Upregulation of CD271 Transcriptome in Breast Cancer Promotes Cell Survival via NFκB Pathway

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Abstract

Background

Biological treatment of many cancers currently targets membrane bound receptors located on a cell surface. We are in a great to need identify novel membrane proteins associated with migration and metastasis of breast cancer cells. CD271, a single transmembrane protein belongs to tumor necrosis factor receptor acts and play its role in proliferation of cancer cell. The purpose of this study is to investigate the role of CD271 in breast cancer.

Methods and Results

In this study we analyzed the expression of CD271 in breast tumor tissue, breast cancer cell line MCF7 and isolated cancer stem cells (MCF7-CSCs) by quantitative real-time polymerase chain reaction (RT-qPCR). CD271 was upregulated among breast cancer patients in all age groups. Within the promoter region of CD271, there is a binding site for NF-κB1 which overlaps a putative quadraplex forming sequence. While CD271 also activates NF-κB pathway, down regulation of CD271 through quadraplex targeting resulted in inhibition of NF-κB and its downstream targets Nanog and Sox2

Conclusion

In conclusion, CD271 and NF-κB are interrelated to each other. Upon CD271 inhibition, the NF-κB expression also reduces which then effected the cell proliferation and migration. These results suggest that NF-κB is regulated by CD271 is playing a crucial role in cancer development and could be a potential therapeutic target.

Introduction

Breast cancer is the serious health issue and 2nd leading cause of mortality among all other cancer accounting for 11.7% of cases worldwide[1, 2] .Breast tumors are known to be composed of diverse group of cells including cancer stem cells (CSCs).CSCs have an ability to control the tumorigenicity and resist conventional therapies. Hence, they are the main cause of the relapse of cancer[3]. Therefore, to prevent breast cancer recurrence, effective treatment involves elimination of CSCs[4]. CD271, also known as nerve growth factor receptor (NGFR) is a transmembrane protein. It belongs to the tumor necrosis factor receptor (TNFR) superfamily [5] and plays a pivotal role in development and regeneration of sympathetic and sensory nervous system [6].It performs a dual role by acting as an antiproliferative agent which control cell apoptosis by the facilitation of cytochrome C release from mitochondria and activation of Caspases 9, 6 and 3. It has also been involved in cell proliferation and promote invasiveness by MAPK and (PI3K)/ AKT signaling pathway[7–10] . In case of carcinoma, it appears to involved in inducing apoptosis in prostate, bladder, stomach and liver cancer[11–14] .Whileits proliferative role has been reported in thyroid carcinoma and melanoma [15, 16]. Several studies have identified CD271 as a neural

crest and mesenchymal stem cell marker while its expression was also reported in other stem cells such as laryngeal epidermal, esophageal and corneal regenerative epithelia [16–21].

In case of breast cancer several studies have shown the induced expression of CD271 in breast carcinoma [5],[22],[23]. The role of CD271 in cancer development and its progression is not fully understood and different results have been reported [10]. Tumor necrosis factor receptor (TNFR) family is known to activates NF-κB pathway along with other factors such epidermal growth factor receptor (EGFR), toll-like receptor (TLR) and cytokines receptors [24]. CD271 being a member of TNFR is involved in the activation of NF-κB pathway to mediate cell proliferation and survival[5, 9, 25] .The signaling pathway of NF-κB is widely associated with cancer development and progression .NF-κB is very important transcription factor which plays crucial role by binding to consensus DNA sequence at the promoter region to transcriptionally regulates target genes.[26]. It is also reported to be involved in controlling the stemness and survival of cancer stem cells [27]. It does so by transcriptional regulating sox2 and Nanog both are very crucial for the maintenance of stem cells [28]. We hypothesized that activation of NF-κB by CD271 could facilitate the cell survival and proliferation through Nanog and Sox2 so can promote carcinogenesis (Fig1).

Methodology

Exclusion and Inclusion Criteria

Patients suffering from breast cancer were included in the study and patients with any infection was excluded. Patients included must be histologically confirmed for different grades of cancer (Grade I, II and III).The samples were taken at the time of surgery, along with the histopathological reports.

Breast Biopsies, Cell Culture and Drug preparation.

Breast tumor confirmed pathologically with adjacent normal tissues as control, were collected from 60 breast cancer patients, immediately after surgery from PIMS (Pakistan Institute of Medical Sciences) and Holy family hospital after taking consent from the patients. The excised tissues were stored in 1X PBS(Gibco) at -20 ℃.

Clinical pathological features studied were age, tumor types, tumor grade and Receptor's profile as indicated in Table 1.

Human Breast cancer cell line (MCF-7) was obtained from American Type Culture Collection (ATCC). The cell line was cultured in RPMI 1640 media (Gibco,USA) supplemented with 10% fetal bovine serum (FBS) and 1 % GPSS (L-Glutamin, pyruvate, streptomycin-pencillin) maintained at 37℃ and 5% CO_2 in a humidified atmosphere in T25 flask. Thymoquinone was purchased from (Sigma-Aldrich) and 2mM stock was prepared in DMSO. Working concentrations were prepared by diluting the stock in freshly prepared RPMI 1640 media.

After the cells were >80% confluent and growing exponentially in T25 flask, they were counted and plated in 96 well plate with variable concentrations of TQ in µM (160, 80, 40, 20,10, 5 and 2.5) to measure the IC50.

Cytotoxicity Assay

The anti-proliferative effect of thymoquinone was determined by MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide] assay. Briefly, 10^3 cells were counted and seeded in triplicates in each well of 96 well microtiter plate followed by overnight incubation. After 24 hours cells were exposed to increasing concentrations of TQ (2.5, 5, 10, 20, 40,80 and 160 µM doses) for the next 24 hours. TritonX-100 (78ppm) was used a positive control and the blank (RPMI 1640 medium) for calculating viability. The cells were treated with MTT (10µl) solution after 24 hours and incubated for 3 hours to reduce the yellow dye to purple formazan crystals. After that medium was removed and 100µl of stopping solution (10% SDS in 1M HCL) was added to dissolve the crystals and further incubated for overnight. The absorbance was recorded at 570nm of each well by using microplate reader (FLUOstar Omega) and viability was calculated.

% cell viability = {(At-Ab) / (Ac-Ab)} x 100

A graph of viability versus drug concentration was used to calculate IC50 values for MCF-7 cell line. The IC50 of TQ was obtained as 70 µM. For expressional analysis $0.5x10^5$/ml MCF-7 cells were seeded in three 6 well plates. After 24hrs of incubation, TQ was added in varying concentrations (40, 50, 60, 70 and 80 µM) in their respective wells in three plates. The next day plates were taken out, trypsinzed and RNA was isolated.

Isolation of Cancer Stem Cells from MCF-7 (MCF7-CSCs)

The breast cancer stem cells were isolated by using the MAG-iso^{TM} (Cat No. - K10103) kit. The MAG-iso^{TM} Human Isolation Kit is designed to isolate BCSCs based on $CD24^{-/low}$ and $CD44^{+/high}$ surface markers. Cultured MCF-7 cells in T75 flask with 80%-90% confluency was trypsinized and counted by hemocytometer. The cancer stem cells were isolated by untreated MCF-7 cells is considered as NTC. For the treated cells doses of thymoquinone were selected as 50µM and 60µM. Initially $1x10^6$ cells were seeded in 6 well plate and followed by overnight incubation. After 24 hours, cells were exposed with TQ doses and further kept for overnight incubation. The next day cells of each well were trypsinized and counted. The starting population of MCF-7 cells for isolation of CSCs should be as low as $1x10^6$/ml according to the protocol. The cells were then resuspended in cold Iso-Mag buffer (2□C-8□C) to proceed kit isolation procedure. Isolated BCSCs were suspended in trizol reagent for further analysis.

Isolation of RNA

Isolation of RNA from breast biopsies, MCF-7 cell line and MCF7-CSCs were carried out by trizol reagent (Invitrogen, USA) according to manufacturer's protocol. The extracted RNA was analyzed on 1X TBE gel

to confirm the isolated RNA. Quantification of RNA was done by UV spectrometer (Implen Nanophotometer) and absorbance was measured at 260/280nm. Extracted RNA was stored in DEPC water at -20□C.

Quantitative Real Time PCR

cDNA was prepared from 500ng of RNA by two step protocol using thermo cycler (Applied Biosystem). The expressional analysis of CD271, NF-κB1, β-actin, Sox2 and Nanog was carried out by 5x SolisGreen® qPCR mix (Solis Biodyne) on Step 1 plus PCR system (Applied Biosystem). Primers were designed by Primer3plus tool with the conditions indicated in (Table 2). β-actin was used as an internal control. The thermal cycler condition was 95□C for 12 mins initial denaturation followed by 35 cycles of 95□C for 45s, annealing temperature of desired primers for 30s followed by extension at 72□C for 30s. The data acquisition was done on extension step. The expressional analysis of CD271, NF-κB1,Sox2 and Nanog relative to endogenous control β-actin was performed by Livak methods (of 2^-delta Ct) [29] .

BrdU proliferation assay

The assay was performed using BrdU Cell Proliferation Assay Kit (Biovision Incorporated) following the manufacturer's instructions. MCF-7 cells were cultured in 96 well plate at 5000 cells/well and treated with TQ concentrations (50μM, 60 μM and 70 μM) for 24 hrs. BrdU solution was added into each well and incubated at 37°C for 3hrs before fixation. BrdU positive cells were detected by measuring the absorbance at 450nm by microplate reader.

Invitro scratch wound healing assay.

This assay was used to study the effect of TQ on the migration of MCF-7 cell line. Cells were cultured in 60mm petri dish under normal cultural condition and allowed to reach confluency. After reaching 89-90% confluency, one plate is taken as control and other one is treated with 70μM TQ. Pre-sterilized 200μl tip was used to create a scratch on both plates. The status of scratch wounds was monitored at the beginning of the assay (0 hr) and after regular intervals (24hr and 48 hrs) and images collected by Keyence bz-x700 microscope.

Computational Analysis

The sequence of CD271 promoter (500bp upstream form transcription start site) was retrieved from Ensemble (ENST00000172229.8). QGRS mapper (G-quadraplex analysis tool) was used to locate G-quadruplex at CD271 promoter [30]. The binding site of transcription factor NF-κB1 at CD271 promoter was identified by ContraV3 bioinformatics tool [31].

Statistical Analysis

IBM SPSS (version 25) was used to apply unpaired student's t test and one-way Analysis of variance (Anova) for evaluating statistical significance of CD271 expression, and the significance between CD271

expression and clinical pathological features in cancer patients. The data analysis of CD271, NF-κB1, Sox2 and Nanog in MCF-7 and MCF7-CSCs was also done by paired student's t test and one-way Anova. R software was used for the regression and correlation analysis. The data withp value less than 0.05 was considered statistically significant.

Results

The regulatory role of CD271 in the incidence of breast cancer has been reported previously[32].To analyze it in our cohort, the expression profile of CD271 was examined in breast cancer patients initially. Further analysis was done to elucidate the mechanism of CD271 in regulating NF-κB pathway.

Expression of CD271 in Breast Biopsies and pathological features

The expression was induced by 4 folds in tumor samples when compared to adjacent controls (p=0.0002) (Fig 2A). The result indicated that CD271 was significantly induced among patients than their adjacent control tissues.

To understand the role of CD271 in the prognosis and onset of disease, the involvement of different clinical parameters in association with the CD271 expression level was taken into consideration. These included analyzing the expression on the bases of age, types of breast carcinoma, tumor grading, and hormonal receptors. In our cohort the expression was consistently induced regardless of different clinical factors. That means the mechanism of induction of CD271 in breast cancer is independent of these factors and is exclusive to breast cancer (Fig2B).

Expression analysis of CD271 in MCF-7 and MCF7-CSCs

CD271 expression was measured in breast cancer cell line (MCF-7) and extracted cancer stem cells MCF7-CSCs. As CD271 is a member of TNFR, so we hypothesized that it has stimulatory role in NF-κB regulation. Being a transcription factor, NF-κB regulates the expression of its downstream targets sox2 & Nanog, which are important factors for maintaining the survival of MCF-7 cell line along with MCF7-CSCs. Therefore, expression of NF-κB1 (subunit of NF-κB) and its regulatory genes sox2 and nanog were also elucidated.

Expression of CD271 mRNA was 2 folds high (p=0.03) in MCF7-CSCs in comparison to MCF-7 cell line. Sox2 and Nanog also showed significant upregulation in MCF7-CSCs then MCF-7 cell line. (Fig 2C). The correlation was also analyzed between CD271 and NF-κB1 by Kendall's rank correlation test in MCF7 cell line. A strong positive correlation (p=0.01, tau=1) was found between CD271 and NF-κB1 indicating the increase of NF-κB1 expression with the increase of CD271 expression (Fig 2D)

Computational Analysis

QGRS mapper was used to identify the G-rich sequences (G-quadruplexes/G4) within the promoter region of CD271 gene, 500 bps upstream to the TTS. Promoter analysis revealed the presence of a putative G-

quadruplex forming sequence (5-GGGGAGGGGTGGGGATGGGG-3) at -452 to -471 position on the promoter of CD271. We searched the binding site of NF-κB subunits on CD271 promoter and found the binding of NF-κB1 (-460 to -472 pb) on the G-quadraplex sequences upstream of transcription start site of CD271 (Fig 3A). Promoter quadraplex has been shown to regulate the transcription in both positive and negative way [33]. CD271 region from (-452 to -471) upstream of transcription start site TSS appears to be very crucial, the folding of DNA strand because of G-quadraplex interfere with the binding of NF-κB1 and effects the transcription of CD271(Figure 3B).

Expression of CD271 in MCF-7 cell line

G-quadruplex structures in the gene promoters is linked with transcriptional activation or repression. Consequently, interaction of small molecules with such G-quadruplexes may modulate transcription. Thymoquinone (TQ) has been reported to bind with G-quadraplex and interfere with DNA structure and inhibits the DNA synthesis, transcription, and viability of cell[35], [36].Therefore, we treated the cells with TQ to block the binding of NF-κB to CD271 promoter (Fig 4A).

The effect of TQ on CD271 as quadraplex binder was studied on MCF-7 cell line. The expression of CD271 was inhibited in dose dependent manner in MCF-7 cell line. NF-κB1 also showed decrease expression with increasing dose of TQ. Thus, TQ has downregulated mRNA expression of CD271 and its downstream target NF-κB1 (Fig 4A).

Expression of CD271 in MCF7-CSCs

The effect of expression repression of CD271 in CSCs isolated from MCF-7 cell line was analyzed. The expression of CD271 was inhibited after the TQ application. The study was further expended towards the downstream regulator of CD271 to check if the lower transcription can affect its downstream targets NF-κB1, sox2 and Nanog. Overall. It was found that TQ downregulated the expression of all the selected genes in a dose dependent manner as illustrated in Fig 4B and 4C.

TQ inhibits proliferation and migration of MCF-7 cells.

The proliferation of BrdU incorporated MCF-7 cells were measured in presence of TQ. TQ has shown the overall significant effect ($p=0.002$) by decreasing the proliferation of MCF-7 cells, while 70μM was measured the most effective dose ($p=0.001$) for controlling the proliferation of MCF-7cells (Fig 4D). In case of migration, untreated MCF-7 cells taken as control have shown the movement towards the center of wound and completely healed the scratch after 48hrs. While the cells treated with TQ showed slow movement towards the center and wound is still not healed after 48hrs (Fig 4E). This shows the migration of cells are affected by the inhibition of CD271 expression by TQ. Hence, CD271 can be considered as a potential therapeutic target to control the cell proliferation and migration.

Discussion

Tumor recurrence is controlled by stem-like cancer cells termed as cancer stem cells [37]. Lots of research is going to find the way to cease the proliferative capability of CSCs to reduce the chance of cancer relapse. CD271, a receptor for nerve growth factor has a greater capacity of tumor initiation. $CD271^{+}$ $CD44^{+}$ subpopulation reported to contain the more stem cell like cells having an enhanced tumorigenic, proliferative and metastatic potential [38–41]

CD271 has dual role being proliferative to stimulate survival or anti-proliferative to stimulate cell death [32, 42]. In this study the expression of CD271 was investigated in breast cancer patients along with cancer stem cells (MCF7-CSCs) to define its role as proliferative or anti-proliferative. We found that CD271 was induced individually of clinical factors in all breast cancer biopsies when compared to adjacent non-cancerous control. It assumed that CD271 might modulate the proliferation of breast cancer cells as consistent with previous studies [6, 17, 21]. The established markers for the breast cancer are specific with certain clinical parameter such as Human epididymis protein 4 (HE4) is associated with ductal carcinoma [43], CD24 with invasive breast carcinoma and early-stage cancer [44, 45], ki-67 is associated with higher stage and higher-grade tumor [46] while CD271 in this study was induced independent with any clinical parameter. By analyzing these results, we can see the role of CD271 as facilitator of cancer incidence which helps in cancer cell survival independent of any clinical parameters.

CD271 controls the proliferation of cells by activation of MAPK, (PI3K)/ AKT and NF-κB signaling pathway [32, 47]. Our data suggests that induced CD271 might plays a contributing role in the proliferation and metastasis of cancer cells through NF-κB pathway and its downstream targets Nanog and Sox2. Correlation study also showed the positive correlation between both CD271 and NF-κB. Induction of CD271 in MCF-7 and MCF7-CSCs can also contributes towards the more enhanced proliferation of these cells. Upon treatment with TQ, a G-quadraplex binder, CD271 expression was reduced which also reduces the cell movement in the wound healing assay. Bioinformatics analysis showed the binding site of NF-κB1 at the promoter region of CD271 which overlaps with G-quadraplex sequences. NF-κB subunits NF-κB1 and NF-κB2 after translocation to the nucleus, dimerize and bind to the promoter sequences of target gene CD271 [34]. One of the previous study reported the inhibitory binding of TQ to the NF-κB2 subunit which do not allow NF-κB2 to bind with NF-κB1 [48]. This ceases the binding of NF-κB dimers to its downstream genes. Reduced CD271 expression by TQ might be due to its inhibitory effect on NF-κB binding to CD271 promoter. There might be a possibility of direct binding of TQ to the quadraplex at CD271 promoter which in turn prevent the interaction of NF-κB1 for transcription activation. Bioinformatic analysis was also performed to identify G-rich sequences or G-quadraplex in the promoter region of Sox2 and Nanog genes. No G-rich sequences were identified with in promoter region of Sox2 and Nanog genes. In our results we have also observed the downregulation of these genes in dose dependent manner. This could be corresponded with the lower expression of CD271 which effected the further cascade including NF-κB1, Sox2 and Nanog. It is therefore suggested that TQ is reducing the expression of CD271 and its downstream target genes by binding with the CD271 G-quadraplex. Thymoquinone also suppress the TNFR induced NF-κB activation by one of the study [49] which more

emphasizes the inhibitory effect of TQ towards the cancer cell survival. This supports our hypothesis that downregulation of CD271 will in turn downregulates NF-κB1 and its downstream targets, effecting the cancer cell growth and migration.

Initially we proposed the stimulating role of CD27l receptor in the activation of NF-κB pathway. By computational analysis later we have found NF-κB1 binding at CD271 promoter. Here we can see NF-κB1 as a transcription factor, is facilitating the transcription of CD271. Hence the positive feedback loop can be suggested by our data as CD271 regulates NF-κB pathway and NF-κB1 as transcription factor facilitate the expression of CD271 as its target gene (Fig 5). Blocking either CD271 promoter or NF-κB1 binding by TQ can alter the expression of target gene of NF-κB pathway which include stemness markers Sox2 and Nanog involved in proliferation and survival of cancer cells and cancer stem cells.

Conclusion

In conclusion this study showed that induced CD271 expression is independent of any clinicopathological feature. It may regulate the most important transcription factor NF-κB and its target genes involved in cancer stemness and proliferation. Inhibiting CD271 can downregulates NF-κB and its targets gene involves in cell survival Sox2 and Nanog. CD271 here seems to be a potential target to treat breast cancer. Further study at protein level is needed to elucidate the results.

Declarations

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Conflicts of Interest

The authors declare no conflict of interest.

Data availability

The data and material analyzed during this study are available from the corresponding author on reasonable request (ramla_shahid@comsats.edu.pk).

Author Contribution:

Ramla Shahid and kehkeshan Mazhar conceptualized and designed the experiment. Nabiha Bashir and Mehreen Isfaq performed the experiments. The manuscript was written by Nabiha Bashir and edited by Ramla Shahid and Kehkeshan Mazhar.

Ethical approval

This study was approved by the ethical committee of COMSATS University Islamabad (CUI) Islamabad and was performed in agreement with the guidelines of PIMS Hospital Islamabad and Holy Family Hospital Rawalpindi Pakistan.

Consent to Participate

A written informed consent was obtained from all participants.

Consent for Publication

Participants have consented to the submission of the data.

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Tables

Table 1: List of primers used for quantitative Real Time PCR Assay

Gene	Primer	Sequence (5' -3')	Annealing Temperature (°C)	Amplicon Size (bp)
CD271	Forward	GTATTCCGACGAGGCCAAC	58	140
	Reverse	TGGACCGTGTAATCCAACG		
NF-κB1	Forward	TTTCTTCCGGATAGCACTGG	56	120
	Reverse	CCAGCTGTCCTGTCCATTCT		
Sox2	Forward	TGATGGAGACGGAGCTGAA	57	103
	Reverse	GGGCTGTTTTTCTGGTTGC		
Nanog	Forward	GATTTGTGGGCCTGAAGAAA	57	140
	Reverse	CTTTGGGACTGGTGGAAGAA		
β-actin	Forward	CTGAACCCCAAGGCCAAC	59	109
	Reverse	AGAGGCGTACAGGGATAGCA		

Note: The amplicon size listed are correspond to the cDNA fragment used for Real Time PCR.

Table 2: Clinical pathological Distribution of samples (*n*=60)

Parameters	Number of Cases (%)
Age	
G1(20-40)	14(23)
G2(41-60)	36(60)
G3(61-80)	10(17)
Cancer Types	
DCIS	8(13)
IDC	44(74)
ILC	8(13)
Grade	
I	0 (0)
II	41(68)
III	19(32)
Estrogen Receptor	
ER+	41 (68)
ER-	19(32)
Progesterone Receptor	
PR+	44 (73)
PR-	16 (27)
Her2 Receptor	
Her2+	19 (32)
Her2-	41 (68)

Figures

Fig 1

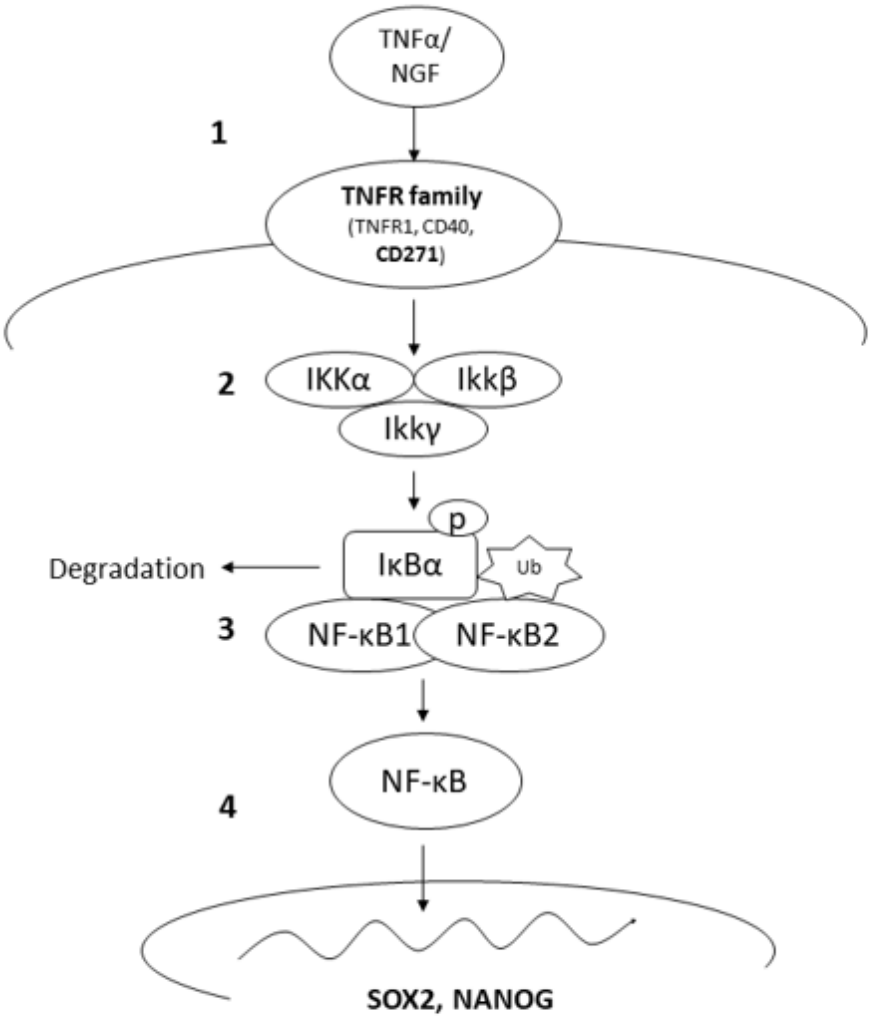

Figure 1

NF-κB pathway: 1. Ligand binds to its tumor necrosis factor receptor (TNFR). 2. It activates IkappaB kinases (IKKs complex) 3. IkappaB Kinase β(Ikkβ) then induces phosphorylation of the inhibitory factor IκBα leading to the ubiquitination and degradation by proteosome, and thus releasing NF-κB factors (p50/p65) 4 Translocation of NF-κB factors (p50/p65) to the nucleus and activation of target genes such as Sox2 and Nanog.

Fig 2

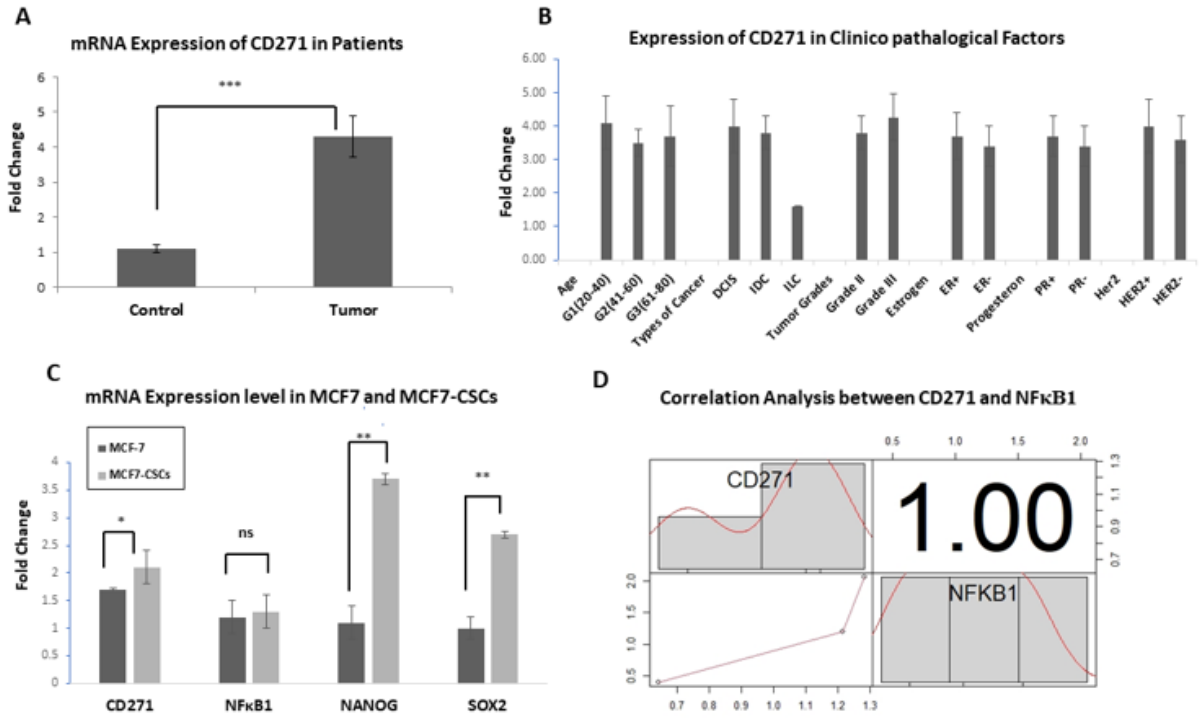

Figure 2

The relative expression of CD271 mRNA in patients as compared to control The bar graph shows fold change (2^- ΔΔCt) of CD271normalized against β-actin (A). The expression of CD271 in relation to different clinic-pathological Factors. The bars show the fold change of CD271 in Age group (p=0.8), Types of cancer: Ductal carcinoma insitu (DCIS), Invasive ductal carcinoma (IDC), invasive lobular carcinoma (ILC) (p=0.5), Tumor grades (p=0.7), Estrogen receptor (p=0.8), Progesterone receptor (p=0.2), and Human epidermal growth factor receptor Her2 receptor (p=0.7) (B). The bar graph shows induced level of CD271 (p=0.01), NFκB1, Nanog (p=0.002) and sox2 (p=0.003) in MCF-CSCs as compared to MCF-7 (A). Correlation analysis of CD271 expression with NF-κB1 expression (C) CSCs Correlation study done by Kendall rank correlation test shows significant (p=0.01) and strong positive (tau=1) correlation between CD271 and NF-κB1expression (D). Error bar represents standard error of means.

Fig 3

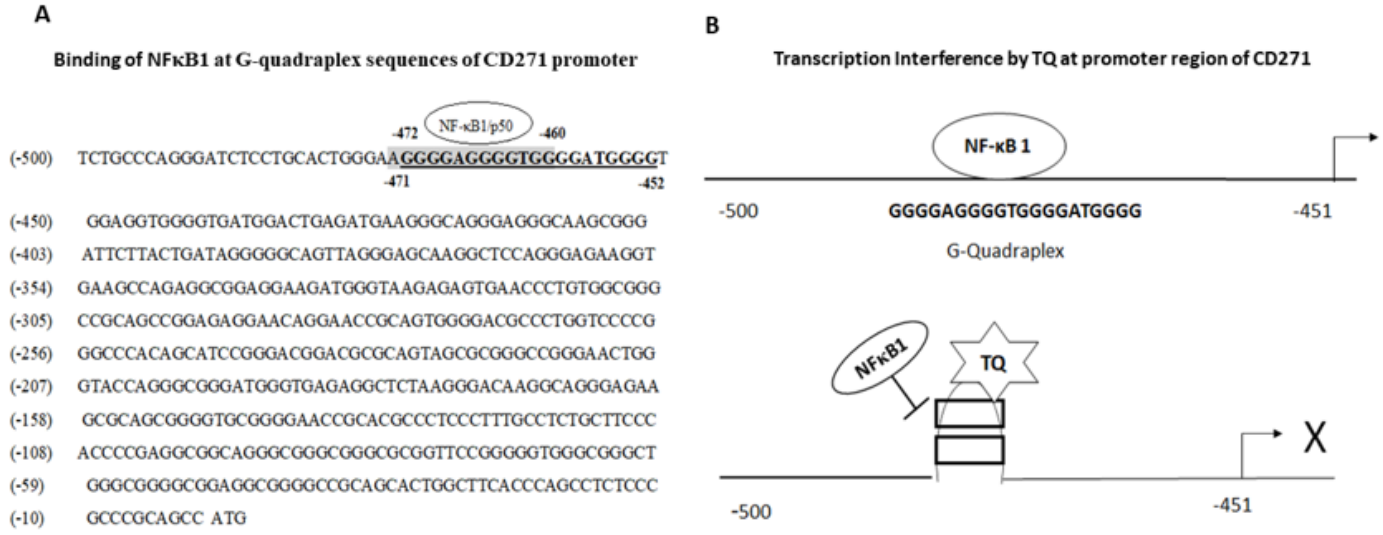

Figure 3

Schematic representation of CD271 promoter region 500bp upstream of transcription start site (TSS). G-quadraplex (G-score 62) is identified at region -452bp to -471bp and indicated by bold letters and line. Binding site of NF-κB1 has been identified at -460 to -472 bp (A). Diagrammatic illustration showing G-quadraplex sequences in CD271 promoter region and binding of thymoquinone at G-quadraplex region of promoter of CD271. TQ binding stabilize the G-quadraplex and cause the hindrance of p50(NF-κB1) to bind to the promoter and halt the transcription of CD271 (B).

Fig4

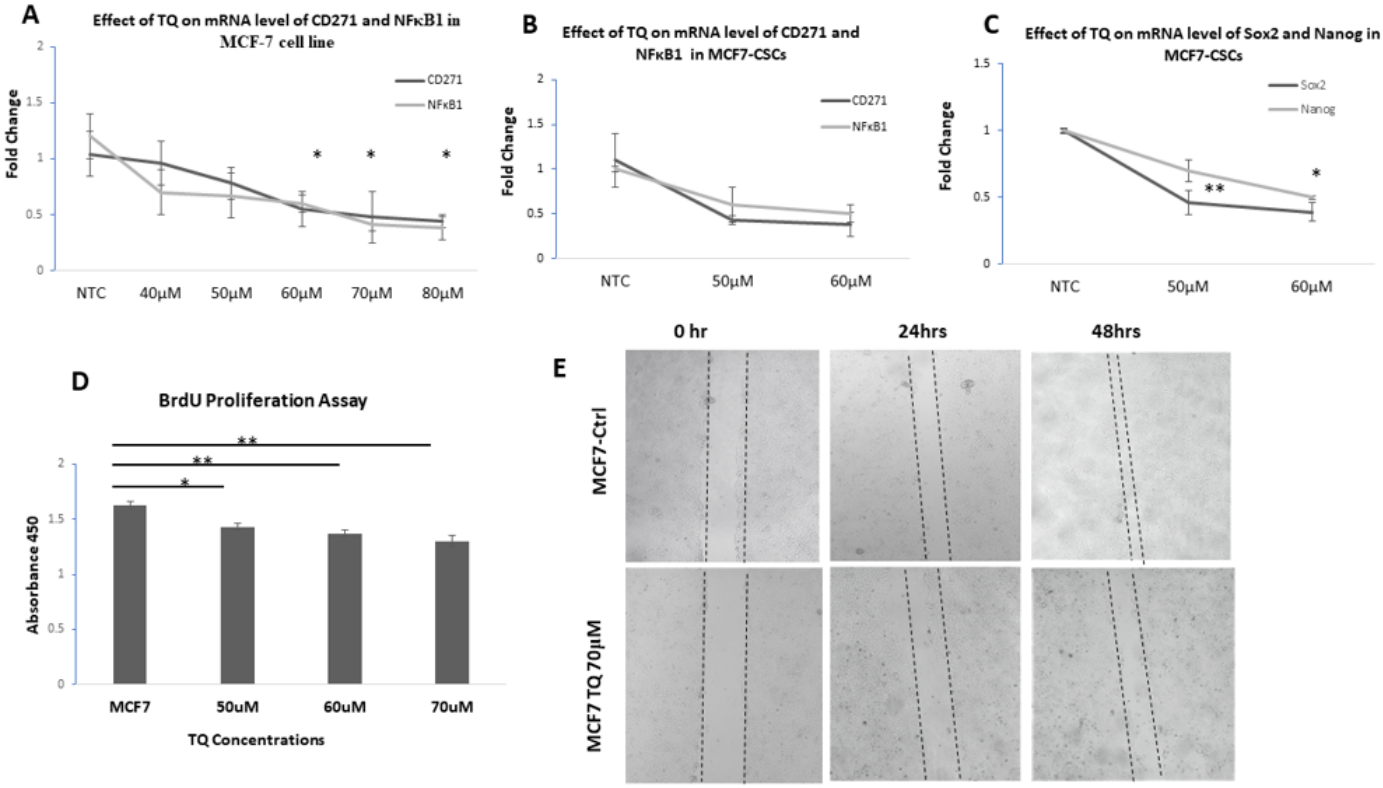

Figure 4

The inhibitory effects of thymoquinone TQ on expression of mRNA in MCF-7 cell line. The line graph shows reduction of CD271 and NFκB1 mRNA level by increasing the concentration of TQ in MCF-7 cell line. The significant down regulation of CD271is shown by 60μM (p=0.01), 70μM (p=0.01) and 80μM (p=0.04) (A). The line graph shows reduction of mRNA level of CD271 and NF-κB1 in MCF7-CSCs (B) The overall reduction of Sox2 (p=0.002) with increasing the concentration of TQ and Nanog (p=0.02) at 60μM TQ (C). The proliferation assay showed the significant negative effect of TQ on the proliferation of MCF-7 cells at 50μM (p=0.03), 60μM (p=0.007) and 70μM (p=0.001) (D). The scratch wound healing assay were performed to assess the effect of TQ on the movement of MCF-7 cells for 48 hrs. (E).

Fig 5

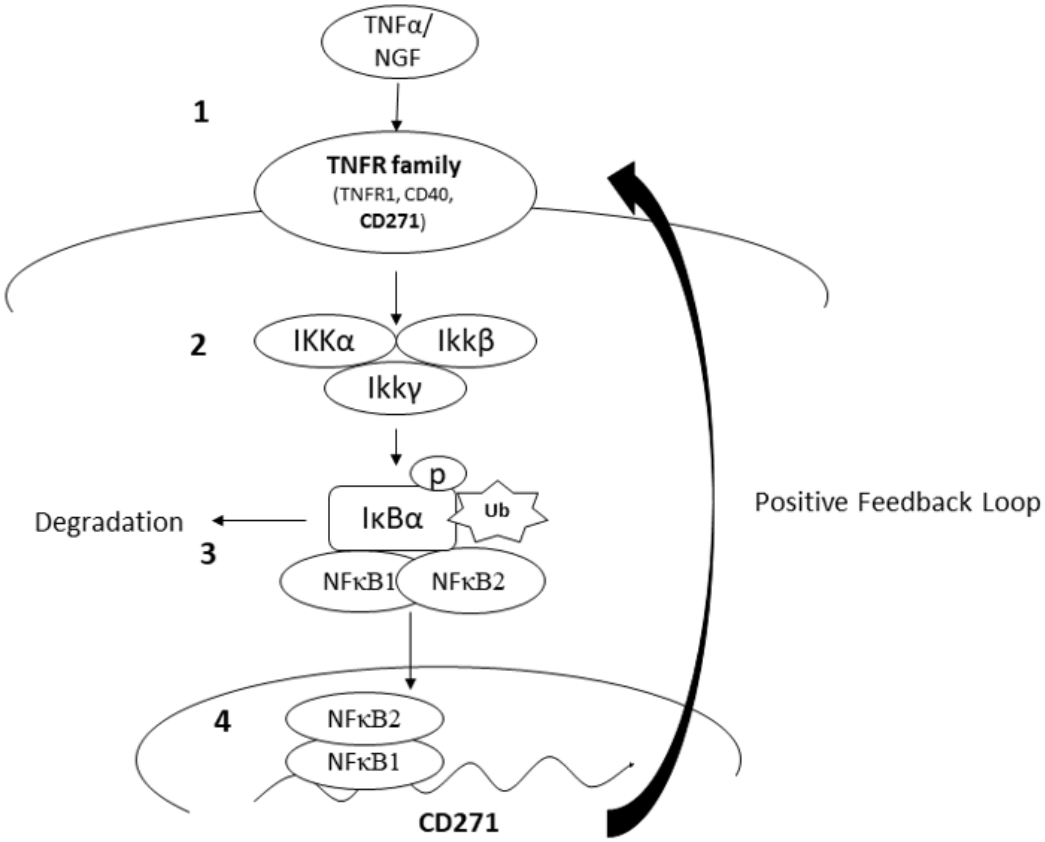

Figure 5

Positive feedback loop of CD271: 1. Ligand binds to its tumor necrosis factor receptor (TNFR). 2. It activates IkappaB kinases (IKKs complex) 3. IkappaB Kinase β(Ikkβ) then induces phosphorylation of the inhibitory factor IκBα leading to the ubiquitination and degradation by proteosome, as a result of which NF-κB factors (NF-κB1 and NF-κB2) is released. 4 NF-κB factors (NF-κB1 and NF-κB2) are translocated to the nucleus and activates CD271.