using GraphPad Prism 5. The expression values of BSG in BSG low expression group and BSG high expression group were 13.190±0.335 and 14.104±0.378 (mean ± standard deviation), respectively.

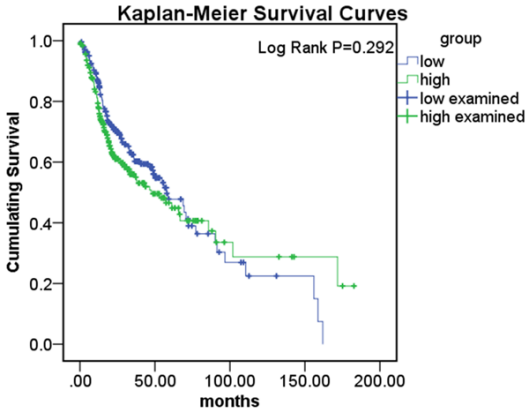


**Figure 12.** Kaplan Meier Survival analysis for BSG expression data. The prognostic data of BSG expression in HNSCC was downloaded from OncoInc. Then, Kaplan-Meier Survival analysis was conducted to evaluate the prognostic significance of BSG in NPC. BSG exhibited no significant effect on the survival of HNSCC patients (P=0.292).

pression in various cancers such as gastric cancer, oral squamous cell carcinoma and renal cell carcinoma were also elaborated in previous studies [40-42]. In NPC, BSG was reported in several articles to participate in the pathogenesis of NPC with its overexpression [43-45]. Despite that, the exact molecular function of BSG in NPC remained elusive. Therefore, we carried out the research for the purpose of identifying the clinical significance of BSG expression in NPC and clarifying the underlying molecular mechanism.

The results from IHC indicated that BSG was statistically associated with tissue types and higher clinical stage of NPC patients. In addition to the relationship of BSG expression and clinico-pathological features in NPC, we also investigated the correlation between BSG expression and EGFR or P53 expression. From the outcome of our study, BSG was predominantly expressed in positive EGFR expression group and positive P53 expression group, which demonstrated that BSG followed the expression trends of EGFR and P53. Based on these results, we postulated that BSG might coordinate with EGFR or P53 to influence the malignant progression of NPC and the combination of BSG, EGFR and P53 may be reliable diagnostic targets for NPC. Recently, there emerged several studies concerning the interaction between BSG, EGFR and P53. Studies from Jin JS et al. and Menashi S et al. both reported that EGFR expression may induce BSG expression and the subsequent release of MMP [46, 47]. In breast cancer, increased expression of BSG from non-transformed, non-invasive breast epithelial cells was able to activate the EGFR-Ras-ERK signaling pathway [48]. Zhu H et al. reported that wild type P53 functioned as a tumor suppressor gene via inhibiting the BSG expression to alleviate the invasion of cancer cells [49]. Thus, we hypothesized that EGFR or P53 might exert similar influence on the expression of BSG in NPC. Further studies are needed to strengthen the comprehension of the underlying molecular mechanism between BSG, EGFR and P53 in NPC.

To verify BSG expression in NPC, we compared BSG expression in NPC tissues and non-cancer tissues with data from public databases including HPA and oncomine as well as meta-analysis. It should be noted that in HPA and oncomine, we acquired BSG expression values in head and neck cancer rather than BSG expression in NPC. Now that NPC was one type of head and neck cancer, we conceived that head and neck cancer and NPC may share similar trend of BSG expression. All the results from HPA, oncomine and meta-analysis supported

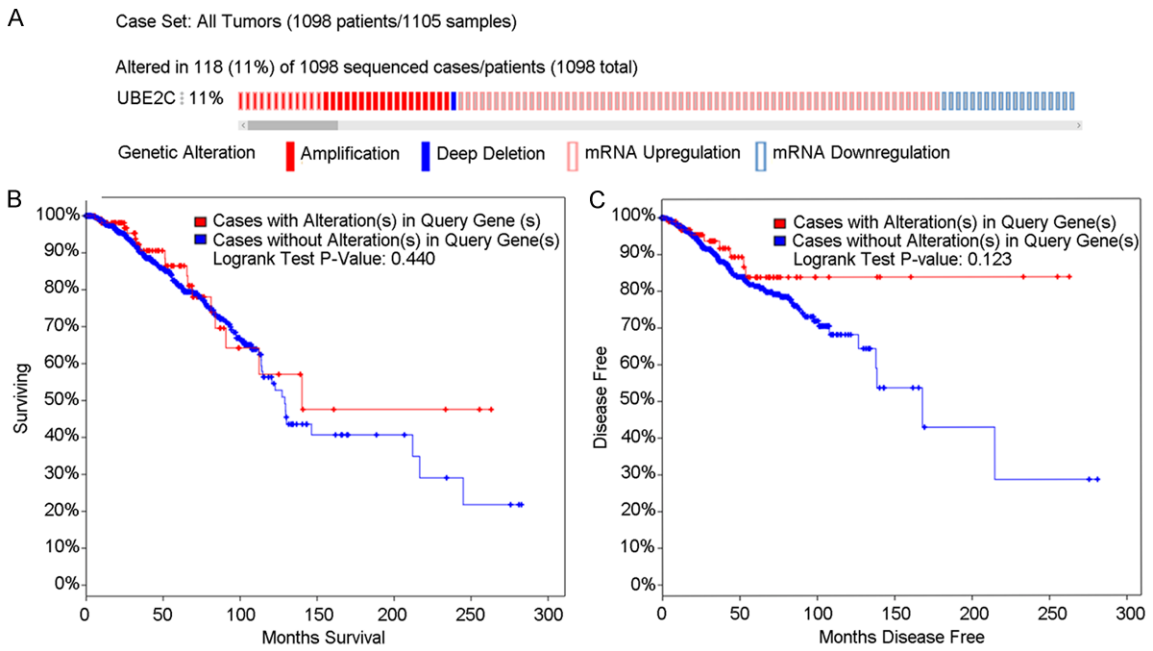


**Figure 13.** Alteration of BSG gene status in NPC and its influence on the survival of NPC patients. A. 5% patients (28/528 cases) exhibited BSG gene alteration, including 4 cases of deep deletion, 22 cases of mRNA up-regulation and 2 cases of mRNA down-regulation. B. No significant difference was found in the overall survival between NPC patients with and without BSG gene alteration. C. There was no significant difference in the disease-free survival between NPC patients with and without BSG gene alteration.

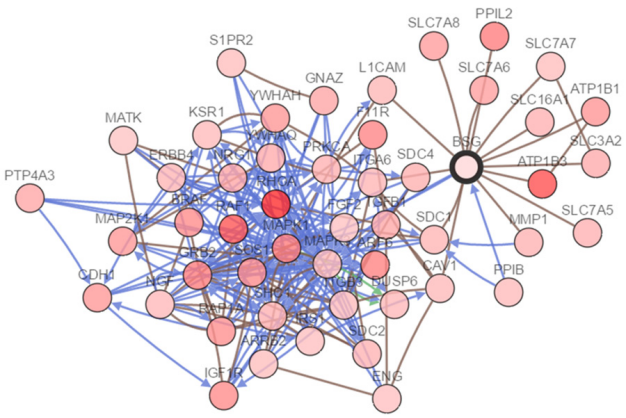


**Figure 14.** Gene co-expression network of BSG and neighboring genes. Gene co-expression network illustrated that BSG was closely associated with some genes including PPIB, L1CAM, SLC7AB, PPIL2, SLC7A7 and ATP1B3. A deeper color of the node represented a more significant interaction and the arrows between nodes reflected the direction of interaction.

the IHC results that BSG was remarkably overexpressed in NPC tissues rather than in non-cancer tissues. Moreover, the result of meta-analysis indicated that BSG expression was linked to lymph node metastasis and TNM stage of NPC. As for the relationship between BSG and TNM stage, sensitivity analysis suggested that the study of Du et al. caused great impact to the overall pooling results. The limited number of studies prevented us from exploring the sources of heterogeneity through subgroup analysis. We speculated that the samples and criteria of positive staining might generate the great heterogeneity between studies. Future study with larger sample size is necessary to validate the relationship between BSG expression and the development of NPC.

Having proved the overexpression of BSG in NPC and its role in the malignant progression of NPC, we investigated the prognostic significance of BSG in NPC through analyzing the data from Oncolnc. Although BSG expression was available in HNSCC instead of in NPC, BSG expression in HNSCC might reflect the prognostic significance of BSG in NPC. The results of the Kaplan Meier survival analysis revealed insignificant prognostic value of BSG in NPC. However, the study from Du et al. reported obvious differences between the five-year overall survival


**Figure 15.** Heat map of BSG-correlative genes. Samples of no value and HNSCC samples were labeled as 0 and 1, respectively. The expression of genes increased from blue to red. Results from co-expression analysis revealed that genes including HCN2, GZMM, RNF126, FGF22, POLRMT, PRSSL1, CDC34, AZU1, PRTN3, ELANE, CFD, SBNO2, STK11, C19orf20, MADCAM1, FSTL3, EFNA2, C19orf24, TMPR559 and UQCR11 were the top 20 genes significantly correlated with BSG.

rates of BSG low-expression and high-expression patients [32]. The contradictory results between our study and the study of Du et al. might be explained by the sample differences. The prognostic value of BSG in NPC needs further investigation in future study.

The results above indicated that BSG may have an effect on the initiation, invasion and metastatic ability of NPC. Several previous studies also focused on the carcinogenic role of BSG in NPC. Huang et al. reported that an increased level of BSG expression promoted lymph node metastasis of NPC [50], which was consistent with the results of our study. The NPC-SVM classifier including BSG proposed by Wang et al. suggested that NPC-SVM classifier effectively enhanced the accuracy of the classification of NPC patients [51], while BSG expression bore no statistical significance in identifying histological types of NPC in our study. This contradictory result may be due to the size of our sample. A larger cohort study with more varied NPC tissues was needed to further validate the outcome.

To achieve a comprehensive understanding of the molecular mechanism underlying BSG expression in NPC, we probed into the alteration of BSG gene status in NPC with data from cBioPortal. According to the results, mRNA up-regulation constituted the major type of alteration in 5% of all the sequenced NPC cases. The up-regulation of mRNA might lead to the BSG overexpression in NPC, as was confirmed in our study. We postulated that the deregulation of BSG mRNA might be attributed to the upstream molecules that regulated the expression of target mRNAs. Particularly, miRNA, a short non-coding RNA, was one of the representatives of the molecule mediating mRNA expression [52]. A growing number of studies have pointed that miRNAs played an essential role in the occurrence and progression of various human cancers through affecting the cellular differentiation, proliferation, metabolism, cell-cycle control, and apoptosis of cancer cells [53-55]. In hepatocellular carcinoma, miR-485-5p was found to suppress the growth of cancer cells via targeting BSG [56]. Similarly, miR-22 also exerted an inhibitory function on the proliferation, migration and invasion of hepatocellular carcinoma cells through down-regulating BSG expression [57]. These findings enlightened us that the down-expression of certain miRNAs might result in the overexpression of BSG in the development of NPC. It would beneficial to carry a profound research on the association between BSG and miRNAs in NPC. To figure out the driving force behind the alteration of BSG genes, we further explored the interactions between BSG and the neighboring genes with frequent alterations. The interaction network suggested that genes such as PPIB, L1CAM, SLC7AB, PPIL2, SLC7A7 and ATP1B3 directly interacted with BSG. We assumed that the interaction between BSG and these genes might interpret the alteration event of BSG gene in NPC. Additionally, we identified the BSG related genes in head and neck cancer by the Co-expression Analysis in TCGA. Top 20 genes including HCN2, GZMM, RNF126, FGF22, POLRMT, PRSSL1, CDC34, AZU1, PRTN3, ELANE, CFD, SBNO2, STK11, C19orf20, MADCAM1, FSTL3, EFNA2, C19orf24, TMPR559 and UQCR11 were screened out, which significantly correlated with BSG. Thus, we hypothesized that the top 20 genes and genes that had direct interaction with BSG in the network might exert an oncogenic influence on BSG to accelerate the initiation and malignant progression of NPC. It is worthwhile to validate the regulatory function of these genes in BSG in the future work.

In conclusion, the present study confirmed that the over-expressed BSG played an oncogenic role in the pathogenesis of NPC and was indicative of the advanced clinical stage. The potential diagnostic and therapeutic value of BSG in NPC awaits further investigation in future studies.

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## Disclosure of conflict of interest

None.

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