Upregulation of CD271 transcriptome in breast cancer promotes cell survival via NFκB pathway

Nabiha Bashir1 · Mehreen Ishfaq1 · Kehkashan Mazhar2 · Jahangir Sarwar Khan3 · Ramla Shahid1

Received: 3 August 2021 / Accepted: 29 October 2021 / Published online: 9 November 2021
© The Author(s), under exclusive licence to Springer Nature B.V. 2021

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 family 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 mRNA expression of CD271 in breast tumor tissue, breast cancer cell line MCF7 and isolated cancer stem cells (MCF7-CSCs) by RT-qPCR. We also measured the protein levels through western blotting in MCF-7 cell line. CD271 was upregulated in breast cancer patients among all age groups. Within the promoter region of CD271, there is a binding site for NF-κB1 which overlaps a putative quadruplex forming sequence. While CD271 also activates NF-κB pathway, down regulation of CD271 through quadruplex targeting resulted in inhibition of NF-κB and its downstream targets Nanog and Sox2.

Conclusion In conclusion, our data shows that CD271 and NF-κB are regulated in interdependent manner. Upon CD271 inhibition, the NF-κB expression also reduces which in turn affects the cell proliferation and migration. These results suggest that CD271 is playing a crucial rule in cancer progression by regulating NF-κB and is a good candidate for the therapeutic targeting.

Keywords CD271 · NF-κB1 · Breast cancer · MCF7-CSCs · Breast cancer stem cells · G-quadruplex

Introduction
Breast cancer is the serious health issue and second 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 3, 6 and 9. 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]. Its proliferative role has been reported in thyroid carcinoma and melanoma [15, 16]. CD271 have also been identified 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].

Several studies have shown the induced expression of CD271 in breast carcinoma [5, 22, 23]. However, the exact role of CD271 in cancer development and its progression

---

1 Department of Biosciences, COMSATS University Islamabad (CUI), Islamabad, Pakistan
2 Institute of Biomedical and Genetic Engineering (IBGE), KRL Hospital Islamabad, Islamabad, Pakistan
3 Department of General Surgery, Rawalpindi Medical University, Rawalpindi, Pakistan
is not fully understood [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 known to be 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 which in turn can promote carcinogenesis (Fig. 1).

**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 Pakistan Institute of Medical Sciences (PIMS) and Holy family hospital after taking consent from the patients. The excised tissues were stored in 1X PBS(Gibco) at – 20 ºC.

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)

| 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)             |

![NF-κB pathway](image_url)
supplemented with 10% fetal bovine serum (FBS) and 1% GPSS (l-Glutamin, pyruvate, streptomyein-pencillin) maintained at 37 °C and 5% CO₂ in a humidified atmosphere in T25 flask. Thymoquine was purchased from (Sigma-Aldrich) and 2 mM 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 thymoquine was determined by MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide] assay. Briefly, 10⁵ cells were counted and seeded in triplicates in each well of 96 well microtiter plate followed by overnight incubation. After 24 h cells were exposed to increasing concentrations of TQ (2.5, 5, 10, 20, 40, 80 and 160 μM doses) for the next 24 h. TritonX-100 (78 ppm) was used a positive control and the blank (RPMI 1640 medium) for calculating viability. The cells were treated with MTT (10 µl) solution after 24 h and incubated for 3 h to reduce the yellow dye to purple formazan crystals. After that medium was removed and 100 µl of stopping solution (10% SDS in 1 M HCL) was added to dissolve the crystals and further incubated for overnight. The absorbance was recorded at 570 nm of each well by using microplate reader (FLUOstar Omega) for calculating viability. The cells were exposed to increasing concentrations of TQ (2.5, 5, 10, 20, 40, 80 and 160 µM doses) for the next 24 h. The anti-proliferative effect of thymoquine was determined by MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide] assay. Briefly, 10⁵ cells were counted and seeded in triplicates in each well of 96 well microtiter plate followed by overnight incubation. After 24 h cells were exposed to increasing concentrations of TQ (2.5, 5, 10, 20, 40, 80 and 160 μM doses) for the next 24 h. TritonX-100 (78 ppm) was used a positive control and the blank (RPMI 1640 medium) for calculating viability. The cells were treated with MTT (10 µl) solution after 24 h and incubated for 3 h to reduce the yellow dye to purple formazan crystals. After that medium was removed and 100 µl of stopping solution (10% SDS in 1 M HCL) was added to dissolve the crystals and further incubated for overnight. The absorbance was recorded at 570 nm of each well by using microplate reader (FLUOstar Omega) and viability was calculated. % cell viability = [(At − Ab)/(Ac − Ab)] × 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 0.5 × 10⁵/ ml MCF-7 cells were seeded in three 6 well plates. After 24 h 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, trypsinized 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™ (Cat No. - K10103) kit. The MAG-iso™ 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 thymoquine were selected as 50 µM and 60 µM. Initially 1×10⁶ cells were seeded in 6 well plate and followed by overnight incubation. After 24 h, 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 1×10⁶/ml according to the protocol. The cells were then resuspended in cold Iso-Mag buffer (2–8 °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 (Invtrogen, 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 260/280 nm. Extracted RNA was stored in DEPC water at −20 °C.

Quantitative real time PCR

cDNA was prepared from 500 ng of RNA by two step protocols using thermo cycler (Applied Biosystem). The expressional analysis of CD271, NF-κB1, β-actin, Sox2 and Nanog was carried by 5×SolisGreen® qPCR mix (Solis Biodyne) on Step 1 plus PCR system (Applied Biosystem). Primers were designed by Primer3plus tool with the conditions indicated in (Supplementary Table 2). β-actin was used as an internal control. The thermal cycler condition was 95 °C for 12 min initial denaturation followed by 35 cycles of 95 °C for 45 s, annealing temperature of desired primers for 30 s followed by extension at 72 °C for 30 s. 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−ΔΔCt) [29].

Protein isolation and western blotting

Cells (MCF-7, MCF7 treated with TQ, and Human Mammary Epithelial cell line HMEC as control) were lysed in RIPA buffer (89900, Thermo scientific) containing protease inhibitor cocktail (11836153001, Roche) in accordance with manufacturer’s instructions and concentration was determined by Bradford Assay. Proteins (5 µg) were separated in 10–15% SDS–polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a poly-phosphorylated difluoride (PVDF) membrane. The membrane was then applied with anti-rabbit monoclonal primary antibodies (all purchased from Cell Signaling Technology (CST), Beverly, MA,USA),
against NFκB1 (#12540S, 1:1000), CD271 (#8238 T, 1:1000), Sox2 (#3579 T, 1:1000), Nanog (#4903 T, 1:1000) and GAPDH (#5174S, 1:500) overnight at 4 °C. After overnight incubation proteins re-probed with anti-rabbit immunoglobulin G (IgG; #7074, 1: 2000) diluent labeled with horseradish peroxidase and incubated for 1 h at room temperature. The ImageJ 1.48u software (National Institutes of Health, Bethesda, Maryland, USA) was utilized for the protein visualization.

**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 (50 µM, 60 µM and 70 µM) for 24 h. BrdU solution was added into each well and incubated at 37 °C for 3 h before fixation. BrdU positive cells were detected by measuring the absorbance at 450 nm by microplate reader.

**In vitro 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 60 mm petri dish under normal cultural condition and allowed to reach confluence. After reaching 89–90% confluence, one plate is taken as control and other one is treated with 70 µM TQ. Pre-sterilized 200 µl tip was used to create a scratch on both plates. The status of scratch wounds was monitored at the beginning of the assay (0 h) and after regular intervals (24 h and 48 h) and images collected by Keyence bz-×700 microscope.

**Computational analysis**

The sequence of CD271 promoter (500 bp upstream form transcription start site) was retrieved from Ensemble (ENST00000172229.8). QGRS mapper (G-quadruplex 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 with p 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.

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 (Table 1). 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 (Fig. 2B).

**Expression analysis of CD271 in MCF‑7 and MCF7‑CSCs**

CD271 expression was measured in breast cancer cell line (MCF-7) and isolated cancer stem cells MCF7-CSCs. As CD271 is a member of TNFR, so it could have a 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'-GGGGAGGGGTGGGATGGGG-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-quadruplex sequences upstream of transcription start site of CD271 (Fig. 3A). Promoter quadruplex 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-quadruplex could interfere with the binding of NF-κB1 and effects the transcription of CD271 (Fig. 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-quadruplex and interfere with DNA structure and inhibits the DNA synthesis, transcription, and viability of cell [34, 35]. Therefore, we treated the cells with TQ to block the binding of NF-κB to CD271 promoter (Fig. 4A). 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). TQ effect on protein level of CD271, NFκB1 and its downstream targets sox2 and Nanog also showed subsequent downregulation at 60 µM
Fig. 3 Schematic representation of CD271 promoter region 500 bp upstream of transcription start site (TSS). G-quadruplex (G-score 62) is identified at region -452 bp to -471 bp 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-quadruplex sequences in CD271 promoter region and binding of thymoquinone at G-quadruplex region of promoter of CD271. TQ binding stabilize the G-quadruplex and cause the hindrance of p50(NF-κB1) to bind to the promoter and halt the transcription of CD271 (B).

Fig. 4 The inhibitory effects of thymoquinone TQ on expression of mRNA in MCF-7 cell line. The line graph shows reduction of CD271 and NF-κB1 mRNA level by increasing the concentration of TQ in MCF-7 cell line. The significant down regulation of CD271 is shown by 60 µM (p = 0.01), 70 µM (p = 0.01) and 80 µM (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 60 µM TQ (C). The protein analysis showed the effect of TQ at 60 µM and 70 µM on the expression of NFκB1, CD271, Sox2 and Nanog (D). The proliferation assay showed the significant negative effect of TQ on the proliferation of MCF-7 cells at 50 µM (p = 0.03), 60 µM (p = 0.007) and 70 µM (p = 0.001) (E). The scratch wound healing assay were performed to assess the effect of TQ on the movement of MCF-7 cells for 48 h (F).
and 70 µM when treated with TQ. HMEC was taken as control cell line (Fig. 4D).

**Expression of CD271 in MCF-7-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 C.

**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 70 µM was measured the most effective dose (p = 0.001) for controlling the proliferation of MCF-7 cells (Fig. 4E). 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 48 h. While the cells treated with TQ showed slow movement towards the center and wound is still not healed after 48 h (Fig. 4F). 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 [36]. 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 more stem cell like cells having an enhanced tumorigenic, proliferative and metastatic potential [37–40].

CD271 has dual role being proliferative to stimulate survival or anti-proliferative to stimulate cell death [32, 41]. In this study the expression of CD271 was investigated among breast cancer patients along with cancer stem cells (MCF7-CSCs) to define its role in cell proliferation. We found that CD271 was induced independent of clinical factors among all breast cancer patients when compared to adjacent non-cancerous control. It suggests 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 [42], CD24 with invasive breast carcinoma and early-stage cancer [43, 44], ki-67 is associated with higher stage and higher-grade tumor [45] while CD271 in this study was induced independent with any clinical parameter. The data in this study points towards a facilitation role of CD271 in cancer cell survival in general.

CD271 affects the proliferation of cells by activation of MAPK, (PI3K)/AKT and NF-κB signaling pathway [32, 46]. Our data suggests that induced CD271 can promoter cell proliferation and metastasis via NF-κB pathway and its downstream targets Nanog and Sox2. Correlation study also showed the positive correlation between both CD271 and NF-κB. Upon treatment with TQ, a G-quadruplex binder, CD271 expression was reduced which corresponded with cell movement in the wound healing assay. The promoter sequence analysis revealed NF-κB binding site overlapping a putative quadruplex forming sequence. NF-κB subunits NF-κB1 and NF-κB2 after translocation to the nucleus, dimerize and bind to the promoter sequences of target gene CD271 [47]. The TQ binds to NF-κB2 and hinder its dimerization with NF-κB1 [48]. This hinders the binding of NF-κB dimers to its downstream genes. The TQ could also interact with the putative promoter G-quadruple of CD271 and downregulate its expression by hindering the NF-κB binding. No G-rich sequences were identified with in promoter region of Sox2 and Nanog genes, which suggest that the inhibition in NF-κB1 expression level has inhibited the expression level of its downstream genes Nanog and Sox2 as well. The data suggest that TQ is reducing the expression of CD271 by binding to its promoter quadruplex which further inhibited the downstream genes. 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 role of CD271 receptor in the activation of NF-κB pathway. By computational analysis later we have found NF-κB1 binding at CD271 promoter. The data is pointing towards a positive feedback loop relation between NFKB and CD271. NF-κB is a transcription factor of CD271 which activates the CD271 genes and higher-grade tumor [45] while CD271 in this study was induced independent with any clinical parameter. The data in this study points towards a facilitation role of CD271 in cancer cell survival in general.
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.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11033-021-06900-1.

Acknowledgements We highly appreciated the efforts of the local hospitals (PIMS and Holy family) for providing breast cancer patient’s samples. Additionally, we are thankful to Dr Muhammad Abubakar National Veterinary Laboratory NARC, Islamabad for providing us the facilities to conduct important part of the research.

Author contributions RS and KM conceptualized and designed the experiment. NB and MI performed the experiments. The manuscript was written by NB and edited by RS and KM.

Funding This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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

Declarations

Conflict of interest The authors declare no conflict of interest.

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.

References

1. Nagai H, Kim YH (2017) Cancer prevention from the perspective of global cancer burden patterns. J Thorac Dis 9:448

2. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F (2021) Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. https://doi.org/10.3322/caac.21660

3. Yu Y, Ramena G, Eible RC (2012) The role of cancer stem cells in relapse of solid tumors. Front Biosci 4:1528

4. Elbaiomy MA, Akl T, Atwan N, Elsayed AA, Elzaafaryan M, Shamaa S (2020) Clinical impact of breast cancer stem cells in metastatic breast cancer patients. J Oncol. https://doi.org/10.1155/2020/2561726

5. Deng X, Xu G, He L, Xu M (2017) p75NTR promotes survival of breast cancer resistant cells by regulating Bcl-2/Bax and MAPK pathway. Int J Clin Exp Pathol 10:11685–11694

6. Tomellini E, Lagadec C, Polakowska R, Le Bourhis X (2014) Role of p75 neurotrophin receptor in stem cell biology: More than just a marker. Cell Mol Life Sci 71:2461

7. Bhakar AL, Howell JL, Paul CE, Salehi AH, Becker EBE, Said F, Bonni A, Barker PA (2003) Apoptosis induced by p75NTR overexpression requires jun kinase-dependent phosphorylation of bad. J Neurosci. https://doi.org/10.1523/jneurosci.23-36-11373.2003

8. Filipp FV, Li C, Boiko AD (2019) CD271 is a molecular switch with divergent roles in melanoma and melanocyte development. Sci Rep. https://doi.org/10.1038/s41598-019-42773-y

9. Descamps S, Toillon RA, Adriaenssens E, Pawlowski V, Cool SM, Nurcombe V, Le Bourhis X, Boily B, Peyrat JP, Hondermarck H (2001) Nerve growth factor stimulates proliferation and survival of human breast cancer cells through two distinct signaling pathways. J Biol Chem. https://doi.org/10.1074/jbc.M010499200

10. Reis-Filho JS, Steele D, Di Palma S, Jones RL, Savage K, James L, Milanesi F, Schmitt FC, Ashworth A (2006) Distribution and significance of nerve growth factor receptor (NGFR/p75NTR) in normal, benign and malignant breast tissue. Mod Pathol 19:307–319. https://doi.org/10.1038/modpathol.3800542

11. Khwaja F, Tabassum A, Allen J, Djakiew D (2006) The p75NTR tumor suppressor induces cell cycle arrest facilitating caspase mediated apoptosis in prostate tumor cells. Biochem Biophys Res Commun. https://doi.org/10.1016/j.bbrc.2006.01.073

12. Tabassum A, Khwaja F, Djakiew D (2003) The p75NTR tumor suppressor induces caspase-mediated apoptosis in bladder tumor cells. Int J Cancer. https://doi.org/10.1002/ijc.11038

13. Jin H, Pan Y, Zhao L, Zhai H, Li X, Sun L, He L, Chen Y, Hong L, Du Y, Fan D (2007) p75 neurotrophin receptor suppresses the proliferation of human gastric cancer cells. Neoplasia. https://doi.org/10.1593/neo.07175

14. Yuanlong H, Hai-feng J, Xiao-yin Z, Jia-yan S, Jie L, Li Y, Huahong X, Jiugang S, Yanglin P, Kaichun W, Jie D, Daiming F (2008) The inhibitory effect of p75 neurotrophin receptor on growth of human hepatocellular carcinoma cells. Cancer Lett. https://doi.org/10.1016/j.canlet.2008.03.038

15. Rocha AS, Risberg B, Magalhães J, Trovisco V, de Castro IV, Lazarovic P, Soares P, Davidson B, Sobrinho-Simões M (2006) The p75 neurotrophin receptor is widely expressed in conventional papillary thyroid carcinoma. Hum Pathol. https://doi.org/10.1016/j.humpath.2005.12.016

16. Truzzi F, Marconi A, Lotti R, Dallaglio K, French LE, Hempstead BL, Pincelli C (2008) The p75 neurotrophin receptor is widely expressed in conventional papillary thyroid carcinoma. J Investig Dermatol. https://doi.org/10.1038/jid.2008.21

17. Betters E, Liu Y, Kjaeldgaard A, Sundström E, García-Castro MI (2010) Analysis of early human neural crest development. Dev Biol. https://doi.org/10.1016/j.ydbio.2010.05.012

18. Tomellini E, Touil Y, Lagadec C, Julien S, Ostyn P, Daingnin F, Bonni A, Barker PA (2003) Apoptosis induced by p75NTR overexpression requires jun kinase-dependent phosphorylation of bad. J Neurosci. https://doi.org/10.1523/jneurosci.23-36-11373.2003
keratinocyte stem cells in vitro. Oncogene. https://doi.org/10.1038/sj.onc.1206525
20. Di Girolamo N, Sarris M, Chui J, Cheema H, Coronoe MT, Wakefield D (2008) Localization of the low-affinity nerve growth factor receptor p75 in human limbal epithelial cells. J Cell Mol Med. https://doi.org/10.1111/j.1582-4934.2008.00290.x
21. Li X, Shen Y, Di B, Li J, Geng J, Lu X, He Z (2012) Biological and clinical significance of p75 NTR expression in laryngeal squamous epithelia and laryngocarcinoma. Acta Otolaryngol. https://doi.org/10.3109/00016489.2011.639086
22. Tsang JYS, Wong KHY, Lai MWH, Lacambra MD, Ko CW, Chan SK, Lam CCF, Yu AMC, Tan PH, Tse GM (2013) Nerve growth factor receptor (NGFR): a potential marker for specific molecular subtypes of breast cancer. J Clin Pathol 66:291–296. https://doi.org/10.1136/jclinpath-2012-201027
23. Aragona M, Panetta S, Silipigni AM, Romeo DL, Pastura G, Mesiti M, Cascini S, La Torre F (2001) Nerve growth factor receptor immunoreactivity in breast cancer patients. Cancer Investig 19:692–697. doi.org/10.1081/CNV-100106144
24. Liu T, Zhang L, Joo D, Sun SC (2017) NF-κB signaling in inflammation. Signal Transduct Target Ther 2:1–9
25. Dolle L, Adriaenssens E, Yazidi-Belkoura I, Bourhis X, Nurcombe V, Hondermarck H (2005) Nerve growth factor receptors and signaling in breast cancer. Curr Cancer Drug Targets. https://doi.org/10.2174/1568009043328553
26. Xia Y, Shen S, Verma IM (2014) NF-κB, an active player in human cancers. Cancer Immunol. Res.
27. Pratt MAC, Tibbo E, Robertson SJ, Jansson D, Hurst K, Perez- Iraxteta C, Lau R, Niu MY (2000) The canonical NF-B pathway is required for formation of luminal mammary neoplasias and is activated in the mammary progenitor population. Oncogene. https://doi.org/10.1038/onc.2009.131
28. Xia L, Tan S, Zhou Y, Lin J, Wang H, Oyang L, Tian Y, Liu L, Su M, Wang H, Cao D, Liao Q (2018) Role of the NFXb-signaling pathway in cancer. Onco Targets Ther. https://doi.org/10.2147/OTT.S161109
29. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2-ΔΔCT method. Methods. https://doi.org/10.1006/meth.2001.1262
30. Kikin O, D’Antonio L, Bagga PS (2006) QGRS mapper: a web-based server for predicting G-quadruplexes in nucleotide sequences. Nucleic Acids Res. https://doi.org/10.1093/nar/gkl253
31. Kreti L, Soete A, Hulpian P, Botzki A, Saeyes Y, De Bleser P (2017) ConTra v3: a tool to identify transcription binding factor sites across species, update 2017. Nucleic Acids Res. https://doi.org/10.1093/nar/gkx376
32. Mochizuki M, Tamai K, Imai T, Sugawara S, Ogama N, Nakamura M, Matsuura K, Yamaguchi K, Sato K, Sato I, Motohashi H, Sugamura K, Tanaka N (2016) CD271 regulates the proliferation and motility of hypopharyngeal cancer cells. Sci Rep. https://doi.org/10.1038/srep30707
33. Huang MC, Te Chul, Wang ZF, Lin S, Chang TC, Chen CT (2018) A g-quadruplex structure in the promoter region of CLIC4 functions as a regulatory element for gene expression. Int J Mol Sci. https://doi.org/10.3390/ijms19092678
34. Khan MA, Tania M, Fu S, Fu J (2017) Thymoquinone, as an anticancer molecule: from basic research to clinical investigation. Oncotarget 8:51907
35. Dastjerdi MN, Mehdibady EM, Irampion FG, Bahramian H (2016) Effect of thymoquinone on P53 gene expression and consequence apoptosis in breast cancer cell line. Int J Prev Med. https://doi.org/10.4103/2008-7802.180412
36. Ayob AZ, Ramasamy TS (2018) Cancer stem cells as key drivers of tumour progression. J Biomed Sci 25:1–18
37. Civenni G, Walter A, Kobert N, Mihic-Probst D, Zipser M, Belloni B, Seifert B, Moh H, Dummer R, Van Den Broeck M, Sommer L (2011) Human CD271-positive melanoma stem cells associated with metastasis establish tumor heterogeneity and long-term growth. Cancer Res. https://doi.org/10.1158/0008-5472.CAN-10-3997
38. Li S, Yue D, Chen X, Wang L, Li J, Ping Y, Gao Q, Wang D, Zhang T, Li F, Yang L, Huang L, Zhang Y (2015) Epigenetic regulation of CD271, a potential cancer stem cell marker associated with chemoresistance and metastatic capacity. Oncol Rep. https://doi.org/10.3892/or.2014.3569
39. Chung MK, Jung YH, Lee JK, Cho SY, Murillo-Sauca O, Uppaluri R, Shin JH, Sunwoo JB (2018) CD271 confers an invasive and metastatic phenotype of head and neck squamous cell carcinoma through the upregulation of slug. Clin Cancer Res. https://doi.org/10.1158/0008-0432.CCR-17-0866
40. Elkashty OA, Elghanam GA, Su X, Liu Y, Chauvin PJ, Tran SD (2020) Cancer stem cells enrichment with surface markers CD271 and CD44 in human head and neck squamous cell carcinomas. Carcinogenesis. https://doi.org/10.1093/carcin/bgz182
41. Redmer T, Welte Y, Behrens D, Fichtner I, Przybilla D, Wruck W, Yaso ML, Lehrach H, Schäfer R, Regenbrecht CRA (2014) The nerve growth factor receptor CD271 is crucial to maintain tumorigenicity and stem-like properties of melanoma cells. PLoS ONE. https://doi.org/10.1371/journal.pone.0092596
42. Gündüz UR, Gundali M, Isiksan N, Gündüz S, Okuturlar Y, Kocoglu H (2016) A new marker for breast cancer diagnosis, human epididymis protein 4: a preliminary study. Mol Clin Oncol. https://doi.org/10.3892/mco.2016.919
43. Kristiansen G, Winzer KJ, Mayordomo E, Bellach J, Schlüns K, Denkert C, Dahl E, Pilsarky C, Altevogt P, Guskii H, Dietel M (2003) CD271 expression is a new prognostic marker in breast cancer. Clin Cancer Res 9:4906
44. Kwon MJ, Han J, Jo H, Lee SH, Lee SY, Shin JH, Kim YJ, Lee SH, Lai CY, Shin YK (2015) CD271 overexpression is associated with poor prognosis in luminal a and triple-negative breast cancer. PLoS ONE. https://doi.org/10.1371/journal.pone.0139112
45. Kanyilmaz G, Benli Yavuz B, Aktan M, Karaagac M, Uyar M, Denkert C, Dahl E, Pilsarky C, Altevogt P, Guskii H, Dietel M (2003) CD271 expression is a new prognostic marker in breast cancer. Clin Cancer Res 9:4906
46. Kwon MJ, Han J, Jo H, Lee SH, Lee SY, Shin JH, Kim YJ, Lee SH, Lai CY, Shin YK (2015) CD271 overexpression is associated with poor prognosis in luminal a and triple-negative breast cancer. PLoS ONE. https://doi.org/10.1371/journal.pone.0139112
47. Kanyilmaz G, Benli Yavuz B, Aktan M, Karaagac M, Uyar M, Denkert C, Dahl E, Pilsarky C, Altevogt P, Guskii H, Dietel M (2003) CD271 expression is a new prognostic marker in breast cancer. Clin Cancer Res 9:4906
48. Oeckinghaus A, Ghosh S (2009) The NF-kappaB family of transcription factors and its regulation. Cold Spring Harb Perspect Biol 1:a000034
49. Sethi G, Kwang SA, Aggarwal BB (2008) Targeting nuclear factor-κB activation pathway by thymoquinone: role in suppression of antiapoptotic gene products and enhancement of apoptosis. Mol Cancer Res. https://doi.org/10.1158/1541-7786.MCR-07-2088
Publisher’s Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.