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Research Article
Curcumin Confer Radiosensitizing Effect in Breast Cancer Cell Lines via Growth and Motility Inhibition

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Abstract

Background and Objective: Breast cancer is a major cause of morbidity and mortality in economically developing countries such as India. Evidential research is explained about the anticancer properties. The present study investigated curcumin along with the γ-irradiation effect on two different breast cancer cell lines. **Materials and Methods:** MDA-MB-231 and MCF-7 cell was irradiated with gamma rays prior to the curcumin treatment. A 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, clonogenic assays and wound healing assays were performed. The results were analyzed with a one-way analysis of variance (ANOVA) with the Bonferroni multiple comparison post-test, using In Stat software version 3.00. **Results:** The results of the MTT assay showed a statistical significance with $p>0.001$, similarly, clonogenic assay revealed that the curcumin reduced the proliferation rate of cells. Correspondingly, wound healing assay resulted in control of the cell-cell interaction and cell migration at two different time points. **Conclusion:** In conclusion, curcumin along with a lower dose (8 Gy) of radiation acted as the best combinatorial drug treatment for breast cancer cell lines. Our findings put up a rationale for further clinical/preclinical analysis of combination treatments with a lower dose and fewer side effects in breast cancer.

Key words: Curcumin, breast cancer, radiosensitizers, MDA-MB-231, MCF-7, growth, motility

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Breast cancer refers to cancers originating from breast tissue, mainly the inner lining of milk ducts or the lobules that supply the ducts with milk. Worldwide, it comprises 10.4% of all cancer incidences among women, making it the second most frequent type of non-skin cancer after lung cancer and the fifth main cause of cancer death[1]. It is the leading cause of death and disability particularly among young women in middle and low-income countries[2].

Surgery, radiation therapy and chemotherapy are commonly employed treatment strategies for the treatment of cancer. Primary tumors are first and foremost treatment by surgical and radiation therapy whereas disseminated metastatic tumors such as breast, prostate and colorectal cancers are mainly treated using chemotherapy[3]. Though these conventional therapies are more efficient, it was reported that the majority of the patients are developing resistant and the disease is relapsing[4]. Apart from these traditional adopted strategies, there have been major advances with respect to cancer treatment including matrix metalloproteinase inhibitors, gene therapy and immunotherapy. However, these approaches need to be evaluated as currently they have been only tested in clinical trials[5].

Curcumin, the principal constituent of the spice turmeric is derived from the rhizome of the East Indian plant *Curcuma longa*. Among the curcuminoids present in turmeric curcumin is the major one comprising approximately 2-5% of turmeric, it confers yellow color to the spice and is accountable for the majority of turmeric's therapeutic effects[6]. Apart from being used as a flavoring and coloring agent in food, turmeric has also been extensively used in Ayurvedic medicine for its antioxidant, antiseptic, analgesic, antimalarial and anti-inflammatory properties. It is being consumed as a dietary supplement since centuries and has been considered pharmacologically safe[7].

This constituent of turmeric has been the subject of intensive study as a chemopreventive agent and as a complement to radiotherapy. One particular study suggested that resistance to radiation is caused by increased expression of NF-κB-induced prosurvival genes, such as Bcl-2 and Bcl-xL, in response to radiation[8]. In that investigation, curcumin along with radiation inhibited NF-κB activation in DU-145 and LNCaP prostate cancer cells and resulted in the down-regulation of Bcl-2 and curcumin alone enhanced caspase activation and cytochrome C release in both cell types, leading to increased apoptosis. Curcumin furthermore sensitized PC3 prostate cancer cells to radiation[9]. It also confers a radiosensitizing effect in rhabdomyosarcoma and cervical tumor cells[10].

Recent preclinical studies have shown that curcumin act as potent radiosensitizers of tumor cells in cervical, prostate, rhabdomyosarcoma and lung cancer. However, it was not known whether curcumin can sensitize breast cancer cells to radiation. Keeping in mind the high mortality rate due to resistant to current treatments the present study was conducted to evaluate if curcumin conferred radiosensitizing effect in breast cancer cell lines by inhibiting cell growth and motility.

MATERIALS AND METHODS

Study area: Study was performed from June, 2012 to May, 2014 in KIMS Foundation and Research Center, Secunderabad.

Reagents: Dulbecco's Modified Eagle's Medium (DMEM), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), dimethyl sulfoxide (DMSO), phosphate-buffered saline (PBS), fetal bovine serum, L-glutamine, penicillin/streptomycin solution, 0.25% trypsin-EDTA, curcumin (purity ≥97%) were all purchased from Sigma-Aldrich and propidium iodide (PI) was purchased from Invitrogen.

Cell culture: MDA-MB-231 and MCF-7 breast cancer cells were purchased from the national center for cell science, Pune and maintained in DMEM medium (Sigma) at 37°C in a 5% CO_2 humidified atmosphere. Breast cancer cell lines were maintained in DMEM with 10% heat-inactivated fetal bovine serum, 5 mM HEPES buffer (pH 7.4), 2 mM L-glutamine, 100 U mL^{-1} penicillin and 100 μg mL^{-1} streptomycin. Cells were maintained at 37°C in 5% CO_2 incubator to 80-90% cell confluence and then used in the assays.

MTT assay: Mitochondrial succinate dehydrogenase activity was used to assess cell viability and as well as growth using a modified MTT assay. Briefly, cells were seeded into triplicates (7×10^4 cells/well) of a 96-well flat-bottom tissue culture plate. After 24 h incubation, cells were then treated with different concentrations of curcumin and DMSO (2.5-10 μM) and drug kinetics were performed for 72 and 96 h. In some experiments, cells were exposed to 2, 4, 6, 8 and 10 Gy of γ radiation prior to the treatment of curcumin (5 μM) and DMSO[11]. After incubation with the drug, media removed at different time points as indicated and cells were treated with MTT solution at concentration 0.45 mg mL^{-1} and incubated for 3 h at 37°C. Formazan crystals that were formed solubilized in DMSO and optical density was determined at 570 nm using a microplate reader.

Colony-forming assay: For clonogenic cell survival studies, MCF-7 cells were left untreated or exposed to an 8 Gy dose of radiation prior to the treatment of curcumin (5 μM) and plated in 10 cm plates. After incubation for 10 or more days, each flask was stained with crystal violet and the colonies were counted. The surviving fraction (SF) was calculated as a ratio of the number of colonies formed and the product of the number of cells plated and the plating efficiency. The curve was plotted using X-Y log scatter (Delta Graphs 4.0) and by using the formula of the SHMT model, the D0 was calculated[12]. D0 is the dose required for reducing the fraction of cells to 37%, indicative of single-event killing. SF2 is the survival fraction of exponentially growing cells that were irradiated at the clinically relevant dose of 8 Gy.

Wound healing assay: MDA-MB-231 cells were seeded in 6-well plates at a concentration of 1×10^4 cells in DMEM after irradiating with 8 Gy. Cells were then cultured for 24 h. A scratch was made to the monolayer with a 1 mL pipette tip across the center of the well[13]. Cultures were washed with DMEM and then cultured in the absence or presence of 5 μM curcumin for 16 and 24 h. Images were captured using an Olympus camera head connected to an Olympus microscope.

Statistical analysis: Statistical analysis was performed by one-way analysis of variance (ANOVA) with the Bonferroni multiple comparison post-test, using In Stat software version 3.00 (Graph Pad Software Inc., San Diego, CA, USA). Differences were considered as statistically significant when at $p<0.05$.

RESULTS

Mitochondrial assay: The mitochondrial assay was performed to evaluate the cell viability of the MDA-MB-231 and MCF-7 breast cancer cell lines after treatment with varying concentrations of curcumin (2.5-10 μM). As shown in Fig. 1, both the cell lines at 2 different time points did not show much variation when compared to control. Different concentrations of curcumin i.e., 2.5, 5, 10 μM could not affect the cancer cells from its rapid division. It illustrates that curcumin when administered alone, did not suppress cell growth even at its higher concentration, in the breast cancer cell lines.

In contrast to the above results, cells were exposed to 2, 4, 6, 8 and 10 Gy of γ radiation prior to the treatment of curcumin (5 μM). It is observed that curcumin suppressed the growth of proliferating cells in a time and dose-dependent

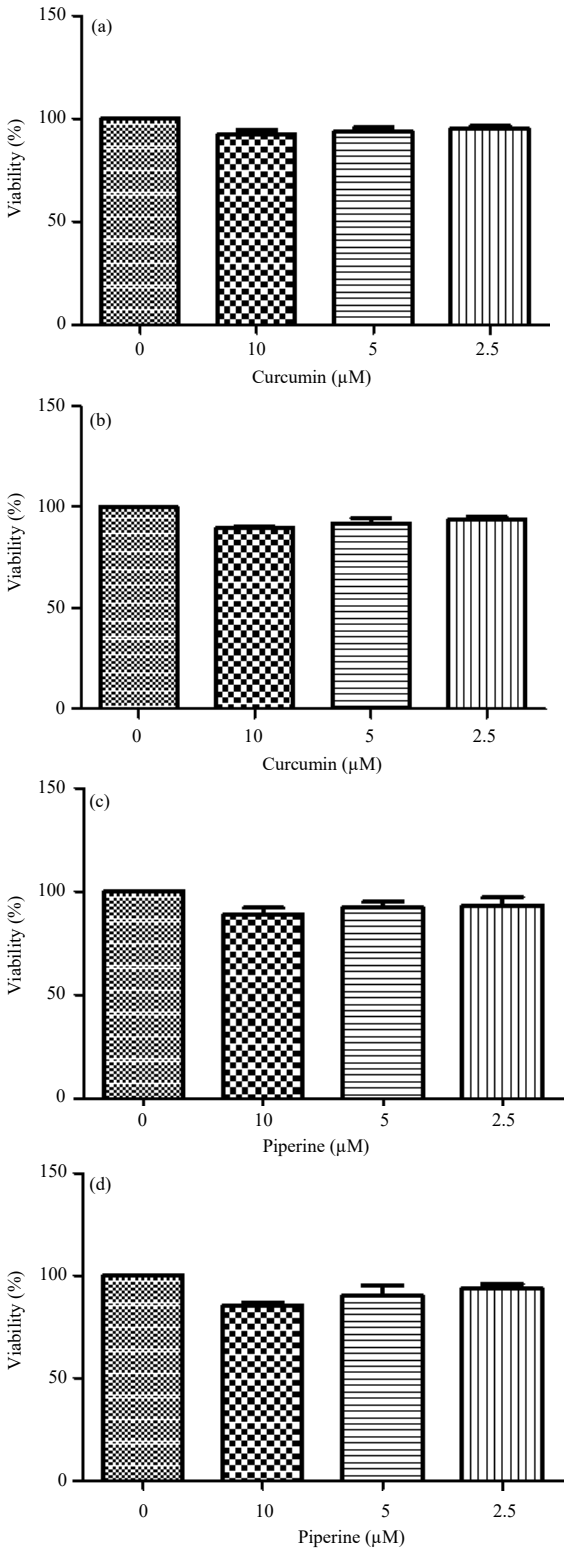

Fig. 1(a-d): MTT assay for MCF 7 and MDA-MB-231 cells treated with curcumin alone, (a) MCF-7 72 h, (b) MCF-7 96 h, (c) MDA-MB-231 72 h and (d) MDA-MB 231 96 h

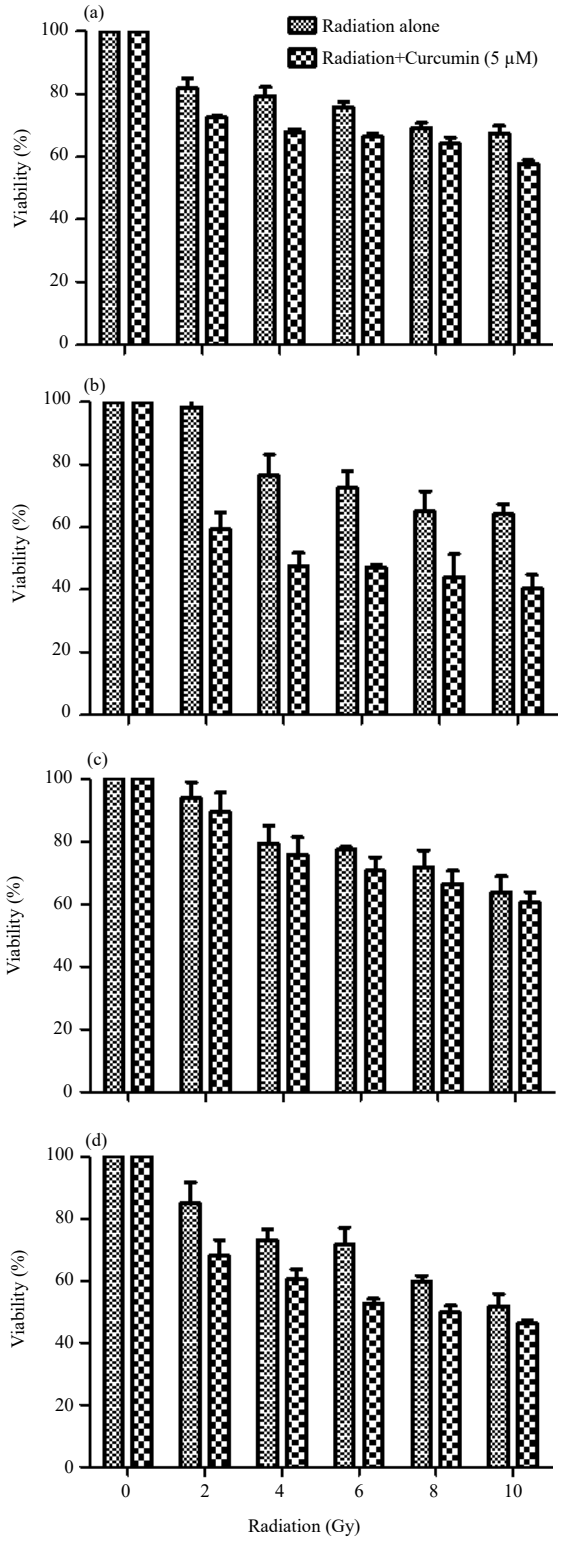

Fig. 2(a-d): Curcumin sensitized the breast cancer cell lines (MCF 7 and MDA-MB-231) to radiation, (a) MCF-7 72 h, (b) MCF-7 96 h, (c) MDA-MB-231 72 h and (d) MDA-MB-231 96 h

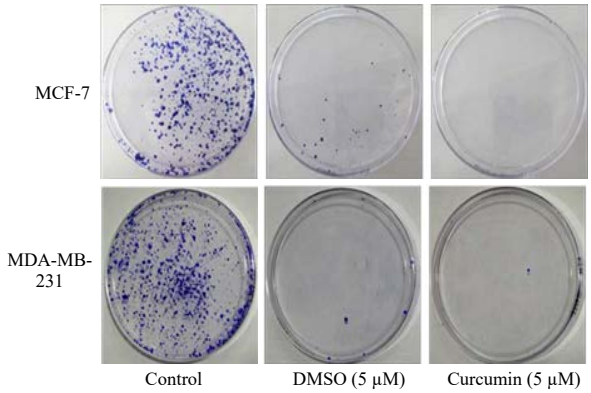

Fig. 3: Clonogenic survival of breast cancer cell lines after treatment with 8 Gy of irradiation plus 5 µM curcumin and DMSO was assessed by crystal violet staining representative testing of three independent experiments is shown

manner. As shown in Fig. 2, a sequential reduction in cell growth with different Gy of γ radiation both breast cancer cell lines. Interestingly, a significant enhancement in the radiosensitizing effect of curcumin was observed at 2 and 4 mM concentrations and suppressed cell growth both in the MDA-MB-231 and MCF-7 cells.

Colony-forming assay: It further confirmed the long-term effects of curcumin on cell growth by clonogenic assay. Colony-forming assay evaluated the proliferation of the MDA-MB-231 and MCF-7 breast cancer cell lines after treatment with DMSO (5 µM) and curcumin (5 µM). In Fig. 3 when compared with the control, DMSO and curcumin treatment for 14 days caused a significant inhibition of colony formation in both breast cancer cell lines. DMSO 5 µM along with 8 Gy radiation could able to regrow and formed colonies after 2 weeks of irradiation dose. But curcumin as combination treatment cooperated well the irradiation and suppressed the colony formation and reduced the long-term survival of treated cells by inducing apoptosis.

Wound healing assay: A wound healing assay was conducted to examine whether curcumin inhibited the migration of the MDA-MB-231 cells. As shown in Fig. 4, it is observed that the treated cells when compared with the control group, 5 µM curcumin along with 8 Gy irradiation significantly inhibited the migration of cancer cell lines at 2 different time points. These results clearly suggest that curcumin possesses an anti-invasive function and interferes with the metastatic potential of the breast cancer cell.

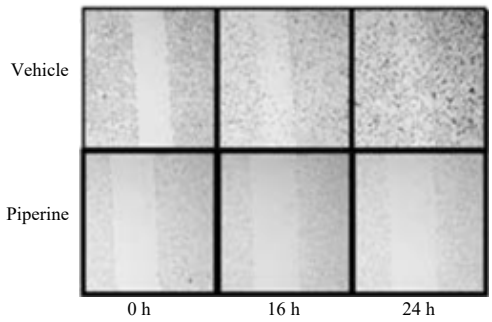

Fig. 4: Cell migration/wound healing assay for MDA-MB-231 cells

DISCUSSION

Radiation therapy (RT) is a treatment modality used for several types of cancer, more than 50% of cancer patients receive RT, often used in combination with surgery and chemotherapy[14]. Radiation stimulates the immune system, which in turn contributes to tumor cell death. These immunological factors suppress tumor development by killing cancer cells on one hand whereas on the other end they induce an immunosuppressive micro environment that contributes to promoting tumor progression[15].

In mammalian cells, ionizing radiation activates many pro-survival pathways that converge to transiently activate key transcription factors (TFs). These include the Nuclear factor kappa B (NF-κB) and signal transducers and activators of transcription members (STATs)[16,17]. These transcription factors regulate several genes that induce cell proliferation, invasion, angiogenesis, metastasis, suppression of apoptosis and treatment resistance in a wide range of tumors[18]. Curcumin a natural polyphenolic compound derived from turmeric has various anticancer properties. It suppresses cancer cell proliferation, down regulates NF-κB target genes, reduces the activity of growth factor receptors and counteracts tumorigenesis[19].

Hence our primary objective was to determine whether curcumin can sensitize breast cancer cells to radiation. We used MDA-MB-231 and MCF-7 breast cancer cell lines for this present study. MDA-MB-231 cell line is an epithelial human breast cancer cell line that is most commonly used for medical research[20]. It is a highly aggressive, invasive and poorly differentiated triple-negative breast cancer cell line lacking estrogen and progesterone receptor expression, as well as HER2 amplification[21,22]. This cell line displays endothelial-like morphology and is distinguished by its invasive phenotype, having stellate projections that often bridge multiple cell colonies[23].

MCF-7 is the other breast cancer cell line that we used for our study as the cell line retains several ideal characteristics particular to the mammary epithelium. These include the ability of MCF-7 cells to process estrogen in the form of estradiol via estrogen receptors in the cell cytoplasm. Apart from this MCF-7 is also progesterone receptor positive and HER2 negative[24]. Using the breast cancer cell lines i.e., MDA-MB-231 and MCF-7 we performed 3 assays that are mitochondrial, colony forming and wound healing assay to evaluate if curcumin confers a radiosensitizing effect in breast cancer cell line by inhibiting the growth, proliferation and motility.

Firstly the mitochondrial assay was performed to evaluate the cell viability of the MDA-MB-231 and MCF-7 breast cancer cell lines after treatment with varying concentrations of curcumin (2.5-10 μM). It was observed that curcumin, when administered alone, did not suppress cell growth in the cancer cell lines but when cells were exposed to gamma radiation prior to treatment of curcumin it suppressed the cell growth in a time- and dose-dependent manner. Furthermore, a significant enhancement in the radiosensitizing effect of curcumin was observed at 2 and 4 mM concentrations. It further confirmed the effects of curcumin on cell growth by clonogenic assay. Compared with the control and DMSO, curcumin treatment caused a significant inhibition of colony formation in both breast cancer cell lines in a dose-dependent manner. In accordance with the mitochondrial assay, the results from our clonogenic assay indicate that curcumin confers a radiosensitizing effect and inhibits cell survival in both MDA-MB-231 and MCF-7 cells.

According to the accumulated evidence over the last few years, most chemotherapeutic agents and radiation therapy activate NF-κB[25]. Resistance to radiation is caused by increased expression of these NF-κB-induced prosurvival genes such as antiapoptotic Bcl-2 family members[26]. Radiation stimulated NF-κB activity in a dose and time-dependent manner while curcumin through inhibition of this NF-κB activation helped sensitize cancer cells to ionizing radiation in our present study[9,27]. These results indicated that the natural compound curcumin significantly enhances the effect of radiation and inhibits the further growth of breast cancer cell lines. Another similar kind of study by Sandur *et al.*[28]. Analyzing the combined effect of curcumin and radiation on colorectal

cancer cells noticed that curcumin inhibited the proliferation and post-irradiation clonogenic survival of multiple colorectal cells.

Finally, the wound healing assay was performed to examine whether curcumin can inhibit the motility of the MDA-MB-231 cells. MCF-7 breast cancer cell lines lack the invasive potential henceforth we restricted the wound healing assay to MDA-MB-231 cells. Compared with the control group, curcumin significantly inhibited the migration of cancer cell lines. These results clearly suggested that curcumin possesses an anti-invasive function and interferes with the metastatic potential of the breast cancer cell. Curcumin inhibited the metastatic progression of breast cancer cell lines in the present study through suppression of urokinase-type plasminogen activator by NF-κB signaling pathways[29]. The identification of curcumin as a radiosensitizer in breast cancer is of considerable interest, because of its afford ability, ease of oral administration and lack of toxicity in clinical use. In human trials of its safety, doses as large as 12 g/day have been tolerated with minimal clinical toxicity[30].

CONCLUSION

Our results proposed that radiation-inducible NF-κB activation provides a pro-survival response to radiation that may account for the development of radioresistance. Curcumin blocks this signaling pathway and potentiates the anti-tumor effects of radiotherapy. The fact that curcumin can achieve efficient radiosensitizing effects without any toxicity makes its development as an adjunct to standard radiotherapy an important goal. Curcumin inhibited growth, proliferation and post-irradiation clonogenic survival of these cell lines. The results from the clonogenic assay indicate that curcumin inhibited cell survival in both MCF 7and MDA-MB 231breast cancer cells.

SIGNIFICANCE STATEMENT

This study discovered the effect of curcumin on radiated breast cancer cell lines that can be beneficial for reducing the high gyration exposure while irradiating the tumor when chemotherapy was given with curcumin at a lower dose. This study will help the researchers to uncover the critical areas of radiation in combination with lower doses of curcumin that many researchers were not able to explore. Thus a new theory on breast cancer with fewer side effects may be beneficial for the cancer bearers.

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