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Novel formulated form of turmeric oleoresin inhibits cell growth and migratory behaviors of breast cancer cells

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ABSTRACT

Background and Objective: Curcumin is a polyphenolic compound derived from Curcumin longa L. There is growing body of data showing the antitumor effect of curcumin in different cancers; however, its efficacy is limited by its low absorption rate. Here, we investigated the antitumor activity of turmeric oleoresin alone or in combination with paclitaxel in MCF-7 cells in monolayer cell cultures and spheroids models.

Methods: The antiproliferative activities of 3 different form, curcumin, phospholipidated curcumin, and turmeric oleoresin were assessed by MTT assay. The migratory behaviors of the cells were determined by migration assay before and after treatment with curcumin. The expression levels of CyclinD1, P65, Nf-kB, and E-cadherin were studied.

Findings: Curcumin suppressed cell growth in MCF-7 cells via modulation of CyclinD1 and NF-KB, which was more pronounced with turmeric oleoresin. Curcumin was able to reduce the invasiveness of MCF-7, compared to control cells through perturbation of E-cadherin.

Conclusion: We demonstrated the antitumor activity of curcumin and curcumin oleoresin in breast cancer cells, supporting further investigations on the therapeutic potential of this novel anticancer agent in in vivo models.

Keywords: Breast cancer, Curcumin, Anti-tumor effect, Spheroid, Oleoresin

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Introduction

Breast cancer is the second most common cause of cancer death among women which contributed to the death of 519,000 people in 2004 (1). Incidence rates of breast cancer vary greatly worldwide, the highest rate was found in North America and western Europe and the low rates were observed in Asia and eastern Europe (2). Taxol is the most commonly used chemotherapeutic agent in the treatment of breast cancer; however, treatment with these drugs results in unwanted side effects and the development of chemo/drug resistance (3, 4). Therefore, it is needed to identify new anticancer agents to increase the efficacy of these drugs and overcome resistance to the current therapy. Curcumin is a polyphenolic compound (1, 7-bis (4-hydroxy-3-methoxyphenyl)-1, 6- heptadiene-3, 5-dione) derived from the rhizomes of turmeric (Curcumin longa L.) It has been reported that curcumin exerts anticancer properties via regulating various signaling pathways, involved in cell proliferation, cell cycle arrest and apoptosis (5-7). In addition it possesses beneficial chemopreventive and chemotherapeutic activity via targeting key signaling pathways, including NF-kB, Signal Transducer and Activator of Transcription (STAT3) (8, 9), extracellular-signal-regulated kinases (ERK1/2) and Epidermal Growth Factor Receptor (EGFR, in different tumor types (10).

Regarding its anticancer properties and safety profile, even in patients who have taken high doses (8 mg/d), several studies has been carried out to investigate the efficacy of combined therapy of curcumin and conventional chemotherapeutic agents. Lev-Ari et al demonstrated that curcumin synergistically enhanced the growth inhibitory effect of celecoxib in colorectal cancer cells through down-regulation of COX-2 expression (10). Curcumin also potentiated the antitumor activities of cisplatin in ovarian cancer cells by suppressing the production of the autocrine IL-6 (11). In addition, curcumin combined with tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) synergistically induced apoptosis in androgen-sensitive prostate cancer cells, suggesting that adjuvant treatment with curcumin could be useful against prostate cancer (12). The combination of chemotherapeutic drugs and curcumin was also effective in cervical cancer (13), colorectal cancer (14), pancreatic cancer (15) and breast cancer. Thus, the aim of the present study was to investigate the antitumor activity of turmeric oleoresin in breast cancer cells in monolayer cell cultures and spheroids models.

Methods

Cell culture

Curcumin was obtained by Sami Labs Ltd. (Bangalore, India). The drugs were dissolved in ethanol or sterile water, and diluted in culture medium before use. MCF-7 cells were obtained from the American Type Culture Collection. The cells were cultured in RPMI-1640, (Gaithersburg, MD) supplemented with 10% heat-inactivated FBS and 1% streptomycin/penicillin (Gaithersburg, MD) followed by incubation at 37 °C. The cells were harvested with trypsin-EDTA in their exponentially growing phase.

Quantitative Reverse-Transcriptase Polymerase-Chain-Reaction (qRT-PCR)

RNAs were extracted from the cells before and after treatment with curcumin at IC50 and 5xIC50 values using the RNXPLOS (CinaColon, Tehran, Iran), according to the manufacturers' protocol. RNAs were reverse-transcribed using the cDNA Synthesis Kit (CinaColon, Tehran, Iran). qRT-PCR was carried out with specific primers for *E-cadherin* (Macrogene co, Seoul, Korea). Gene expression values were normalized to GAPDH (16).

Growth inhibition studies

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The cell growth inhibitory effects of curcumin were explored in the cells after treatment for 72 hours. The plates were processed for the MTT assay, as described previously (17).

Effect of curcumin in multicellular spheroids

MCF-7 spheroids were established by seeding 10^5 cells per ml in RMPI/F12+GlutaMAX-I (1:1) in 24-well coated by agarose. The cytotoxic effects were evaluated for 10 days with the inverted phase contrast microscope Leica-DMI300B (Leica, Wetzlar, Germany) (18).

In vitro invasion assay

Transwell chambers with polycarbonate membranes and 8-µm pores were used for invasion assays (19). These assays were carried out via coated transwell filters, with 100 µl of 0.1 mg/mL collagen I solution. A total of 10^5 cells were seeded on the upper side of the filter and incubated with curcumin at IC50 and 5xIC50 in medium free FBS medium. The cells migrated into the lower side were fixed with paraformaldehyde after 24 hours and then stained with Giemsa. The filters were photographed and then counted.

Statistical analysis

All experiments were performed in triplicate. Data were expressed as mean values±SEM. and analyzed by Student's t-test or ANOVA followed by Tukey's multiple comparison test. Data were analyzed using SPSS v.20 (IBM, Chicago). Statistical significance was set at $P<0.05$.

Results

Curcumin inhibits cell growth

The cell growth inhibitory activity of curcumin in MCF7 cells is shown in Figure 1A. Curcumin inhibited the cell growth in dose dependent manner in MCF7 cells using MTT assay. It has been found that that three-dimensional (3-D) culture models are generally more chemo-/radio-resistant, compared to two-dimensional monolayer cell cultures, indicating the use of 3-D models for drug testing. To assess whether curcumin would be active in 3-D MCF7 models, we determined the activity of curcumin in spheroid cultures of MCF7 cells. Our data showed tumor shrinkage in spheroids after 5 days, compared with the untreated spheroids (Figure 2).

Curcumin inhibits cell invasion and modulates E-cadherin expression

Next, we evaluated the effect of curcumin on breast cell invasion. This data showed that treatment of MCF7 cells with curcumin decreased the invasive behavior of these cells (Figure 3). Since previous studies suggested the lower level of E-cadherin expression in cancer cells with respect to its highly metastatic behavior, we investigated whether curcumin could affect the level of this target. Curcumin increased E-cadherin expression ($P<0.05$; Figure 3).

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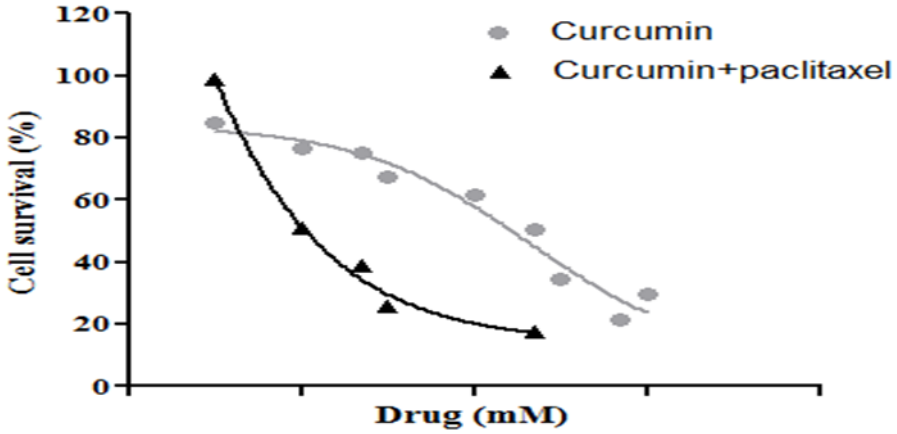

Figure 1. Inhibition of cell proliferation in MCF-7 cells. Growth inhibitory effects after 72 hours; C*olumns* or *Points*, mean values obtained from three independent experiments; *bars*, SEM.
*significantly different from controls.

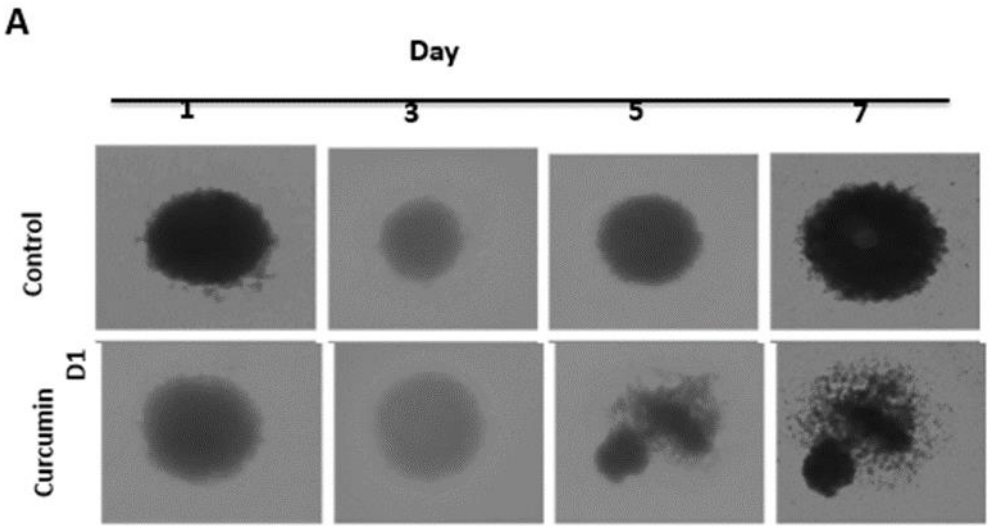

Figure 2. Inhibition of cell proliferation in MCF-7 cells. Effect of curcumin on the PDAC spheroids

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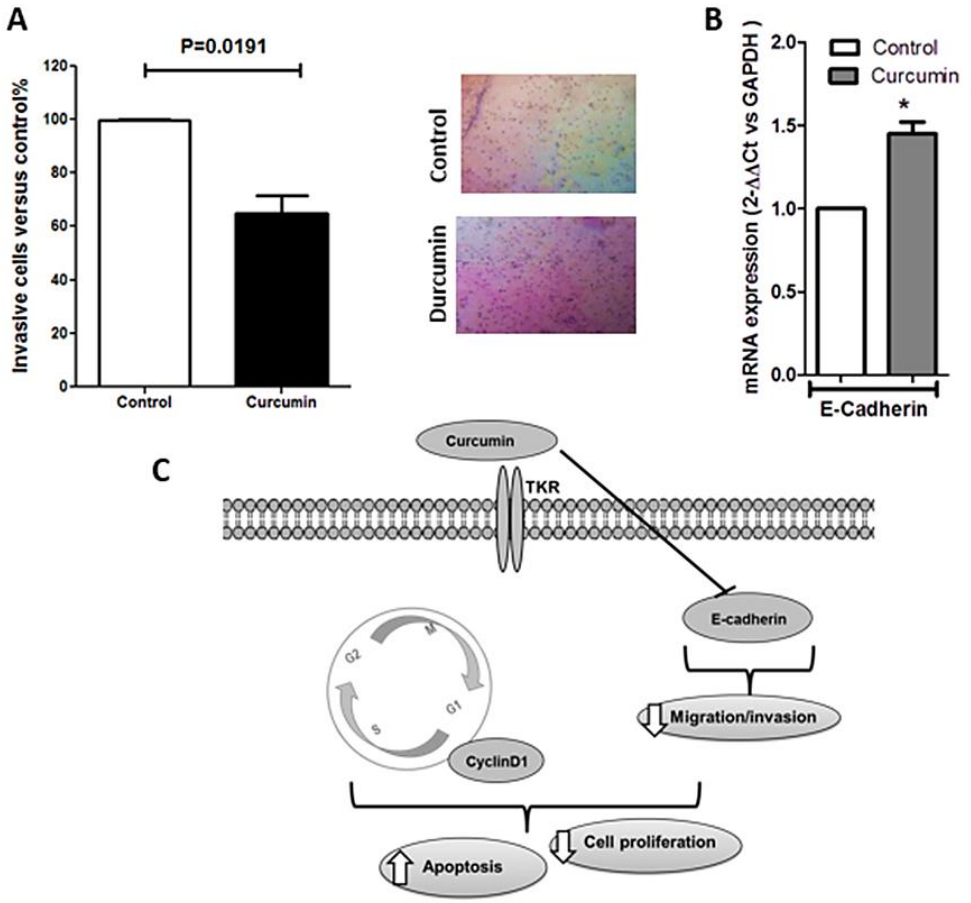

Figure 3. Effects of curcumin on PDAC cells invasion. (A) Results of invasion experiment in the MCF-7 cells exposed for 24 hours to curcumin at 5xIC50 values (insert: representative picture at 24 hours); **(B)** Modulation of *E-cadherin* mRNA levels after 24 hours exposure to curcumin as determined by q-RT-PCR. *Columns* or *Points*, mean values obtained from three independent experiments; *bars*, SEM. *Significantly different from controls. **(C)** curcumin inhibits Wnt and enhances the growth inhibitory effects through its pronounced pro-apoptotic, anti-invasive effects, as well as by inhibiting the cell proliferation.

Discussion

In the present study we evaluated the anticancer effect of turmeric oleoresin curcumin in breast cancer cells. Our findings showed that turmeric oleoresin curcumin inhibited cell growth in breast cancer cells. it has been reported that genistein, a nontoxic flavonoid compound, enhanced the antitumor activity of chemotherapeutic agents (8) by blocking NF-κB activation in prostate, breast, lung, and pancreatic cancer cells (20-23). It has been reported that NF-κB pathway can be inhibited by curcumin and further enhance the antitumor activity of different anticancer drugs that activate NF-KB (7, 24). Ajaikumar et al. indicated that curcumin potentiates the antitumor effects of gemcitabine in pancreatic cancer by suppressing proliferation, NF-κB, and NF-κB–regulated gene products in an orthotopic model of pancreatic cancer (15). Bava et al showed that curcumin enhanced taxol-sensitization in cervical cancer cell via suppression of NF-κB and serine/threonine kinase Akt (18). Notarbartolo et al demonstrated that combination of curcumin with cisplatin or doxorubicin in hepatic cancer HA22T/VGH cell line resulted in a synergistic antitumor activity through blocking NF-kB activation (17). Aggarwal et al showed that curcumin inhibited paclitaxel-induced NF-κB activation in breast cancer cells. Moreover, the administration of dietary curcumin suppressed breast cancer metastasis to the lung in nude mice which was correlated with the down-regulation of NF-κB, cyclooxygenase 2, and matrix metalloproteinase-9 (18). In accordance with these reports, our results are consistent showing curcumin inhibited the NF-kB activation in breast cancer cells and enhanced the antiproliferative activity of doxorubicin and Taxol.

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In addition, Wnt/β-catenin signaling pathway is among the key signaling pathways in breast cancer, which has been reported to be inhibited by curcumin (19, 25). The Wnt-signaling pathway is an evolutionarily conserved and sophisticated signaling cascade which has critical roles in cell proliferation and migration, cell cycle arrest and differentiation and controlling tumor progression. Following the activation of Wnt pathway, β-catenin is released from a cytoplasmic inhibitory complex and accumulates in the cytoplasm which can then translocate to the nucleus and bind to T-cell factor (Tcf) and stimulate the transcription of Wnt target genes (26-28). In this line, PRASAD et al demonstrated that curcumin suppressed Wnt/β-catenin signaling in MCF-7 and MDA-MB-231 breast cancer cells through downregulating the expression of Wnt/β-catenin pathway components including disheveled, β-catenin, cyclin D1 and slug (29). In our study, we demonstrated that curcumin down-regulates the Wnt/β-catenin-signaling and its downstream gene *Survivin*. Curcumin also inhibits proliferation and induces apoptosis by suppressing the Wnt/β-catenin pathway in other human malignancies including hepatocellular carcinoma (30), prostate (31), and colon cancer (32). Furthermore, activation of the Wnt/β-catenin-signaling is correlated with chemoresistance which suggests that the synergistic interaction of curcumin with doxorubicin and Taxol might be mediated by inhibition of Wnt/β-catenin-signaling pathway.

In addition to the effects of curcumin on cell growth, we evaluated whether curcumin can affect breast cancer cell migration and explored its possible mechanism. We found that treatment of breast cancer cells with curcumin decreased migratory behavior of these cells. Our result is in line with previous reports demonstrating that curcumin suppresses migration and invasion of human endometrial carcinoma cells (33), gliomas (34), breast (35), lung (36) and colon cancer (37). This effect may be related to increased expression of cell-cell adhesion molecule, E-cadherin, which has been shown to have invasion-suppressing properties (38). In support of this hypothesis, Mukherjee et al demonstrated curcumin inhibited the breast cancer stem cell migration by restoring the expression of the, E-cadherin (39). Consistently, we observed that exposure of breast cancer cells with curcumin significantly increased the mRNA level of E-cadherin. These results reveal that the anti- migratory effect of curcumin is correlated with up-regulation of E-cadherin.

Conclusion

In conclusion, we have shown that curcumin potently inhibits breast cancer cells proliferation, and invasion via perturbation of Wnt and NF-κB signaling pathways. Thus, targeting these proliferative signaling pathways by curcumin might have a great clinical significance in terms of the treatment of breast cancer and enhance the effect of chemotherapy. However, further studies are required to determine the exact molecular mechanisms and therapeutic values of different formulations of curcumin in breast cancer.

Compliance with Ethics Guidelines

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Consent for Publication

Informed consent was obtained from all individual participants included in the study.

Competing Interests

The authors have no conflict of interest to disclose.

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