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BTF3-Promoter Based Screening of Anti- Human Breast Cancer Compounds

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Abstract

Basic Transcription Factor 3 (BTF3) is a Transcription factor known to differentially express in different forms of cancer; where in gastric cancer, silencing of BTF3 induced apoptosis. Previously, we reported Human β casein fragment 54-59 (NS) down modulates BTF3 expression in Human THP-1 cells. In the present study, we developed an *in vitro* model targeting BTF3 Promoter for screening of compounds down regulating BTF3 and to study the impact of BTF3 down regulation on apoptosis in MCF-7 cell line. We further confirmed the efficacy of NS to down regulate BTF3 in MCF-7 cells through western blotting. NS and its analogs AN1, AN2 and AN3 were screened on BTF3 promoter-reporter construct during transient transfection in MCF-7 cell line, where the expression of reporter was found to be down regulated upon treatment. This study was further confirmed by immunoblotting which produced similar results in MCF-7 cells. To determine the biological function on suppressing BTF3 expression by NS and analogs, cell viability assay and Annexin-V-FITC staining were performed, the results clearly demonstrated an increase in apoptosis on BTF3 down regulation in MCF-7 cells. In conclusion, BTF3 expression is a crucial biomarker in breast cancer therapy and our model can be an asset for fast screening of compounds modulating BTF3 expression in different cancer forms, where it is found to be overexpressed.

Keywords: BTF3; β-casein; Analogs; Luciferase; Down-regulation; MCF-7; Apoptosis

Introduction

Breast cancer is one of the most frequently diagnosed forms of metastasis in women worldwide and ranks second after lung cancer [1]. Studies performed in year 2014 revealed more than 3.1 million breast cancer cases in U.S women [2]. Nearly 458,000 deaths per year from breast cancer were reported making it the most common cause of female cancer death in both the developed and developing world [3]. BTF3 (Basic Transcription Factor 3), is a 27 kDa protein, first studied in HeLa cell where it was discovered as a general transcription factor and its role was defined in transcription initiation by RNA polymerase B from proximal promoter elements such as TATA box and CAAT box sequences [4,5]. Apart from its role in Transcription, later studies revealed that BTF3 is also involved in protein regulation during translation and is therefore also known as Nascent- polypeptide associated complex β-subunit (βNAC) [6]. BTF3 has been studied for its importance embryonic development in mouse embryos, where mice homozygous for mutant allele died after 6.5 embryonic day post implantation [7]. Involvement of BTF3 in alveolar growth also has been reported in Mice in which expression of BTF3 was found strongly overexpressed [8]. Icd-1, homolog of BTF3 (βNAC) in *C.elegans* is reported as an important factor required in apoptosis, where icd-1 over expression leads to inhibition of apoptosis in cells that are normally programmed to die [9]. BTF3 is known to be differentially expressed in different types of Cancer and its expression has been associated with cell cycle regulation and apoptosis. In Burkitt's lymphoma and Jurkat T cells, BTF3 participates in anti-IgM antibody-mediated apoptosis and its elimination was observed within 6 hours after apoptosis induction [9,10]. BTF3 was found overexpressed in colorectal cancer, gliobastomas, and hepatocellular carcinomas [11-13]. In Human gastric cancer tissues, BTF3 expression was found significantly higher than control tissues and on silencing BTF3 there was significant increase in apoptosis in SGC7901 cells [14]. The transcriptional targets of BTF3 were studied in pancreatic cancer, where silencing of BTF3 leads to the reduced expression of EPBH2 (ephrin receptor B2), HPSE2 (Heparanase 2), ABL2 (V ABL ablation murine leukemia viral oncogene homolog 2) and ATM (ataxia-telangectasia mutated gene) genes well known for their tumor promoting functions [11]. In a study performed to measure gene expression profiles of Taiwanese breast cancer patients, when compared with U.K datasets, BTF3 was found differentially expressed in both Taiwanese and U.K patients [15]. BTF3 gene expression could be efficiently measured by promoter activity analysis in response to external stimuli, thus a promoter – reporter construct is made where the expression of reporter gene is regulated by BTF3 promoter. Firefly Luciferase gene as reporter is highly sensitive and is very efficient to measure translated reporter gene product in cell lines. Immunostimulatory peptides are being used as a viable adjunct to established therapeutic modalities in treatment of cancer and microbial infections nowadays because ability potentiate the immune system of the host [16,17]. Human β casein corresponding to fragment 54-59 (NS) havingVal-Glu-Pro-Ile-Pro-tyr amino acid sequence is capable of exerting strong immunostimulatory effects including resistance to certain bacterial infections [17]. In our previous work, using NS we have shown the clearance of M.bovisBCG from THP-1 cell line *in vitro*. Using proteomic approach we found the Basic Transcription Factor, BTF3, was down regulated in THP-1 cell line after peptide treatment [18]. In this study, we identified BTF3 promoter *in silico* and developed a fast screening *in vitro* model targeting BTF3 to screen anti-cancer compound in MCF-7, Human breast cancer cells [19-22]. Further, using the promoter driven reporter gene model by transient transfection in MCF-7 cells, NS and its analogs AN1(Val-Glu-D-Pro-Ile-Pro-Tyr), AN2 (Ala-D-Glu-Pro-Ile-Pro-Tyr)andAN3 (Val-D-Glu-Gly-Ile-Pro-Tyr) have been tested on this BTF3 promoter based assay system so as to establish its applicability as a rapid and effective means to evaluate BTF3 down modulation inducing apoptosis in MCF-7 cells [23].

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Material and Methods

Prediction of Core Promoter, Transcription Factor Binding Sites in the Upstream Region to BTF3 A 2.5-kb sequence upstream from the transcription start site of the Human BTF3 gene was analyzed using genomic analysis software (Gene2Promoter; Genomatix).Further confirmation of the sequence identified was done by promoter prediction software(Promoter Inspector; Genomatix, Munich, Germany), which predicts the genomic context of eukaryotic polymerase II promoter regions with high specificity in mammalian genomic sequences, based on equivalence classes of International Union of Pure and Applied Chemistry words. The identified region was marked as a true positive if a transcription start site was located within or up to 200 bp downstream of the predicted putative promoter sequence. Eukaryotic transcription factor binding sites were identified with transcription factor analysis software (MatInspector; Genomatix) [24]. The putative BTF3 promoter sequence was compared for homology with its orthologous sequences using T-coffee multiple sequence alignment program (http://www. ebi. ac.uk/Tools/t-coffee/) [23-26].

Cloning of the BTF3 Promoter and Validation Luciferase Reporter Constructs Amongst different promoter sequences given by the software, a putative promoter segment from -1027 to +103 was selected, as it contains maximum transcription binding sites. Putative promoter region from -1027 to +103 was amplified usingForward Primer: 5' AACTCGAGACGCGTTGTCTCTCCAAACACAGCAGG 3' and Reverse Primer: 5' ATAAGCTTGGTGGAGGTTGAGACAGCGTCGCCT 3'from Human genomic DNA and was cloned in PGL4.17 (Promega, U.S.A), upstream to Luciferase gene. 2×106 THP-1 cells were suspended in incomplete culture medium (without serum and antibiotics) mixed with 20 µg plasmid DNA (reporter construct) in a total volume of 0.4 ml, transfered to cuvette (4 mm gap) and electroporated at 290 LV using the electroporator (BTX). After 2 hrs of incubation in incomplete medium the cells were replenished with 20% FBS containing medium and 30 nM PMA for differentiation and incubated for 48 h. After incubation THP-1 cells were trypsinized and washed twice with 1x PBS and the luminescence in lysates of harvested cells was measured 48 hours after transfection with the Oneglow luciferase reporter assay protocol as recommended by Promega. Percentage change in RLU/sec was calculated by comparing the RLU of reporter construct transfected cells with respect to RLU of un-transfected and vector transfected control.

THP-1 cells were transfected with BTF3 promoter-reporter construct and differentiated as described earlier. PMA Differentiated THP-1 cells were given treatment with Human β-casein fragment (NS) at 20 µM concentration (NS) for 24 h. Cells were harvested 24 hours post treatment and luciferase activity was analysed. Percent change in RLU/sec was calculated by comparing RLU of NS treated cells with respect to RLU of control without treatment.

Synthesis of peptides

Synthesis of human β-casein fragment and its analogs were carried out by step wise chain elongation using solution phase method of peptide synthesis. Boc group [16,19] and benzyl group were employed for the protection of α-amino and carboxyl functions respectively except Val and Alaat position 1 where Z group [16 ,20] was used for α-amino protection in order to achieve simultaneous removal of Z and Benzyl group by catalytic hydrogenation [16,21] in the last step of the synthesis. DCC/HOBt was used as a coupling reagent for preparation of peptide bond. Boc group was replaced by treating the peptide derivatives with HCl/dioxane in the presence of thioanisole. Z and benzyl groups were removed by catalytic hydrogenation.

Cell Culture and Transfection

THP-1 and MCF-7 cell lines obtained from in-house cell and tissue culture facility of Central Drug Research Institute were cultured routinely inRPMI 1640 complete media and DMEMmedia (Sigma), supplemented with 10% FBS (GIBCO Invitrogen corporation), 100 U/mL penicillin and 100 µg/mL streptomycin. Cultures were maintained at 37°C in a humidified atmosphere with 5% CO_2 saturation. During transfection 2×106MCF-7 cells were suspended in DMEM media mixed with 25 µg plasmid DNA in a total volume of 0.4 ml, transfered to cuvette (4 mm gap) and electroporated at 190 LV using the electroporator (BTX).

Screening of NS and its analogs on reporter construct

MCF-7 cells transfected with reporter construct were treated with Human β casein fragment (54-59), NS and its Analogs AN1, AN2 andAN3 at a concentration of 20 µM. After 48 hours of treatment the cells were harvested and using Oneglow Luciferase Substrate (Pomega U.S.A) cells were lysed and the luciferase activity was measured in cell lysate. Down regulation of BTF3 expression was checked by comparing the RLU of treated cells with respect to RLU of untreated control.

Western blotting

Seeding of 0.5×106 /well MCF-7 cells was done in 12-well plate and treatment with NS, AN1, AN2 and AN3 was given after 24 hours of seeding at 20 µM concentration respectively,. Cells were harvested after 48 hours of treatment and washed with 1x PBS twice. The pellet was resuspended in 1x Passive Lysis Buffer (Promega, U.S.A) and sonicated in ice at 10 amplitude giving 4 pulses of 10 seconds on and 5 second off. After sonicated the samples were centrifuged at 13000×g (4°C for 10 min) and using Bradford protein assay, protein concentration was determined in each sample. 12% SDS Polyacrylamide gel was prepared to separate total protein after running the gel, the protein was transferred to PVDF membrane (Millipore), Membranes were treated for 1 hour at room temperature in 1x PBS containing 5% bovine serum albumin (Sigma) and 0.1% Tween-20. Blots were then overnight incubated with anti-BTF3 antibodies (abcam, UK) at 4°C, followed by washing and 2 hour incubation with peroxidase conjugated secondary antibody. Immunoreactive bands were visualised using enhanced chemiluminescence (ECL, Pierce-Thermo ScientifiUSA). Blots were then stripped and reprobed with an anti α- Tubulin monoclonal antibody (Sigma) to assess the equal loading of all protein samples.

Cell Viability Assay

Viability assay in MCF-7 cells was performed using 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Briefly 1×104cells were treated with NS and analogs AN1, AN2 and AN3 at 20 µM concentration. After 48 hrs of treatment in 96-well plate, 20 µl of MTT solution (2.5 mg/ml) was added to each well and incubated for 4hrs. Media was removed and 200 µl DMSO was added to each well and incubated for 30 min at room temperature with shaking to dissolve the Formazon crystal completely. The colorimetric assay is examined at an absorbance of 570 nm and 690 nm in microplate reader (Biotek instruments, USA).

Annexin-V-FITC Assay

The induction of early and late apoptosis on down modulation of BTF3 expression by NS, AN1, AN2 and AN3 was further studied via an

Annexin-V-FITC/PI staining assay. Briefly, MCF7 cells (0.5 × 106/well) were plated in a 6-well plate in duplicates and treated with NS, AN1, AN2 and AN3 at 20 μM concentration for 48 hours. The adherent and suspended cells were harvested and washed twice with PBS. Then, the MCF7 cells were then re-suspended in Annexin-V binding buffer (BD Biosciences, San Jose, CA, USA) and stained with Annexin-V-FITC (BD) and PI (Sigma) according to the manufacturer's instructions. The fluorescent intensity of MCF7 cells was then examined using flow cytometry (BD FACSCanto™ II) and quadrant statistics for necrotic and apoptotic cell populations. Detection of early and late apoptosis was done by Annexin-V, while PI was responsible for the detection of late apoptosis and necrosis.

Stastical Analysis

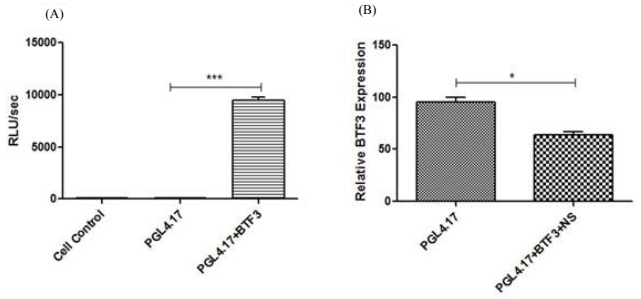
Each test was performed in triplicate. Statistical analysis was performed by GraphPad prism (version 4.0 Graphpad software Inc, San Diego, CA, USA). Analyses of variance were performed using the one-way ANOVA test followed by Bonferroni'st test. The results were expressed as the mean value ± SD.

Results

Identification of the Putative CTRP5 Promoter and its functional validation

We evaluated the upstream region of Human BTF3 gene to find potential promoter region, the genomic sequence was retrieved from NCBI database (NC000005.10).The promoter region thus identified using *in silico* tools as mentioned in materials and methods section is transiently transfected in THP-1 cells, 24 hours post transfection cells were harvested and luciferase value measured was compared with the vector and cell control, where we find a significant increase in luciferase expression of cells expressing BTF3 promoter driven luciferase gene reflecting the activity of BTF3 promoter identified *in silico* (Figure 1A).

Since NS is known to suppress endogenous BTF3 expression in THP-1 cell line, we used NS to validate the activity of the promoter in THP-1 cell line upon transfection with BTF3 promoter –reporter construct. THP-1 cells on transfection with BTF3 promoter-reporter construct were treated with NS, after 24 hours post transfection there was a significant suppression in Luciferase expression when compared to control without NS treatment. The reduced activity of BTF3 promoter on treatment with NS in THP-1 cell line is similar to NS treatment effect on endogenous BTF3, validating this model *in vitro*.



(A) THP-1 cells were transfected with BTF3-reporter construct and the expression was checked after 24 hours, change in expression was taken in terms of RLU/sec.

(B) Relative BTF3 expression was taken as percent change in Luciferase expression on transfection of THP-1 cells with BTF3 promoter reporter construct following NS treatment for 24 hours.

Figure 1: Analysis of BTF3 promoter driven luciferase expression in THP-1 cell line

Human β casein fragment (54-59), NS down regulates expression of BTF3 in MCF-7 cell line.

Human β casein fragment (54-59), NS is known to down regulate BTF3 expression in THP-1 cell line, leading to Mycobacterial clearance [17]. In this study the efficacy of NS to down regulate the differentially expressed transcription factor, BTF3 in Human Breast cancer was studied by treating MCF-7, Human Breast cancer cell line with NS (20 μM) at two different time points of 24 hours and 48 hours, following western blotting using anti-BTF3 antibody. A significant down regulation of BTF3 expression was found at 48 hours of treatment in MCF-7 cell line (Figure 1), therefore showing the potential of NS to down regulate BTF3 expression in MCF-7 cell line.

BTF3 Promoter driven Reporter activity is repressed in MCF-7 cell line on treatment with Human β casein fragment (54-59) and its analogs

NS was found efficient to down regulate endogenous BTF3 in MCF-7 cells at 48 hours, therefore NS and its analogs AN1, AN2 and AN3 were screened on the BTF3 promoter-reporter model in MCF-7 cell line. The BTF3 promoter-reporter construct expressing firefly luciferase gene was transfected in MCF-7 cell through electroporation and treatment of NS, AN1, AN2 and AN3 was given for 48 hours. Percent change in RLU/sec was recorded as the modulation in expression of reporter gene under the impact of BTF3 promoter on treatment with NS and its analogs. Our data demonstrated that NS and its analogs (AN1, AN2 and AN3) were able to down regulate the expression of reporter gene, when compared to control MCF-7 cells without peptide treatment by 31.7%, 57.8%, 31.2% and 62.3% respectively (Figure 2).

These results demonstrated that similar to NS, its analogs are also capable of modulating reporter gene expression that was under the control of BTF3 promoter.

NS and analogs downregulates BTF3 in MCF-7 cell line

Western blotting was performed to check the downregulation of BTF3 on treatment with NS and analogs AN1, AN2 and AN3 for 48 hours at 20 μM concentration using anti-BTF3 antibody. BTF3 was found down modulated by 33.1%, 57.7%, 28.8% and 35.75% respectively, on treatment with NS, AN1, AN2 and AN3 respectively when compared to control without treatment (Figure 3).

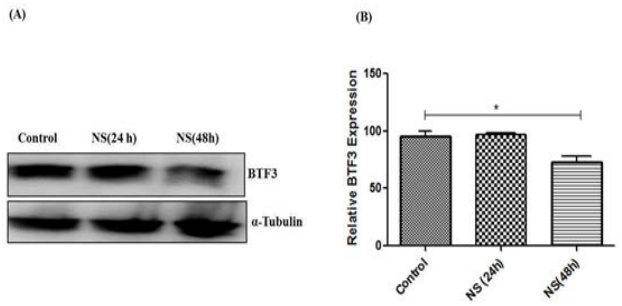
These results show that the peptides NS and its analogs are modulating the expression of endogenous BTF3 expression with respect to the activity of Luciferase reporter gene upon treatment. Therefore, our model has potential to screen compounds that influence BTF3 expression in different cancer cell lines (where its overexpression is studied) by analyzing expression of reporter gene regulated by regulatory regions of BTF3. This model helps for fast screening of compounds.

BTF3 downregulation is associated with cell death and apoptosis in MCF-7 cell line.

We next performed experiments to determine whether BTF3 down regulation modulates cell death in MCF-7 cell line, as reported ingastric cancer cases, where silencing of BTF3 lead to increase in cell death. Cell viability test was performed by MTT assay, which demonstrated cell death in MCF-7 cells on treatment with NS, AN1, AN2 and AN3 (20 μM) for 48 hours respectively (Figure 4a), Confirmation of

apoptosis following MTT assay was performed by Annexin/PI staining employing flow cytometry, where Annexin-V, a phospholipid binding protein has high affinity for externalized Phosphotidylserine on the cell membrane and therefore serves as a marker for early apoptotic cells. MCF-7 cells on treatment with NS and its analogs (20 µM) for 48 hours were checked for apoptosis using Annexin/PI staining by flow cytometry revealed 14.5%, 18.04%, 13.34% and 21.11% apoptosis in NS, AN1, AN2 and AN3 treated samples respectively, when compared to control sample with 2-3% apoptosis rate (Figure 4b).

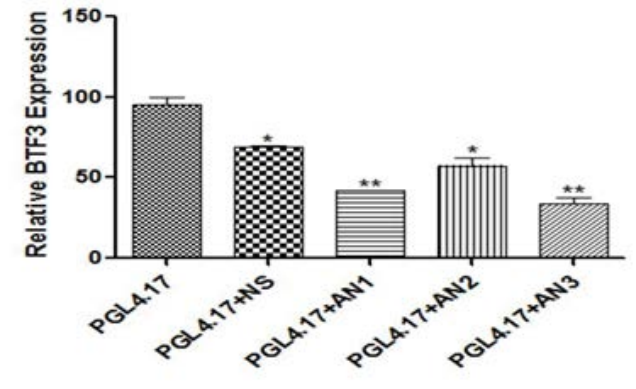
The results clearly suggests that the suppression of BTF3 expression in MCF-7 cells by NS and its analogs leads to cell death and apoptosis and therefore, screening of compounds using our BTF3 promoter based model could verify compounds capable of inducing BTF3 mediated apoptosis in MCF-7 cells and strongly demonstrates the therapeutic role of Human β casein peptide and its analogs in Breast cancer.



(A) MCF-7 cells were treated with β casein (54-59) peptide (NS-20µM). Change inBTF3 expression was shown by western blotting using anti-BTF3 antibody, loading was done as-**Lane 1:** control**, Lane 2**: Expression after 24 hr and **Lane 3**: Expression after 48 hr. Lower panel shows expression of α-Tubulin which was detected using anti-Tubulin monoclonal antibody to check equal loading of protein.

(B) Densitometric studies showing fold change in expression of BTF3 during treated conditions is presented in respect to control. Results are shown as mean fold change ± SEM (n=3) and change was considered significant at $P \leq 0.05$ (*).

Figure 2: Effect of NS on BTF3 Expression.



(A) MCF-7 cells were transfected with BTF3 promoter-reporter construct, following treament with NS and analogs AN1, AN2 andAN3 at 20µm concentration. RLU was measured after 48 hours of treatment. Decrease in RLU reading after 48 hours is taken as the effect of NS and analogs on BTF3 promoter based reporter expression. The values and error bars represent average and standard deviations of three independent set of experiments. Student T test was performed to find out significant difference between control and treated conditions and comparisons were considered significantly different at $P \leq 0.0005$ (***)

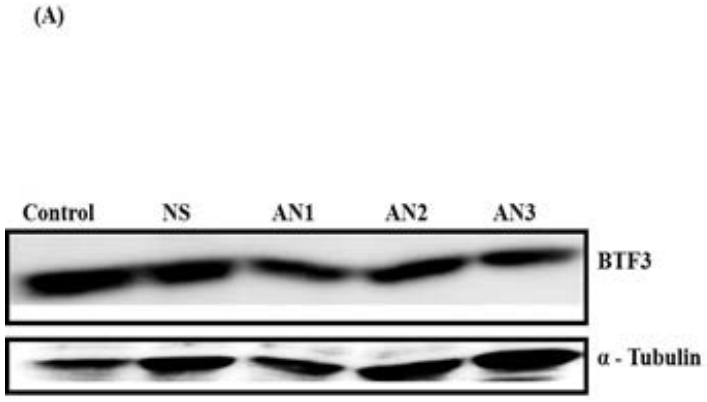
Figure 3: Effect of NS and analogs on BTF3 promoter-reporter expression.

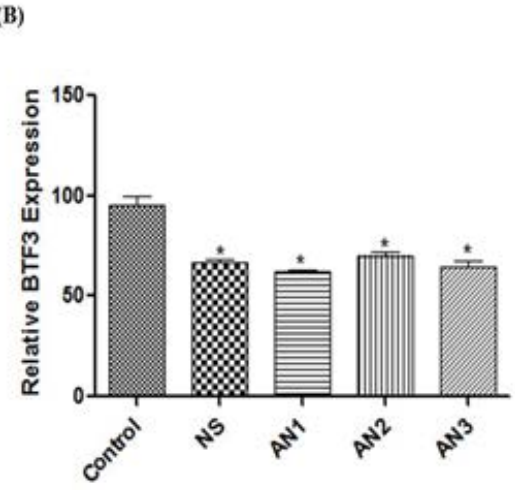
Discussion

RNA Polymerase B transcription factor 3, also known as Basic Transcription factor 3 (BTF-3) or Nascent Polypeptide associated complex β (NACβ) is a transcription factor and is involved in RNA Pol B mediated initiation of transcription [11,14]. Studies have been performed time to time on its functional relevance in *C.elegans,* Drosophila, and Mice [7-9,11], which came out with its importance in early stages of development. In Humans, BTF3 has two isoforms BTF3a and BTF3b, due to alternative splicing. BTF3b lacks the first 44 amino acids of BTF-3a, which is the transcriptionally active form [4,5,7,11]. BTF3 has been found to be over expressed in cancer cells like sporadic colorectal cancer, glioblastomamultiforme and pancreatic cancer cells [11,12], and it was argued that the down regulation of BTF3 gene in cancer cell may result in increased apoptosis of some cancer cells [14], but this aspect is still under investigation. Considering anti-apoptotic nature of BTF-3, its down regulation can initiate apoptosis in cancer cell lines, where it is overexpressed. BTF3 overexpression has been checked in gastric cancer samples, where its siRNA mediated silencing leads to apoptosis [14]. In Pancreatic Cancer, downregulation of BTF3 using siRNA approach leads to downregulation of several cancer associated, genes highlighting its role in cancer [12].

BTF3 being a transcription factor which may be associated with the transcription regulation of genes involved in tumourigenesis, where its overexpression may lead to deregulation of factors downstream . BTF3 has been shown to bind specifically with AF-1 domain of nuclear receptor Estrogen Receptor α *in vitro* and *in vivo* in both Ligand-dependent and Ligand–independent manners, respectively. In MCF-7 cells, BTF3 was shown to exert a positive, dose-dependent effect on reporter gene transcription only in the presence of activated ER α. It therefore suggests that BTF3 may be a component of the mechanism by which the AF-1 function of ERα stimulates gene transcription [27].

Different studies revealed the differential expression of BTF3 in most of the cases of Breast Cancer. Since, BTF3 is known to be anti-apoptotic in nature; we targeted BTF3 in our study using MCF-7, Human Breast Cancer cell line. In this paper, using *in silico* techniques we identified the promoter of Human BTF3 containing all regulatory sites essential to mediate transcriptional regulation of reporter gene to develop an alternative *in vitro* model based on BTF3 promoter mediated expression of Luciferase gene [23-26]. In our previous work, Human β casein fragment (54-59), NS has shown down modulation of BTF3 [18], we therefore validated our model by transfecting BTF3 promoter reporter construct in THP-1 cell line and further treatment with NS showed downregulation of reporter expression similar to our previous study in THP-1 cell line . Expression of BTF3 was checked in MCF-7 cell line and using NS, its downregulation was found, which encouraged us to screen its analogs on the promoter-reporter construct in MCF-7 cell line. AN1, AN2 and AN3 along with NS were found effective to downregulate BTF3 expression in MCF-7 cells upon screening on the promoter-reporter model through transient transfection and this study was validated through endogenous BTF3 expression study in MCF-7 cells on treatment with NS, AN1, AN2 and AN3, respectively. Downregulation of BTF3 has been correlated with apoptosis in different cancer forms, Cell viability assay was performed on treatment with NS and its analogs in MCF-7 cells to check the cell viability on downregualtion of BTF3, where we found significantly low cell viability in comparison with control cells without treatment. BTF3 downregulation upon treatment with NS and its analogs leads to

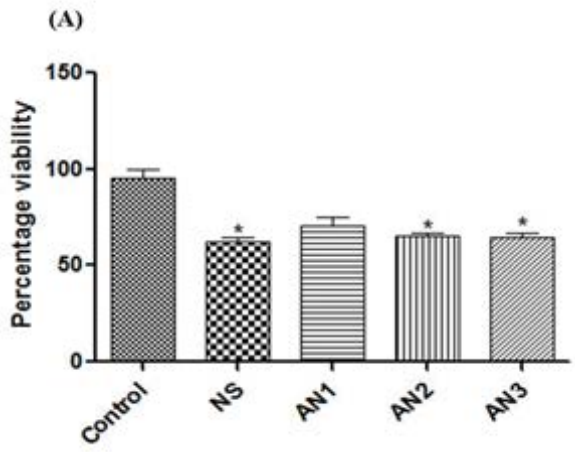


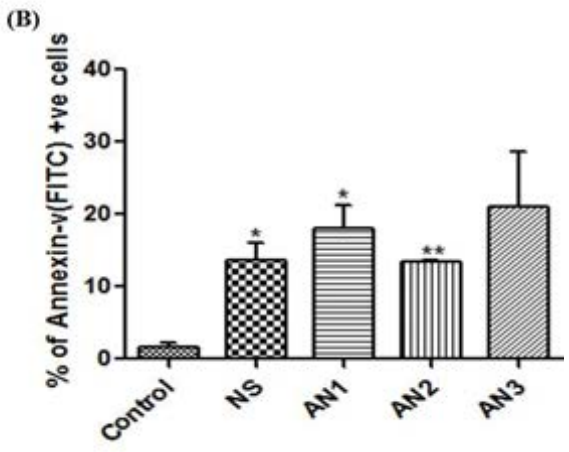


(A) MCF-7 cells were treated with β-casein (54-59) peptide and its analogs (20μM) AN1, AN2 and AN3 for 48 hrs. Change in BTF3 expression was shown by Western Blotting using anti-BTF3 antibody Lower panel shows expression of α-Tubulin which was detected using anti-α-Tubulin monoclonal antibody to check equal loading of protein.

(B) Densitometric studies showing fold change in expression of BTF3 during treated conditions is presented in respect to control. The values and error bars represent average and standard deviations of three independent set of experimentsand change was considered significant at P ≤ 0.05 (*) , P ≤ 0.01 (**) and P ≤ 0.001 (***).

Figure 4: NS and Analogs downregulates the expression of BTF3.





(A) MTT Assay was employed to check cell viability in MCF-7 cell line on treatment with NS and analogs AN1, AN2 andAN3 at 20μM concentration for 48 hours.The values and error bars represent average and standard deviations of the independent set of experiments. Student T test was performed to find out significant difference between control and treated conditions and comparisons were considered significantly different at P ≤ 0.05 (*).

(B) MCF-7 cells were incubated 48 hours with NS and analogs AN1,AN2 and AN3 and the rate of apoptosis was measured by flow cytometry using Annexin-V/PI. The analysis was performed with the BD FACSDiva software. The data are Results are shown as mean fold change ± SEM (n=3) and change was considered significant at P ≤ 0.05 (*) and P ≤ 0.01 (**).

Figure 5: BTF3 down regulation induces cell death and apoptosis in MCF-7 cells.

apoptosis in MCF-7 cells was shown by Annexin-V-PI staining, which indicates the importance of these peptides in therapeutics (Figure 5).

Conclusion

The major application of this system would be high-throughput identification of compounds modulating BTF3 expression in different cancer cell lines, where BTF3 is overexpressed and to further study the mechanism of apoptosis and other BTF3 mediated functions in different cancer cell lines.

Acknowledgement

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