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Dendrosomal Nano-Curcumin Downregulates *CCAT2* Expression Levels and Promotes Cell Cycle Arrest and Apoptosis in Tamoxifen-Resistant *MCF-7* Cells

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ABSTRACT

Objectives: Tamoxifen (TAM) is routinely used for the treatment of estrogen-positive breast carcinoma. Approximately 40% of patients with metastatic breast cancer will develop resistance to TAM. TAM therapeutic failure has been a major challenge in the treatment of TAM-resistant breast cancer cells. Therefore, finding a way to eliminate TAM resistance is very valuable. Curcumin is a polyphenol extracted from the rhizomes of *Curcuma longa* and has extensive biological and pharmacological effects on many cancers. The purpose of this study was to look into the effects of dendrosomal nano-curcumin (DNC) on cell growth and apoptosis, as well as the effects of DNC on the expression levels of long non-coding RNA *CCAT2* in TAM-resistant MCF-7 cells (TAM-R). Methods: TAM-R cells were created, and *CCAT2* expression was evaluated in TAM-R compared to TAM-sensitive MCF-7 cells (TAM-S). Forty eighth hours after TAM-R treatment with 20 μM of DNC, Q-RT-PCR, and flow cytometry cell cycle and Annexin V-PI assays were performed. P-value < 0.05 was defined as statistical significance. Results: *CCAT2* was significantly upregulated in TAM-R compared to TAM-S. DNC administration downregulated *CCAT2* expression, and markedly suppressed cell cycle and induced apoptosis in TAM-R. Furthermore, DNC decreased the anti-apoptosis gene (*BCL-2*) and increased the apoptotic gene (*BAX*) expression levels respectively in TAM-R. Conclusion: DNC promoted cell cycle arrest and apoptosis, eventually by *CCAT2* downregulation in TAM-R. However, the probable mechanisms of how DNC affects *CCAT2* expression levels are unknown and need future studies.

Keywords: Dendrosomal nano-curcumin, *CCAT2*, Tamoxifen-resistant MCF-7, Cell cycle, Apoptosis

INTRODUCTION

Breast cancer is the most frequent cancer in women and the second-leading cause of cancer death worldwide [1]. It is estimated that at least two-thirds (70%) of patients with breast cancer are estrogen-positive (ER+) [2]. In addition to surgery, endocrine gland therapy, such as the medication tamoxifen (TAM), has improved patients' overall survival rates and quality of life for those with breast cancer. The most popular hormone therapy, TAM, operates as an estrogen antagonist in the cases of breast cancer [3]. TAM is used to treat the majority of patients with ER+ breast cancer, but 40% of patients have recurrence due to TAM resistance [4,5]. TAM therapeutic failure has been a major challenge in the treatment of TAM-resistant breast cancer cells. Therefore, it is crucial to fully comprehend the components and mechanisms underlying the emergence of TAM-resistant cells. TAM resistance can be mediated by a variety of mechanisms, including ER depletion, the induction of abnormal estradiol levels, and changes in nuclear proteins such as histone de-acetylation and non-coding RNAs deregulation (long non-coding RNAs and microRNAs) [3,5-9]. The dysregulated expression of long non-coding RNAs, which do not code for any proteins and were originally believed to be a part of the junk genome, is one of the mechanisms of TAM resistance. The relevance of these lengthy non-coding RNAs, however, has only recently come to light. As a result, their capacity to control the tumor's recurrence and resistance to chemotherapy and medication has been assessed [7,10]. Long non-coding

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RNAs, which are approximately longer than 200 nucleotides [11] are gradually becoming significant biomarkers and therapeutic targets in a variety of human malignancies [12-14]. The long non-coding RNA colon cancer-associated transcript-2 (*CCAT2*), which has a 1752 base pair size, was found on chromosome 8 (8q24.21). It has been reported that *CCAT2* increases tumorigenesis and tumor progression in multiple cancers, such as colorectal cancer [15], esophageal squamous cell carcinoma [16], and lung cancer [17]. *CCAT2* also promotes tumor growth and metastasis [17,18] while decreasing sensitivity to chemotherapy [19]. According to reports, *CCAT2* also possesses a trait linked to cancer stem cells (CSCs) [20].

There is evidence that *CCAT2* may act biologically as an oncogene in breast cancer cells. For instance, Y. Cai *et al.* (2015) confirmed that *CCAT2* is highly expressed in breast cancer tissues and cell lines [21]. R.S. Redis *et al.* (2013) found higher levels of *CCAT2* in breast tumor samples compared to the non-tumor ones [19]. It has been demonstrated that *CCAT2* is overexpressed in TAM-resistant cells. Y. Caia *et al.* (2016) established two TAM-resistant cell models (MCF-7 and T47D) and showed higher levels of *CCAT2* expression compared to TAM-sensitive cells [22]. Similarly, F. Moradi *et al.* (2022) created a model of TAM-resistant MCF-7 cells and confirmed CCAT2 upregulation in TAM-resistant MCF-7 cells compared to TAM-sensitive MCF-7 cells [23].

Curcumin is a hydrophobic polyphenol that was isolated from *Curcuma longa* plant roots in 1815, and its chemical formula was determined as $C_{21}H_{20}O_6$ in 1910. However, curcumin's advantages are diminished by its extremely poor water solubility, minimal absorption, quick metabolism, and quick removal from the body. Dendrosomes as carriers of curcumin are micelle- or polymersome-like polymeric structures that were first described by M.N. Sarbolouki *et al.* (2000) [24,25]. In recent years, M. Tahmasebi-Mirgani *et al.* (2014) proposed a unique formulation of dendrosomal nanoparticle (OA400 carrier), which was produced by esterification of oleic acid and polyethylene glycol in the presence of chloroform as solvent [26,27] (Fig. 1). This highly effective nanocarrier increased the curcumin substance's water solubility and its bioavailability by enclosing almost 87% of curcumin in spherical micellar or polymersome structures. Dendrosomal curcumin with this nanoformulation, due to its tiny size (roughly 142 nm), proved appropriate for systemic delivery and provided enough physical and chemical stability [27].

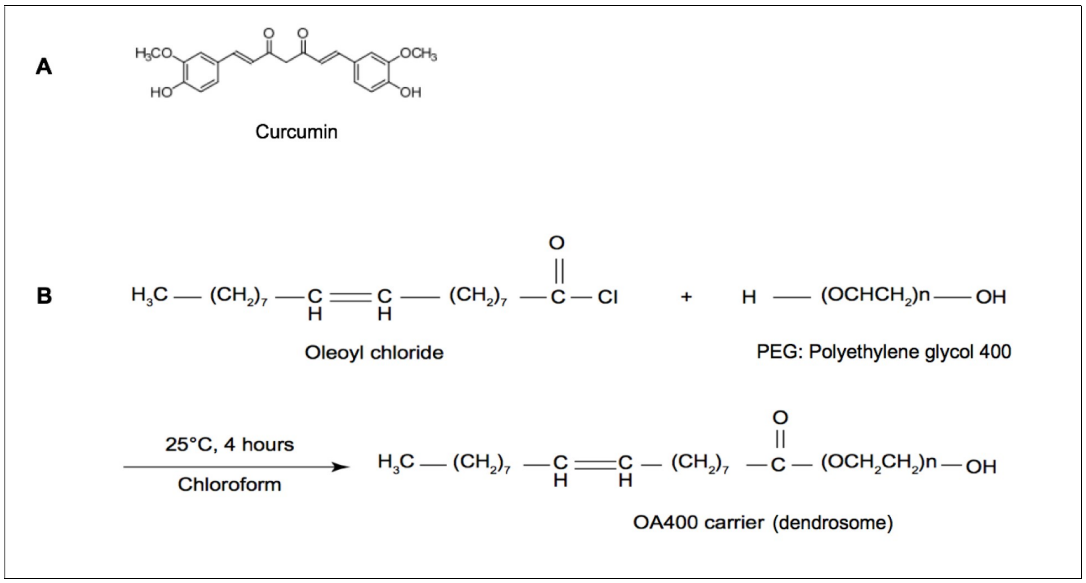

Fig. 1. A) Structure of free curcumin and B) structure of dendrosome. The synthesis method of dendrosomal nanoparticles called OA400 carrier, which is synthesized from the esterification of oleic acid and polyethylene glycol residues in the presence of chloroform as a solvent. Figure is adapted from reference [26].

Curcumin has been shown to possess a variety of anti-tumor effects, including the ability to suppress tumor growth and development by preventing angiogenesis, invasion, metastasis, and proliferation, as well as by inducing apoptosis [26,28,29]. E. Babaei *et al.* (2012) published the first report on the effects of dendrosomal nano-curcumin (DNC), in which the anticancer potential of DNC was investigated on the fibrosarcoma mouse model [29]. DNC has been shown to have anticancer properties in vitro and *in vivo* in a variety of mouse and human cancer cells, including glioblastoma [27,30], bladder [31], breast [32] and hepatocellular carcinoma [33-35]. It has also been shown that dendrosomal nanocarriers have no cytotoxic effects on normal cells [27,29]. Finally, these results introduce DNC as a potent antitumor agent.

Additionally, growing data suggests that curcumin is crucial in the development of cancer through changing the expression of particularly long non-coding RNAs in a range of malignancies [26,30,36-38]. According to clinical trials, curcumin, either by itself or in combination with other medications, exhibits anti-cancer properties in breast cancer patients without causing any negative side effects. Numerous studies demonstrate that curcumin overcomes chemoresistance and makes breast cancer cells more sensitive to chemotherapy and targeted therapy [32,38,39] and resistant to TAM in breast cancer cells [40-42]. However, whether curcumin could regulate the expression of long non-coding RNA *CCAT2* in TAM-resistant cells remains largely unknown. The purpose of this study was to look into the effects of DNC on cell growth and apoptosis, as well as the effects of DNC on the expression levels of long non-coding RNA *CCAT2* in TAM-resistant MCF-7 cells.

MATERIALS AND METHODS

Study Design

Approximately 70% of breast cancer patients are estrogen receptor positive (ER+). Among all drugs, tamoxifen (TAM) is the most widespread hormone therapy and acts as an estrogen antagonist in breast cancer. Although most patients with ER+ breast cancer are treated with TAM, many tumors recur due to resistance to TAM. One of the mechanisms that leads to patients' resistance to TAM is the aberrant expression of long non-coding RNAs. Investigating a method or a drug that can specifically sensitize resistant cells to TAM can promise to stop the resistance and lead resistant cells to apoptosis. Therefore, studying the role and expression relationships of important long non-coding RNAs for the management of TAM resistance is very valuable. On the other hand, investigating the effects of curcumin, which is a yellow-orange component of turmeric, on modulating the aberrant expression of long non-coding RNAs involved in TAM resistance, can help to better understand the mechanisms of resistance. According to studies conducted so far, curcumin has anticancer and detoxifying properties in many cancers. The effects of curcumin in sensitizing drug-resistant cancer cells have been reported, but the effects of curcumin on the expression levels of long non-coding RNA *CCAT2* in TAM-resistant cells have not been reviewed yet. Therefore, the present research was necessary to evaluate the anticancer effects of DNC on the expression levels of *CCAT2* in TAM-resistant MCF-7 cells and also to assess the impacts of DNC on the cell cycle and apoptosis of TAM-resistant MCF-7 cells.

Cell Culture

MCF-7, a human breast cancer cell line, was purchased from the Iranian cell bank at the Pasteur Institute in Tehran, Iran. The Gibco DMEM cell culture medium was used for the cell culture, and it was supplemented with 10% fetal bovine serum (FBS) and 1% penicillin and streptomycin (all purchased from Thermo Fisher Scientific). MCF-7 cells were incubated at 37°C in a humid incubator that supplied 5% CO_2. The cells were subcultured each time they reached 80% confluence.

Tamoxifen (TAM) (CAS number: 54956-24-1) was purchased from the Xian Mellon Chemical Technology Company in Xian, China. TAM is continuously given to MCF-7 cells to create TAM-resistant MCF-7 cells. TAM was added to the culture initially at a concentration of 0.35 µg ml^{-1}. The cells received the same dose of TAM twice weekly, with a slight increase in dose the following weeks. The TAM concentration was gradually increased for four months, reaching a maximum of 16 µg ml^{-1}. The procedures were carried out as previously mentioned [42,43].

DNC was obtained from Dr. Sadeghizadeh's lab (Lab 4408 from the Genetics Group at Tarbiat Modares University, Tehran, Iran). TAM-resistant MCF-7 cells were exposed to 20 µM of DNC in addition to their natural medium of 25 ml flasks for 48 h. The concentration of DNC was determined as described in previous investigations [26,42].

Primer Design, Extraction of RNA, Synthesis of cDNA, and Q-RT-PCR

Using Trizol (Invitrogen), total RNA was extracted from the grown cells and then subjected to RNase-free DNase treatment (Fermentase, Lithuania). After that, the quality and purity of the isolated RNA were examined using spectrophotometry and gel electrophoresis. Then, using PrimeScript reverse transcriptase (TAKARA, Japan) and random hexamers, the manufacturer's instructions were followed to make cDNA from RNA (1 µg). The quantitative evaluation of the study genes was carried out using quantitative real-time reverse transcriptase-polymerase chain reaction (Q-RT-PCR) in the ABI Step One Sequence Detection System (Applied Bio-Systems, CA, USA). SYBR® Premix Ex TaqTM II (TAKARA, Japan) was used in this procedure in accordance with the manufacturer's instructions. Primers were designed using Oligo7 software and were checked by PrimerBLAST tools for specificity. There is a list of specific sequences of primers used in this investigation (Table 1). An internal reference was made using the *GAPDH* gene. The relative expression of genes was calculated using the $2^{-\Delta\Delta Ct}$ method. GraphPad Prism (version 7) was used to describe the results.

Cell Cycle Analysis

TAM-resistant MCF-7 cells were taken out 48 h after being exposed to DNC therapy, centrifuged for 5 min at 1,300 rpm, and then washed twice with PBS. The cells are then fixed in 500 µl of 70% cold ethanol for two hours. Next, they were stained with 500 µl of RI/RNase staining solution and kept at 37 °C in the dark for 30 min. The cells were immediately passed through a flow cytometer (FACS Calibur, USA). The FlowJo software was used for evaluation and analysis (version 10, TreeStar, USA).

Apoptosis Analysis

Forty-eight hours after DNC treatment, the apoptosis of TAM-resistant MCF-7 cells was examined using the Annexin V-FITC/PI staining kit (Roche, Germany). The cells were cultured into 24-well plates of full DMEM medium with serum. The cells were then suspended in a binding buffer, washed in cold PBS, and stained for 15 min at room temperature with Annexin V-FITC/PI in the dark. Next, the levels of apoptosis and necrosis cells in the cells were determined by flow cytometry (FACS Calibur, USA). The results were evaluated using the FlowJo software (version 10, TreeStar, USA).

Statistical Analyses

Each experiment was done in triplicate, independently. The student t-test was run using the GraphPad Prism (version 7) program to see whether there were any significant differences in the assessed variables across groups. P-value < 0.05 was defined as statistical significance.

Table 1. The Sequences of Primers Used in this Study

Gene	Forward (5'→3')	Reverse (5'→3')	Size (bp)
CCAT2	ATGAAGGCGTCGTCCAAATG	TGGAGCTGGAAGGGAAATCA	162
BAX	GCAAACTGGTGCTCAAGG	CAGCCACAAAGATGGTCA	183
BCL-2	GTGGATGACTGAGTACCTGA	GCCAGGAGAAATCAAACAGA	119
GAPDH	TGGATGCCACTGGCGTCTT	TTGCTGATGATCTTGAGGCTGT	160

RESULTS

CCAT2 Expression Levels were Elevated in TAM-Resistant MCF-7 Cells

To assess the expression levels of *CCAT2* in TAM-resistant MCF-7 cells, Q-RT-PCR was performed. The results showed that *CCAT2* was noticeably elevated by 1.7-fold in TAM-resistant MCF-7 cells compared to TAM-sensitive MCF-7 cells (p-value < 0.05, Fig. 2).

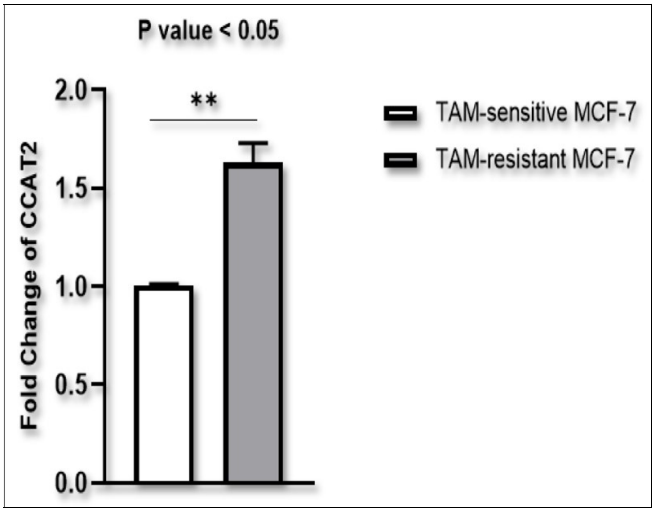

Fig. 2. *CCAT2* expression in TAM-resistant and TAM-sensitive MCF-7 cells. Q-RT-PCR results of the expression levels of *CCAT2* in TAM-resistant MCF-7 cells compared to TAM-sensitive MCF-7 cells. Means ± SEM was displayed. The student t-test was used for statistical analysis. P-value < 0.05 was defined as statistical significance.

Dendrosomal Nano-curcumin (DNC) Decreased the Expression Levels of *CCAT2* in TAM-resistant MCF-7 Cells

In order to evaluate the anticancer effects of DNC on *CCAT2* expression levels, TAM-resistant MCF-7 cells were treated with 20 µM DNC. The results of Q-RT-PCR showed that *CCAT2* expression was dramatically reduced by 0.6-fold after 48 h by 20 µM DNC treatment in TAM-resistant MCF-7 cells compared to untreated control cells (p-value < 0.05, Fig. 3).

Dendrosomal Nano-curcumin (DNC) Suppressed the Cell Cycle of TAM-resistant MCF-7 Cells

In order to evaluate the anticancer effects of DNC on the cell growth rate, TAM-resistant MCF-7 cells were treated with 20 µM DNC for 48 h. DNC treatment significantly increased the number of cells in the sub-G1 phase from 7.3 to 41.58 percent and at the same time decreased the number of cells in the S phase from 31.05 to 22.92 percent and also in the G2/M phase from 13.2 to 10.05 percent compared to untreated control cells (p-value < 0.0001, Figs. 4A, B).

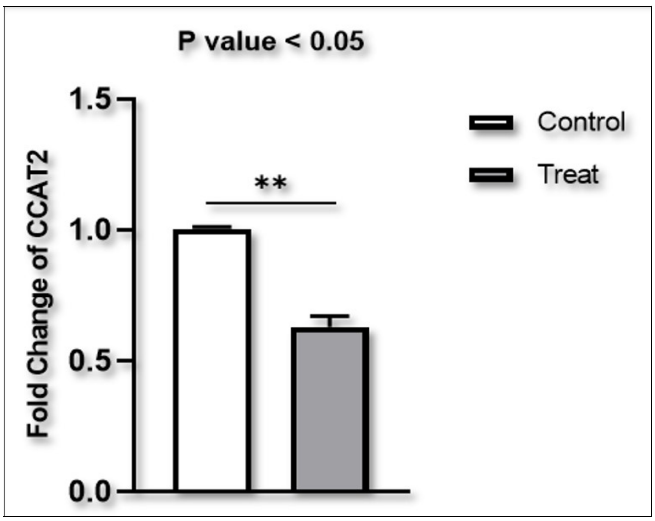

Fig. 3. The effects of DNC on the expression levels of *CCAT2* in TAM-resistant MCF-7 cells. Q-RT-PCR results of *CCAT2* expression levels in TAM-resistant MCF-7 cells after treatment with DNC compared to untreated control cells. Means ± SEM was displayed. The student t-test was used for statistical analysis. P-value < 0.05 was defined as statistical significance.

Dendrosomal Nano-curcumin (DNC) Promoted the Apoptosis of TAM-resistant MCF-7 Cells

In order to evaluate the anticancer effects of DNC on the cell apoptosis rate, TAM-resistant MCF-7 cells were treated with 20 µM DNC for 48 hours. After TAM-resistant MCF-7 cells were administrated with DNC, early and late apoptosis rates were considerably enhanced from 5.56 to 45.78 percent compared to untreated control cells (p-value < 0.001, Figs. 5A, B).

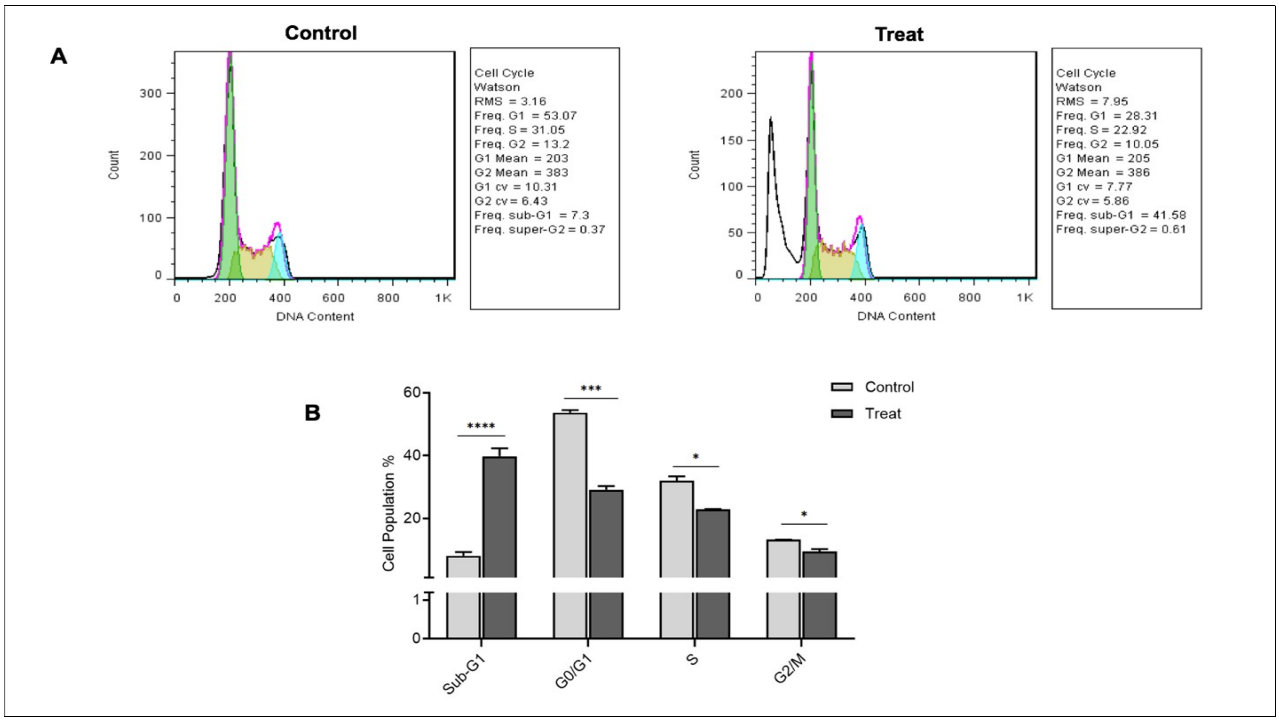

Fig. 4. The effects of DNC on the cell cycle phases of TAM-resistant MCF-7 cells. A) histogram and B) bar plot analysis of the cell cycle phases in TAM-resistant MCF-7 cells, 48 h after being exposed to DNC treatment, compared to untreated control cells. The student t-test was used for statistical analysis. P-value < 0.05 was defined as statistical significance.

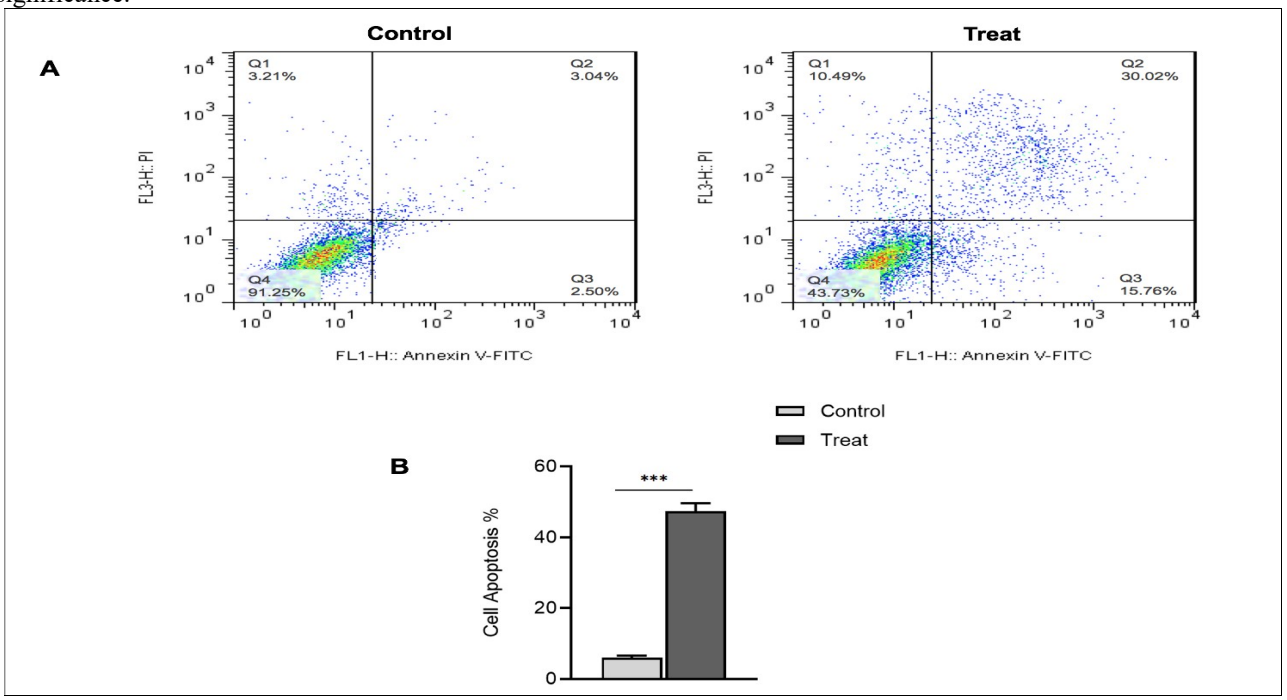

Fig. 5. The effects of DNC on the apoptosis rate of TAM-resistant MCF-7 cells. A) histogram and B) bar plot analysis of the apoptosis rate in TAM-resistant MCF-7 cells, 48 h after being exposed to DNC treatment, compared to untreated control cells. Means ± SEM was displayed. The student t-test was used for statistical analysis. P-value < 0.05 was defined as statistical significance.

Dendrosomal Nano-curcumin (DNC) Regulated the Expression of *BAX* and *Bcl-2* Genes, Favoring Apoptosis

To assess the anticancer effects of DNC on apoptotic (*BAX*) and anti-apoptotic (*BCL-2*) genes in TAM-resistant MCF-7 cells, the expression levels of these two genes were evaluated by Q-RT-PCR. The results of Q-RT-PCR showed that *BAX* expression was significantly increased by 2-fold and *BCL-2* expression was dramatically decreased by 0.5-fold after 48 h of exposure to 20 µM DNC in TAM-resistant MCF-7 cells compared to untreated control cells (p-value < 0.05, Figs. 6A, B).

DISCUSSION

The most widely utilized treatment medicine for treating ER-positive breast cancer patients is tamoxifen (TAM), one of the selective estrogen receptor modulators. However, 40 percent of ER+ patients with metastatic illnesses develop therapeutic resistance to TAM, and many initial responders subsequently relapse [4,5]. As a result, the main difficulty in treating breast cancer with TAM is TAM resistance. Numerous molecules have been discovered over the past 20 years as indicators of response to TAM therapy or as mediators of TAM resistance. To increase patient survival, it is essential to identify the molecules that underlie drug-resistant tumors as well as novel therapeutic approaches to overcome patients' resistance to TAM.

Researchers' focus has recently turned to long non-coding RNAs, a family of regulatory RNAs lacking a protein-coding function that can influence the levels of their target genes' expression [6]. The long non-coding RNA *CCAT2* was previously reported to contribute to TAM resistance in breast cancer cells, as Y. Caia *et al.* (2016) demonstrated that *CCAT2* was increased in TAM-resistant T47D and MCF-7 cells [22]. Increasing evidence has shown that curcumin exerts anticancer effects on TAM-resistant cells by acting on a variety of components involved in resistance to TAM. [40,41,44,45]. In the present study, it was found that curcumin could significantly inhibit the cell cycle and promote apoptosis in TAM-resistant MCF-7 cells by downregulating the long non-coding RNA *CCAT2*.

Curcumin has been found to have a wide range of biological qualities as a natural active ingredient, including

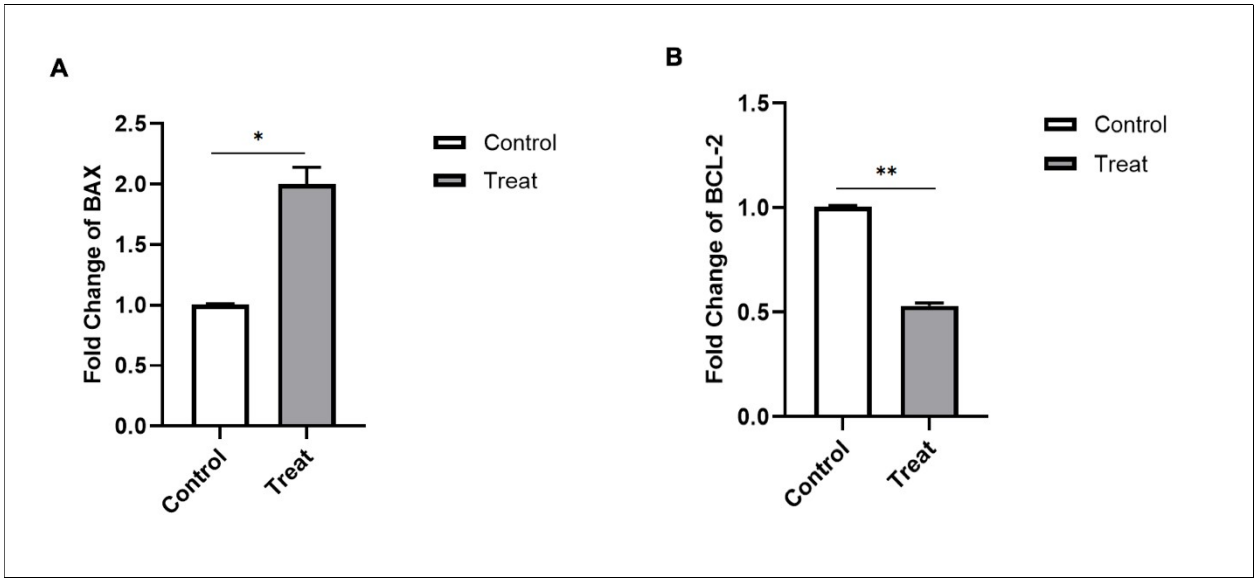

Fig. 6. The effects of DNC on the expression levels of *BAX* and *BCL-2* in TAM-resistant MCF-7 cells. A) Q-RT-PCR results of *BAX* expression levels in TAM-resistant MCF-7 cells after treatment with DNC compared to untreated control cells. B) Q-RT-PCR results of *BCL-2* expression levels in TAM-resistant MCF-7 cells after treatment with DNC compared to untreated control cells. Means ± SEM was displayed. The student t-test was used for statistical analysis. P-value < 0.05 was defined as statistical significance.

anti-inflammatory, anti-oxidative, anti-infection, anti-liver fibrosis, and anti-atherosclerosis capabilities [26]. Curcumin has been utilized in herbal remedies and food coloring for ages. Evidence has focused on the several anticancer effects of curcumin, including reduction of proliferation, invasion, metastasis, angiogenesis, and induction of apoptosis, showing that curcumin has a powerful therapeutic promise in a variety of cancers through controlling tumor growth [26,28,29]. Additionally, growing data suggest that curcumin plays a crucial role in the suppression of cancer development by changing the expression of particularly long non-coding RNAs in a range of malignancies [26,30,36-38]. Clinical studies have demonstrated that curcumin alone, or in combination with other drugs exhibits anti-cancer properties in ovarian cancer [37], prostate cancer [46], glioma cancer [30], and gastric cancer [47] without any negative side effects. Numerous studies have shown that curcumin restores chemoresistance and sensitizes cancer cells to chemotherapy and targeted therapy in breast cancer [32,38,39] and also in TAM-resistant breast cancer cells [40-42].

T. Choudhuri *et al.* (2002) revealed that curcumin increases the percentage of breast cancer cells with sub-G1 and G0/G1 DNA content [48]. Additionally, M.J.D. Esmatabadi *et al.* (2017 and 2018) demonstrated that DNC could stop cell growth and induce the apoptosis of breast cancer cells [32,38]. Furthermore, S. Hajigholami et al. (2018) reported that DNC suppressed the cell cycle and promoted the apoptosis of TAM-resistant MCF-7 cells, while DNC in combination with nano-tamoxifen had a synergistic effect on suppressing the cell cycle and promoting the apoptosis of MCF-7 cells resistant to TAM [42]. Additionally, M. Jiang *et al.* (2013) demonstrated that curcumin induced cell death, inhibited cell growth, and restored TAM sensitivity in TAM-resistant MCF-7/LCC2 and MCF-7/LCC9 breast cancer cell lines [40]. Similar to previous studies, in the present study, we demonstrated that DNC treatment significantly stopped the cell cycle and induced the apoptosis of TAM-resistant MCF-7 cells, implicating DNC as a crucial drug for the improvement of TAM-resistant breast cancer cells.

S. Hajigholami *et al.* (2018) showed that *BAX* and *BCL-2* genes were increased and decreased, respectively, by DNC treatment in TAM-resistant MCF-7 cells [42]. Furthermore, M. Jiang *et al.* (2013) demonstrated that curcumin suppressed development genes (c-Myc and cyclin D1) and *BCL-2* gene expression at both protein and mRNA levels in the TAM-resistant cells [40]. A.J Butt *et al.* (2005) and M.S Larsen *et al.* (2012) reported that breast cancer cells can produce TAM resistance by upregulating c-Myc, cyclin D1, and *BCL-2* gene expression [49,50]. Similar to previous studies, we found that DNC downregulates the expression levels of *BCL-2* (an anti-apoptotic gene) and upregulates the expression levels of *BAX* (an apoptotic gene) in breast cancer cells resistant to TAM. These results indicate that DNC can eventually modulate the apoptosis rate of TAM-resistant MCF-7 cells.

Data recently suggested that some long non-coding RNAs can serve as biomarkers or therapeutic targets in TAM-resistant cells [10,41]. Colon cancer-associated transcript-2 (*CCAT2*) is associated with a number of cancer types. For instance, H. Ling *et al.* first discovered *CCAT2* in 2013 and showed it has an oncogenic role in colorectal cancer cells by promoting tumor growth and metastasis. Additionally, they demonstrated that *CCAT2* was expressed at high levels in microsatellite-stable colorectal cancer tissues but at extremely low levels in normal colon tissues [18]. C.Y Wang *et al.* (2015) found that stomach cancer tissues expressed *CCAT2* at higher levels in comparison to nearby non-tumor tissues. They claimed that a poor prognosis for gastric cancer is linked to higher expression levels of *CCAT2* [51]. M. Qiu *et al.* (2014) indicated that non-small cell lung cancer tissues had considerably higher levels of *CCAT2* expression compared to their paired adjacent normal tissues. They also demonstrated that *CCAT2* induces non-small cell lung cancer invasion [17]. X. Zhang *et al.* (2015) showed that *CCAT2* was found to be increased in esophageal squamous cell carcinoma tissues, particularly in instances with lymph node metastases, advanced TNM stages, and MYC amplification. Additionally, they stated that high levels of *CCAT2* expression are linked to a poor prognosis in esophageal squamous cell carcinoma [16]. Y. Cai *et al.* (2015) found *CCAT2* to be highly expressed in breast tumor samples and cell lines [21]. Furthermore, R.S. Redis *et al.* (2013) reported a *CCAT2* relationship with clinical, histomorphological, and distant metastasis risks in breast cancer. They also showed that breast cancer tissues had

much higher levels of *CCAT2* expression than non-tumor tissues [19]. Moreover, Y. Caia *et al.* (2016) discovered that *CCAT2* was upregulated in TAM-resistant T47D and MCF-7 cells [22]. Similar to this, CCAT2 upregulation in TAM-resistant MCF-7 cells as opposed to TAM-sensitive MCF-7 cells was validated by F. Moradi *et al.* in 2022 [23].

Evidence suggests that CCAT2 plays an oncogenic role in breast cancer and TAM-resistant cells. In line with the previous studies, in the current study we verified that *CCAT2* expression was higher in TAM-resistant cells compared to TAM-sensitive cells. We found that DNC can downregulate the expression levels of the long non-coding RNA *CCAT2* oncogene. However, the probable mechanisms by which curcumin regulates the expression and function of *CCAT2* are unknown.

CONCLUSIONS

In the current research, it is demonstrated that DNC treatment significantly induces cell cycle arrest and apoptosis rate and also upregulates the expression levels of the apoptotic gene (*BAX*) and downregulates the expression levels of the anti-apoptotic gene *(BCL-2*) in TAM-resistant MCF-7 cells. We also found that DNC treatment downregulates the expression levels of long non-coding RNA *CCAT2* oncogene, which has a high expression levels in TAM-resistant MCF-7 cells. These results indicate that DNC can biologically control the cell cycle and apoptosis eventually by affecting the expression levels of the *CCAT2* oncogene in MCF-7 breast cancer cells resistant to TAM. However, the probable mechanisms of how DNC downregulates the expression levels of *CCAT2* are unknown and need future studies.

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