



Clinicopathological and prognostic significance of elevated BTF3 expression in gastric cancer

Pu Zhang¹, Chaochen Wang¹, Haiqing Wang¹, Xiuzhong Zhang^{2*}

¹Xuzhou Medical University, Department of General Surgery, Xuzhou Hospital of Traditional Chinese Medicine, Xuzhou, China

²Department of General Surgery, The Affiliated Hospital of Xuzhou Medical University, Xuzhou, China

ARTICLE INFO

Original paper

Article history:

Received: June 22, 2023

Accepted: November 03, 2023

Published: December 20, 2023

Keywords:

Gastric cancer (GC), Basic transcription factor 3 (BTF3), Clinicopathological parameters, Prognosis

ABSTRACT

The purpose of this paper was to explore the significance of basic transcription factor 3 (BTF3) in the process and clinicopathological parameters of gastric cancer (GC) patients. GC tissues were collected in our hospital to detect the mRNA expression of BTF3 by quantitative real-time polymerase chain reaction (Q-PCR). Western blot analysis was performed to detect the protein expression of BTF3. Kaplan-Meier method and Log-rank analysis were used to analyze the progression-free survival time and overall time of GC patients, while the Chi-square test was used to investigate the association between BTF3 and clinicopathological parameters of GC patients. SiRNA was designed to suppress the expression of BTF3. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay and transwell assay were conducted to determine the viability and invasion ability of GC cells. BTF3 was found abnormally up-regulated in GC tissues and cells and was related to the Grade, Lymph node metastasis and stage of GC patients, as well as the poor progression-free survival and overall survival of them. Besides, inhibition of BTF3 in GC cells could trigger the reduction of cell viability and invasion ability. Our results demonstrated that BTF3 played an important role in the process of GC and could be regarded as a new target for the diagnosis and therapy of GC.

Doi: <http://dx.doi.org/10.14715/cmb/2023.69.14.34>

Copyright: © 2023 by the C.M.B. Association. All rights reserved.

Introduction

Gastric cancer (GC), the fourth most common malignant tumor and the second leading cause of tumor-related death, seriously threatens human health (1,2). Although the early detection rate of GC is rising due to the improvement in gastroscopy and other diagnostic methods in recent years and certain progress has been made in combination therapies such as surgery, chemoradiotherapy and immunotherapy (3-6), most patients still have tumor metastasis due to the characteristics of GC, including high heterogeneity, insidious onset and rapid development, resulting in the undesirable prognosis of the GC patients (7). As the understanding of the etiology, pathology and molecular mechanism of the disease is gradually deepened (8), some progress has been achieved in the selection of treatment protocols for GC (9,10), but no significant effects have been obtained yet. Therefore, further elaborating the pathogenesis of GC, exploring new molecular targeted drugs and enhancing the patient's quality of life are still the key issues in the treatment of GC to be solved urgently.

Basic transcription factor 3 (BTF3) is a kind of protein separated from Hela cells at first, it forms stable complexes by interacting with RNA Pol II to participate in transcription initiation (11). BTF3, a molecular chaperone-like protein, can exert its biological function by means of interaction with different proteins, with the cytoplasm as the main site because the proportion of BTF3 in the cytoplasm is much higher than that in the nucleus (12,13). In

recent years, some studies have successively discovered that BTF3 is closely associated with multiple tumors. The earliest proteomic analysis on Burkitt lymphoma cell line BL60-2 indicated that BTF3 is an anti-apoptotic factor (14). Wang et al. (15) proposed that BTF3 overexpression is an early event of colorectal cancer and may become a molecular marker of early diagnosis of the disease. Moreover, the overexpression of BTF3 is also reported one after another in prostate cancer (16) lung cancer (17) and pancreatic cancer (18).

Given the fact that no documents have reported the association of BTF3 with GC, we thus aimed to investigate the significance of BTF3 in the process and clinicopathological parameters of GC patients.

Materials and Methods

Tissues collection

All samples were collected from patients diagnosed with GC in our hospital from April 2016 to December 2018. The patients did not receive anti-tumor treatment such as chemotherapy or radiotherapy before surgery. Follow-up of patients was performed according to the local clinical recommendation. The study was approved by the Ethics Committee of our hospital, and all patients signed informed consent before participating in the study.

Culture of cells

The human gastric mucosal epithelial cell line GES-1 and the human GC cell lines SGC-7901, BGC-823, and

* Corresponding author. Email: 13605229119@163.com

MGC80-3 were derived from the American model culture collection (ATCC) (Manassas, VA, USA). Cells were supported with 10% fetal conventional culture in Roswell Park Memorial Institute 1640 (RPMI 1640) medium (HyClone, South Logan, UT, USA) containing bovine serum and 1% penicillin-streptomycin. The condition of the constant temperature incubator was 37°C, containing 5% CO₂. When the cell fusion degree reached 80% to 90%, the cells were washed 3 times with phosphate-buffered saline (PBS), and finally digested with 0.25% trypsin containing 0.02% EDTA (ethylenediaminetetraacetic acid) and passaged (in a ratio of 1:3) or subjected to cell experiment.

Cell transfection and grouping

When the cell density in the 6-well plate reached 50%, transfection was performed according to the reagent manufacturer's instructions. Briefly, BTF3-siRNA/si-RNA NC and lipo3000 were separately mixed with serum-free medium and allowed to stand at room temperature for 5 minutes. Then, the above two mixtures were added together and allowed to stand at room temperature for 20 minutes. Then add 2 mL per well and change to normal fresh medium after 6 h. Cells under transfection were thus divided into two groups: NC group (transfected with si-RNA NC) and si-BTF3 group (transfected with BTF3-siRNA).

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay

When the cells were in the logarithmic growth phase, they were digested and counted, then 2000 cells per well, and 3 replicate wells per group were seeded in 96-well plates. The plate was placed in an incubator containing 5% CO₂ and cultured at 37°C for 4 days. From the 2nd day of plating, 10 µL of MTT (Sigma-Aldrich, St. Louis, MO, USA) at a concentration of 5 mg/mL was added to each well of a 96-well plate and placed in an incubator. After 4 h, the culture solution was discarded, 150 µL of DMSO (dimethylsulfoxide, dimethyl sulfoxide) was then added to each well to terminate the reaction, and the mixture was gently shaken and removed. The absorbance (A) was measured at 490 nm using a microplate reader. The A value of the cells was statistically analyzed and plotted.

Transwell assay

50 µL of Matrigel was added to the chamber of a 24-well plate, incubated for 30 min at 37°C in a 5% CO₂ incubator, then 600 µL of medium containing 20% fetal bovine serum (FBS) (Gibco, Rockville, MD, USA) was added to the chamber. Next, 20,000 cells of each group were added into the upper chamber, and incubated for 48 h in a 5% CO₂ incubator at 37°C. Then the chamber was taken out, cells in the upper were wiped off with a cotton swab, washed 3 times with PBS, followed by fixing in 4% paraformaldehyde for 30 min. After washing with PBS three times, cells were stained with Giemsa stain for 30 min. Then, five fields of view were randomly selected under an inverted microscope, and the number of cells was counted by ImageJ software (NIH, Bethesda, MD, USA).

Quantitative real-time polymerase chain reaction (q-PCR) analysis

RNA from tissues and cells was extracted with TRIzol reagent (Invitrogen, Carlsbad, CA, USA), and the concentration and purity of RNA were determined using

Nanodrop. After reverse transcription, 1 µg of cDNA was taken from each group, and 3 replicate wells were set for Q-PCR reaction. The expression level of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was regarded as an internal reference, and the value calculated by the 2^{-ΔΔCT} method was regarded as the relative gene expression level in each group.

Western blot analysis

After washing three times with cold PBS, the cells were added with the lysate (radioimmunoprecipitation assay (RIPA) and phenylmethylsulfonyl fluoride at 100:1) and lysed on ice for 30 min (Beyotime, Shanghai, China). Then, the mixture was collected and centrifuged at 15°C for 15 min, followed by transferring the supernatant to a new 1.5 mL tube. Later, the protein concentration in each group was determined by the bicinchoninic acid (BCA) Protein Quantitation Kit (Beyotime, Shanghai, China) and added to the sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) gel at 30 µg/well for electrophoresis and membrane transfer. After these, the polyvinylidene fluoride (PVDF) membranes (Millipore, Billerica, MA, USA) were placed in tris buffered saline-tween (TBST) + 5% skim milk powder for 2 h, and washed with TBST three times. Then, the PVDF membranes were separately incubated in the corresponding primary antibody overnight, and the secondary antibody was 37°C for 30 min, respectively. Finally, the PVDF membranes were placed on a chemi-luminescence imager for imaging.

Statistical analysis

Statistical analysis was performed using Statistical Product and Service Solutions (SPSS) 22.0 software (IBM, Armonk, NY, USA). Data were represented as mean ± SD (Standard Deviation). The t-test was used for analyzing measurement data. Differences between the two groups were analyzed by using the Student's t-test. Comparison between multiple groups was done using a One-way ANOVA test followed by Post Hoc Test (Least Significant Difference). Survival analysis was performed using the Kaplan-Meier method and Log-rank analysis. The association between BTF3 and clinicopathological parameters of GC patients was performed by Chi-square test. P<0.05 indicated that the difference between the groups is statistically significant.

Results

The expression level of BTF3 in gastric cancer tissues and cells

We first extracted RNA from GC tissues and adjacent normal tissues, then detected BTF3 expression using Q-PCR. The results showed that the expression level of BTF3 in GC tissues was significantly higher than that in normal tissues (Figure 1A, P<0.01). Consistent with this, in contrast to GES-1 cells, an increased expression level of BTF3 was also detected in GC cell lines, including SGC-7901, BGC-823, and MGC80-3 (Figure 1B), suggesting that the abnormal expression of BTF3 might be implicated in mediating the pathogenesis of GC.

Clinical significance of BTF3 in human gastric cancer

According to the expression level of BTF3 detected by Q-PCR, 72 cases of GC tissues were divided into BTF3

Table 1. BTF3 expression and clinical features of patients with GC.

Features	No.	BTF3 expression		p-value
		high	low	
Gender				0.6324
Male	39	25	14	
Female	33	19	14	
Age(years)				0.6287
< 60	38	23	15	
≥ 60	34	21	13	
HP				0.4762
Positive	37	21	16	
Negative	35	23	12	
Grade				0.0032**
Low	28	11	17	
Medium, High	44	33	11	
Lymph node metastasis				0.0030**
No	30	12	18	
Yes	42	32	10	
Stage				0.0143*
I - II	28	12	16	
III - IV	44	32	12	

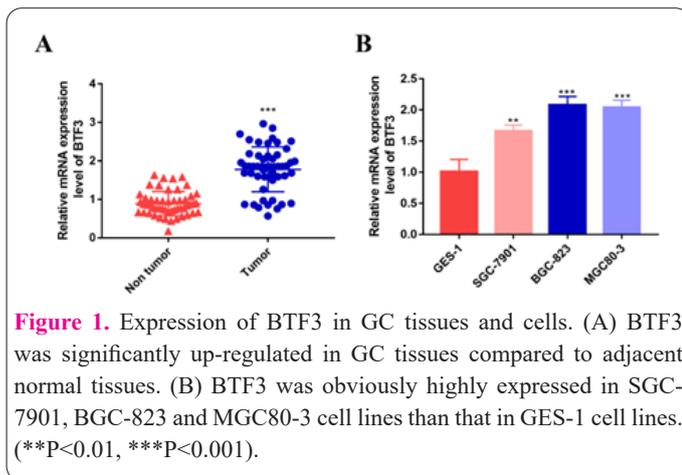


Figure 1. Expression of BTF3 in GC tissues and cells. (A) BTF3 was significantly up-regulated in GC tissues compared to adjacent normal tissues. (B) BTF3 was obviously highly expressed in SGC-7901, BGC-823 and MGC80-3 cell lines than that in GES-1 cell lines. (** $P < 0.01$, *** $P < 0.001$).

low expression group (28/72) and BTF3 high expression group (44/72) according to the mean expression. Subsequently, the correlation between BTF3 expression level and clinicopathological parameters was analyzed. As shown in Table 1, there was no correlation between the expression level of BTF3 and the patient's age ($P=0.6324$), gender ($P=0.6287$), and Helicobacter pylori (HP) positive ($P=0.4762$) in the included GC samples. However, it was related to the Grade ($P=0.0032$), Lymph node metastasis ($P=0.0030$) and stage ($P=0.0143$) of GC. In addition, 72 patients with GC were followed up in this study. The follow-up results in Figure 2A, B showed that patients with low expression of BTF3 had significantly improved progression-free survival ($P=0.0088$) and overall survival ($P=0.0457$) than those with high expression of BTF3. These results provided certain evidences that BTF3 was involved in the progression of GC.

Transfection of BTF3-siRNA suppressed BTF3 expression

Since BTF3 was found abnormally overexpressed in

GC tissues and cells, we thus aimed to knock down its expression to further investigate the effect of BTF3 on GC cells. Thereby, we selected BGC-823 and MGC80-3 cell lines for BTF3-siRNA transfection according to the Q-PCR results. As shown in Figure 3, compared to untreated cells, both the mRNA and protein expression levels of BTF3 were clearly suppressed in BGC-823 and MGC-80-3 cells after transfection of siRNA, respectively.

Inhibition of BTF3 impeded the viability and invasion ability of GC cells

After suppressing the expression of BTF3, the viability was evaluated in BGC-823 and MGC-80-3 cells by performing MTT assay. As shown in Figure 4A, 4B, in contrast to normal cells, an impeded cell viability in 72 h and 96 h triggered by knockdown of BTF3 was observed in BGC-823 and MGC-80-3 cells, respectively. Meanwhile, the invasion ability in GC cells determined by transwell assay showed that BGC-823 and MGC-80-3 cells with BTF3-siRNA treatment also exhibited restrained invasion ability than those normal cells without transfection, respectively (Figure 4C-4D).

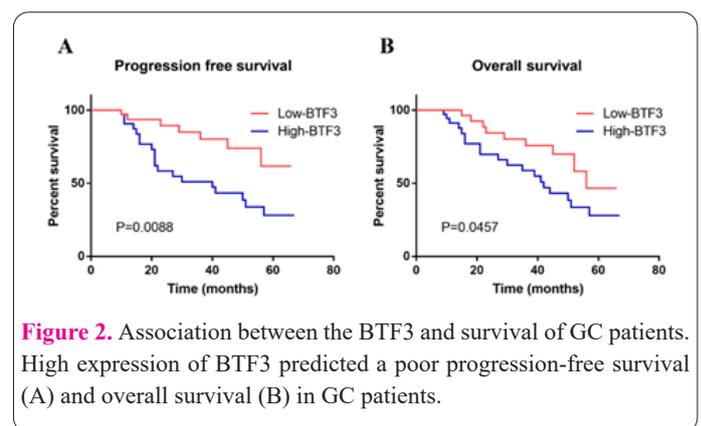


Figure 2. Association between the BTF3 and survival of GC patients. High expression of BTF3 predicted a poor progression-free survival (A) and overall survival (B) in GC patients.

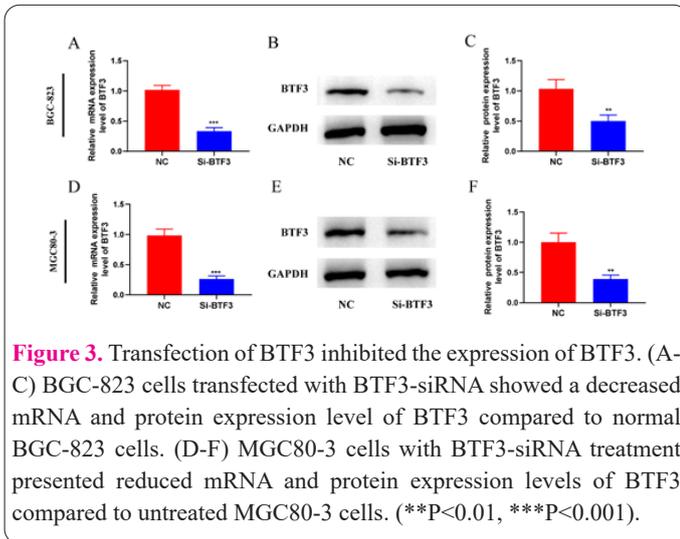


Figure 3. Transfection of BTF3 inhibited the expression of BTF3. (A-C) BGC-823 cells transfected with BTF3-siRNA showed a decreased mRNA and protein expression level of BTF3 compared to normal BGC-823 cells. (D-F) MGC80-3 cells with BTF3-siRNA treatment presented reduced mRNA and protein expression levels of BTF3 compared to untreated MGC80-3 cells. (**P<0.01, ***P<0.001).

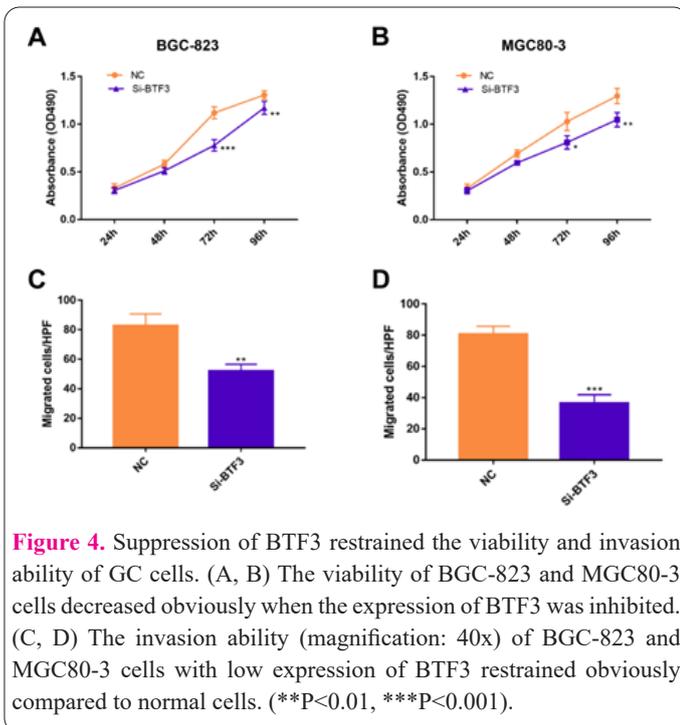


Figure 4. Suppression of BTF3 restrained the viability and invasion ability of GC cells. (A, B) The viability of BGC-823 and MGC80-3 cells decreased obviously when the expression of BTF3 was inhibited. (C, D) The invasion ability (magnification: 40x) of BGC-823 and MGC80-3 cells with low expression of BTF3 restrained obviously compared to normal cells. (**P<0.01, ***P<0.001).

Discussion

Currently, surgical procedures are still the preferred treatment protocols of GC patients in the early stage, but majorities of them lost the opportunity due to high invasiveness and lack of predictive biomarkers with strong sensitivity and specificity of GC (19,20). Besides, most of the GC patients have proceeded to the intermediate and advanced stages when definitely diagnosed, leading to poor prognosis and a 5-year overall survival rate of about 20-35% (21,22). As for the GC patients who are unable to receive surgical resection and have distant metastasis, the median survival time is only 10-14 months (23). So far, there have been only a few targeted drugs at the molecular level for GC treatment, which have limited efficacy and inconspicuous effects on improving prognosis (24). Therefore, it is essential to further elucidate the occurrence and development mechanisms of GC for developing new targeted drugs and promoting the clinical treatment of GC patients.

Existing functional analyses have manifested that BTF3 played crucial roles in the occurrence and development of

various tumors, including pancreatic cancer (18), osteosarcoma cancer (25), colorectal cancer (15) and prostate cancer (PCa) (16). For instance, researchers have found that inhibition of BTF3 in PCa cells could reduce the stem cell-like phenotype and expression of stem cell-associated markers, further affecting the malignant biological behaviors of PCa such as proliferation and migration (16). In addition, Zhang et al. (26) revealed that upregulation of BTF3 affected the proliferation, apoptosis, and cell cycle regulation in hypopharyngeal squamous cell carcinoma. Moreover, Ding et al. (27) demonstrated that inhibition of BTF3 sensitized luminal breast cancer cells to PI3K α inhibition through the transcriptional regulation of ER α .

Although a large number of reports indicated that BTF3 was widely involved in the development and progression of tumors and played an important role, whether BTF3 was related to GC remains unknown. In this paper, we initially compared the expression levels of BTF3 in tumor tissues and adjacent normal tissues of GC patients. Consistent with other literatures (25,28), BTF3 was highly expressed in GC tissues. Subsequently, we verified the abnormal expression pattern of BTF3 in GC cell lines, indicating the important molecular role of BTF3 in the development of GC.

The prognosis of a tumor patient depends on the characteristics of the tumor at a large extent, including tumor size, lymph node metastasis, distant metastasis, etc. (23,24,29). To this end, we analyzed the clinicopathological features of BTF3 and GC patients in order to better explore its potential biological significance. We found a clear correlation between BTF3 and the Grade, Lymph node metastasis and stage of GC, whereas no association was observed between BTF3 and GC patient's age, gender, and HP. In addition, we analyzed the follow-up results and found that GC patients with high expression of BTF3 had shorter progression-free survival and overall survival than those with low expression of BTF3. Based on these findings, we later performed MTT and transwell assay *in vitro*. Not surprisingly, we found that both the cell viability and invasion ability in the BTF3-siRNA group exhibited different levels of decline compared to the NC group, implicating that inhibition of BTF3 in GC cells impaired their viability and invasion ability.

Conclusions

In this paper, we demonstrated that BTF3 was abnormally highly expressed in GC tissues and cells, and was clearly related to the Grade, Lymph node metastasis and stage of GC patients, as well as the poor progression-free survival and overall survival of them. In addition, we illustrated that suppression of BTF3 could trigger the impairment of the viability and invasion ability of GC cells, suggesting that BTF3 could be regarded as a new target for the diagnosis and therapy of GC.

Conflict of Interest

The authors declared no conflict of interest.

References

- Gasenko E, Isajevs S, Camargo MC, et al. Clinicopathological characteristics of Epstein-Barr virus-positive gastric cancer in Latvia. *Eur J Gastroen Hepat* 2019; 31(11): 1328-1333.
- Sun Y, Hu C, Li D. MiR-17-5p Inhibits the Proliferation and

- Metastasis of Gastric Cancer Cells by Targeting PTEN Protein. *Altern Ther Health M* 2022; 28(8): 23-29.
3. Xiao M, Zhang Z, Liao G, Liu M, Chen X. Analysis of the Value of Helicobacter pylori Test in Combination with the Determination of Plasma Propepsin and Gastrin 17 in Screening the Precancerous Status of Gastric Cancer. *Cell Mol Biol* 2022; 68(8): 110-115.
 4. Fard SS, Saliminejad K, Sotoudeh M, et al. The Correlation between EGFR and Androgen Receptor Pathways: A Novel Potential Prognostic Marker in Gastric Cancer. *Anti-Cancer Agent Me* 2019; 19(17): 2097-2107.
 5. Morales-Borges RH, Gonzalez MJ, Duconge J, Minich DM. N-Acetyl Cysteine and Glutathione in Health and Cancer-Pharmacogenomics, Research, and Clinical Practice: Hypothesis and Review. *Altern Ther Health M* 2022; 28(7): 169-177.
 6. Wang G, Jiang X. Effect of PRECEDE-PROCEED-Model-Based Health Education on the Life Quality and 5-HTT Gene Expression of Patients with Gastric Cancer after Surgery. *Cell Mol Biol* 2021; 67(6): 242-248.
 7. Koh JS, Joo MK, Park JJ, et al. Characteristics of proximal early gastric cancer differentiating distal early gastric cancer. *Plos One* 2019; 14(9): e223284.
 8. Kim IH. Current status of adjuvant chemotherapy for gastric cancer. *World J Gastro Oncol* 2019; 11(9): 679-685.
 9. Kasurinen A, Gramolelli S, Hagstrom J, et al. High tissue MMP14 expression predicts worse survival in gastric cancer, particularly with a low PROX1. *Cancer Med-Us* 2019; 8(16): 6995-7005.
 10. Yamamoto M, Kurokawa Y, Kobayashi N, et al. Prognostic Value of the Combined Index of Plasma Fibrinogen and the Neutrophil-Lymphocyte Ratio in Gastric Cancer. *World J Surg* 2020; 44(1): 207-212.
 11. Zheng XM, Black D, Chambon P, Egly JM. Sequencing and expression of complementary DNA for the general transcription factor BTF3. *Nature* 1990; 344(6266): 556-559.
 12. Kanno M, Chalut C, Egly JM. Genomic structure of the putative BTF3 transcription factor. *Gene* 1992; 117(2): 219-228.
 13. Parthun MR, Mangus DA, Jaehning JA. The EGD1 product, a yeast homolog of human BTF3, may be involved in GAL4 DNA binding. *Mol Cell Biol* 1992; 12(12): 5683-5689.
 14. Thakur D, Saxena R, Singh V, et al. Human beta casein fragment (54-59) modulates M. bovis BCG survival and basic transcription factor 3 (BTF3) expression in THP-1 cell line. *Plos One* 2012; 7(9): e45905.
 15. Wang CJ, Franbergh-Karlson H, Wang DW, Arbman G, Zhang H, Sun XF. Clinicopathological significance of BTF3 expression in colorectal cancer. *Tumour Biol* 2013; 34(4): 2141-2146.
 16. Symes AJ, Eilertsen M, Millar M, et al. Quantitative analysis of BTF3, HINT1, NDRG1 and ODC1 protein over-expression in human prostate cancer tissue. *Plos One* 2013; 8(12): e84295.
 17. Jeon YJ, Bang W, Cho JH, et al. Kahweol induces apoptosis by suppressing BTF3 expression through the ERK signaling pathway in non-small cell lung cancer cells. *Int J Oncol* 2016; 49(6): 2294-2302.
 18. Kusumawidjaja G, Kayed H, Giese N, et al. Basic transcription factor 3 (BTF3) regulates transcription of tumor-associated genes in pancreatic cancer cells. *Cancer Biol Ther* 2007; 6(3): 367-376.
 19. Yu L, Zhou GQ, Li DC. MiR-136 triggers apoptosis in human gastric cancer cells by targeting AEG-1 and BCL2. *Eur Rev Med Pharmacol* 2018; 22(21): 7251-7256.
 20. Ding Z, Jiang L, Zhang K, Huang R. Short- and long-term outcomes of conversion in laparoscopic gastrectomy for gastric cancer. *J Buon* 2018; 23(4): 1004-1012.
 21. Monig SP, Bonino MA. Delay in initiation adjuvant S-1 monotherapy for gastric cancer: important prognostic factor. *Transl Gastroent Hep* 2019; 4: 61.
 22. Joubert KS, George BP, Razlog R, Abrahamse H. A Review of Preclinical Research on the Effects of Photodynamic Therapy and Homeopathic Medicine on Cancer Cells. *Altern Ther Health M* 2021; 27(6): 40-50.
 23. Zubarayev M, Min EK, Son T. Clinical and molecular prognostic markers of survival after surgery for gastric cancer: tumor-node-metastasis staging system and beyond. *Transl Gastroent Hep* 2019; 4: 59.
 24. Hsieh HL, Tsai MM. Tumor progression-dependent angiogenesis in gastric cancer and its potential application. *World J Gastro Oncol* 2019; 11(9): 686-704.
 25. Liu Q, Jiang L, Wang W, Jiang T. BTF3 Silencing Inhibits the Proliferation of Osteosarcoma Cells. *J Cancer* 2019; 10(8): 1855-1861.
 26. Zhang Y, Gross N, Li Z, et al. Upregulation of BTF3 affects the proliferation, apoptosis, and cell cycle regulation in hypopharyngeal squamous cell carcinoma. *Biomed Pharmacother* 2019; 118: 109211.
 27. Ding J, Wang X, Zhang Y, et al. Inhibition of BTF3 sensitizes luminal breast cancer cells to PI3Kalpha inhibition through the transcriptional regulation of ERalpha. *Cancer Lett* 2019; 440-441: 54-63.
 28. Hu J, Sun F, Chen W, et al. BTF3 sustains cancer stem-like phenotype of prostate cancer via stabilization of BMI1. *J Exp Clin Canc Res* 2019; 38(1): 227.
 29. Ullrich CI, Aloni R, Saeed M, Ullrich W, Efferth T. Comparison between tumors in plants and human beings: Mechanisms of tumor development and therapy with secondary plant metabolites. *Phytomedicine* 2019; 64: 153081.