Original Article

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Multi-functional effects of curcumin on breast cancer cells

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Abstract

The rate at which cancer develops is significantly influenced by angiogenesis. The multifunctional effects of curcumin on breast cancer cells were examined in this study at the level of gene expression. Using the MTT assay, the cytotoxicity impact of curcumin was assessed. Real-time PCR was used to quantify the expression of a few genes involved in the angiogenesis, and apoptosis of MDA-231-MB cells. The results indicated that curcumin inhibited cancer proliferation in a dose- and time-dependent manner. The IC50 curcumin was about 40 µM and 15 µM after 48 h, and 96 h culture time respectively. Also, the quantitative analysis of gene expression showed that curcumin suppressed angiogenesis by decreasing the expression of VEGF and induced apoptosis by upregulation of Bax and down-regulation of Bcl-xl at the initial dose of toxicity concentration.

Keywords: Curcumin, Breast Cancer, Angiogenesis, Apoptosis

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1. Introduction

Neovascularization is associated with the ability of tumor progression to invasion, and metastasis (1). The growth of new blood vessels within malignant tumors is essential for their development and spread. One of the key processes involved in the development of cancer is angiogenesis, which is dependent on the expression and activation of some angiogenic supporting genes such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), and angiogenin (2). The expression of VEGF was considerably reduced in curcumin-treated cells (3). The dependence of cancer progression on angiogenesis has revealed a clear path for the effective targeting of this disease (4). Among all conventional methods in cancer treatment, the use of herbal medicines is expanding (5). It has been discovered that curcumin, the main bioactive component derived from turmeric, has several biological activities such as inhibition of glycolysis (6), angiogenesis (3), metastasis (1), and epithelial-mesenchymal transition process (7).

By influencing cell signaling pathways, curcumin changes the biological activity of cancer cells and inhibits cancer progression. Curcumin increased autophagic activity while at the same time decreasing Akt expression in MDA-MB-231 breast cancer cells in a dose- and time-dependent manner (8). The relationship between hypoxia-inducible factor 1-alpha (HIF-1α) and VEGF expression and their impact on tumor angiogenesis has been clearly defined. The decrease in Beclin1 expression occurs following the increase in curcumin concentration that shown this polyphenol regulates the production of Beclin1 and blocks the hypoxia-inducible factor to prevent the proliferation of pancreatic cancer cells (9).

Induction of apoptosis and cell cycle arrest are two mechanisms that occur in curcumin-

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treated cancer cells (10). Curcumin triggered apoptosis via a p53-mediated mechanism, upregulation of pro-apoptotic proteins Bax, and cell cycle arrest during the S phase by inhibiting cell cycle-related protein production (11).Curcumin-induced pyroptosis through an increase in expression of ISG3 transcription factor complex in leukemia cells U937 (12)

In this study, we showed that curcumin at the initial dose of toxicity concentration had multifunctional effects on triple-negative breast cancer (TNBC) cells. Curcumin decreased the expression of Bcl-xl and up-regulated Bax expression. Also, curcumin suppressed angiogenesis through reducing VEGF expression.

2. Material and Methods

2.1. Materials

Dulbecco's Modified Eagle's medium (DMEM), Fetal bovine serum (FBS), streptomycin, penicillin, and trypsin were purchased from GIBCO (InvitrogenTM, Grand Island, USA). 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) was purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemical reagents used in buffer preparation and solution were purchased from Merck company (Huhenbrunn, Germany).

2.2. Cell culture and media

The Iranian biological resource center (Tehran, Iran) provided the human breast cancer cell line MDA-231-MB. Two T-25 culture flasks with DMEM as the basal medium and 10% (v/v) FBS, 100 µg/ml streptomycin, and 100 IU/ml penicillin as supplements were used to cultivate the cells. The cell culture flasks were kept at 37 °C and 5% CO_2 in a humidified incubator.

2.3. Assessing the cytotoxicity

After 72 hours of incubation, the cells were collected from the T-75 culture flask using trypsin solution (0.25% w/v). The suspended cells were centrifuged at 90 g for 5 min to precipitate cells. The Re-suspended MDA-231-MB cells were subsequently transferred to a 96-well plate with a flat bottom at the same seeding density (10000 cells/cm2). Throughout the growth period, the cytotoxicity of curcumin on MDA-231-MB cells was examined at various doses. The 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) test was used to measure cell growth. The MTT solution (5 mg/ml in PBS) was applied to each well of the 96-well plate at the end of both periods of culture (48 and 96 h), and the plate was then incubated for 3 hours at 37 degrees °C in a humidified environment (95%) with 5% CO_2. The MTT solution was aspirated, and DMSO was then applied to the culture wells. A multi-well scanning spectrophotometer was then used to measure the optical density of the cells at 570 and 620 nm.

2.4. Real-Time Polymerase Chain Reaction

The cells were twice rinsed with PBS after aspirating the medium from a 6 cm^2 culture dish. After 48 hours of incubation, the cells were extracted from the culture dishes using a 0.25% w/v solution of trypsin. Centrifuging at 90 g for 5 min precipitated the suspended cells. When arrived time to extract the RNA, cell pellets were re-suspended in 200 µl of PBS and stored at -80 oC. A high pure RNA isolation kit (Roche Ap-

Table 1. Primer sequences used in real-time PCR.

Primer	Sequence: 5′ → 3′
HPRT1	ATGCTGAGGATTTGGAAAGGG
	ACAGAGGGCTACAATGTGATGG
TB	GATAAGAGAGCCACGAACCACG
	TTGAAGTCCAAGAACTTAGCTGG
Bax	TGCCAGCAAACTGGTGCTC
	AACCACCCTGGTCTTGGAT
Bcl-xL	GAGACTCAGTGAGTGAGCAGGTG
	GCTTGTAGGAGAGAAAGTCAACC
VEGF	GGAGGGCAGAATCATCACGAAG
	ATCGCATCAGGGGCACACAG

plied Science, Germany) was used to extract total RNA in accordance with the manufacturer's instructions. A Nanodrop spectrophotometer from Thermo Fisher Scientific was used to measure the amount and quality of RNA. Thermo Fisher Scientific's RevertAid First Strand cDNA Synthesis Kit was used in accordance with the manufacturer's instructions to synthesize cDNA from an RNA template. In Table 1, the primer sequences used in real-time PCR were given. The StepOneTM 48-Well Real-Time PCR System from Applied Biosystems and SYBR® Green Real-Time PCR Master Mix were used to perform the qRT-PCR study. HPRT1 and TBP were used as reference genes for relative quantification utilizing the comparative Ct ($2^{-\Delta\Delta Ct}$) approach.

2.5. Statistical analysis

The majority of the experiments were done in triplicate. Using the Excel 2016 program, the data were computed as the mean of two experiments. SPSS software (version 16) was used to perform all statistical computations. The statistical analysis was performed using the independent-samples T-test, and a P value of 0.05 was accepted as significant. Using REST 2009 software (V2.0.13), the significance level for the expression of the target genes was examined.

3. Result

3.1. Cytotoxicity of curcumin

The outcomes demonstrated that curcumin's cytotoxic effects on MDA-231-MB cells were time- and dose-dependent. Figure 1 shows that the viability was obtained by approximately 90% and 38% ($p < 0.05$) at the concentration of 20 µM after 48- and 96-hours culture time, respectively. The growth inhibition threshold of cancer cells treated with curcumin was observed at a concentration of 10 µM, and 72 hours after this treatment (96 h culture time), the cell viability reached 86%. Figure 2 depicts the morphology of MDA-231-MB cells at initial toxic concentration of curcumin.

3.2. Multifunctional effects of curcumin on genes expression

As shown in Figure 3, curcumin in the initial toxic concentration (10 µM) had multifunctional effects on the expression of some genes involved in the process of apoptosis and angiogenesis. The decrease in the expression of Bcl-xl at the same time as the increase in Bax proved that curcumin induces apoptosis in the treated cells at the initial toxic concentrations. The expression ratio of Bax to Bcl-xl has increased by about 2 times compared to the control group. Also, the reduction

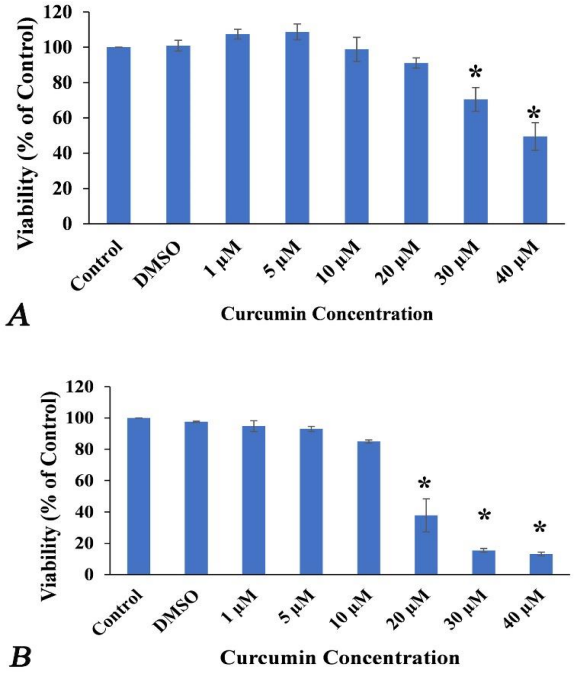

Figure 1. Cytotoxicity effect of curcumin on MDA-MB 231 at a dose of 1-40 µM. (A) at 48 h culture time, (B) at 96 h culture time.

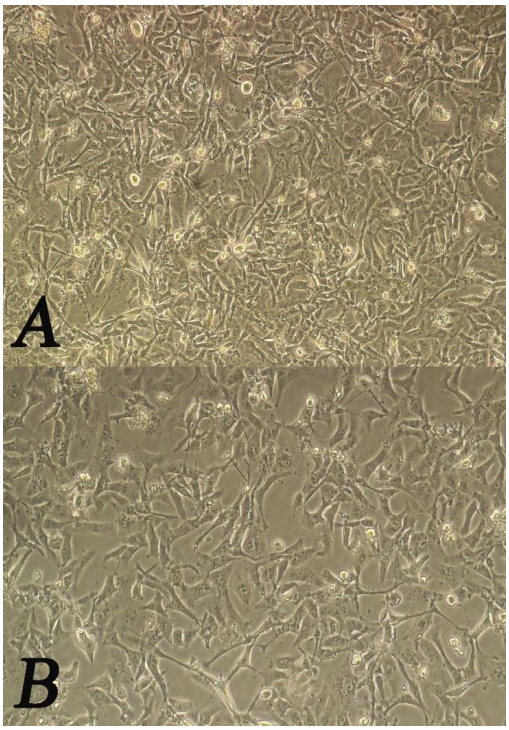

Figure 2. Cellular morphology of the MDA-MB 231 cancer cell line after exposure to initial toxic concentration of curcumin. (A) Control group at 96 h culture time, (B) treated cells at 10 µM concentrations of curcumin at 96 h culture time.

of VEGF expression to 0.6 compared to the control group shows that curcumin inhibits the angiogenesis process.

4. Discussion

The most significant angiogenic factor in the development of breast cancer is VEGF. Also, the most aggressive form of breast cancer, triple-negative breast cancer (TNBC), has high levels of vascular endothelial growth factor (VEGF) expression (13). VEGF can therefore be one of the targets for treating TNBC. Among all conventional methods in cancer treatment, the use of herbal medicines is expanding. Using the MTT assay, the cytotoxic effects of curcumin was examined on triple-negative breast cancer (TNBC) at different concentrations. Cancer cells were treated with gentle doses of chemicals that have little harmful effects in order to promote biocompatibility and minimize the impact on healthy cells. At 48 and 96 hours of cultivation, the results revealed a significant, dose- and time-dependent decline in the viability of MDA-MB-231 cells.

Curcumin significantly increased ataxia-

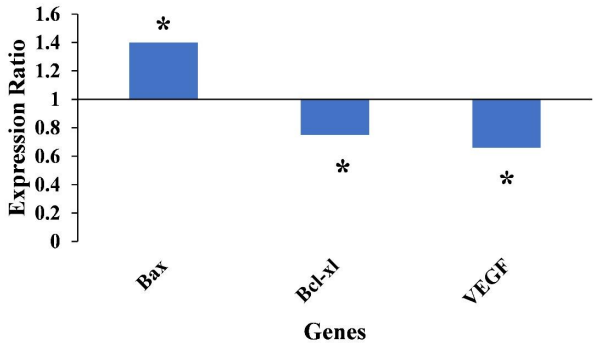

Figure 3. The expression of genes involved in apoptosis and angiogenesis. The cells were treated with curcumin at 10 µM concentration.

telangiectasia mutated (ATM) expression while decreasing HIF-1 expression. Subsequently, Curcumin inhibits the G2/M cell cycle and promotes apoptosis in head and neck squamous cell carcinoma cells in vitro and in vivo via an ATM/Chk2/p53-dependent mechanism (14). In a dose- and time-dependent manner, curcumin suppressed proliferation in pancreatic cancer cells. Curcumin has the ability to induce cell cycle arrest at the G2/M phase as well as apoptosis through increasing in protein expression of Bax and LC3II, and down-regulation of Bcl2 protein (10).

Curcumin not only induces apoptosis in sensitive cancer cells, but also triggers apoptosis signaling in drug-resistant cells (15). In cancer cells treated with curcumin, a series of cellular responses occur, such as the reduction of CDC25 and CDC2 and the increase of P21 protein levels, as well as the inhibition of the phosphorylation of Akt/mTOR and the induction of the mitochondrial apoptotic pathway, which ultimately induced G2/M phase cell cycle arrest and apoptosis (16).

5. Conclusion

The obtained results proved that curcumin has the potential to be developed as a treatment for triple-negative breast cancer because it has a biphasic effect on the MDA-MB-231 cell line. According to our research, curcumin can prevent the spread of breast cancer by inducing apoptosis, which is the result of upregulation of Bax and down-regulation of Bcl-xl. Inhibition of angiogenesis through reduction of VEGF expression and induction of apoptosis in initial toxic concentrations can introduce curcumin as a candidate in cancer treatment.

Conflict of Interest

The authors declare no conflict of interest.

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