Efficient Anti-proliferation of Aggressive Breast Cancer Cells using Curcumin-Encapsulated Nanoparticles

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***Abstract*—The aim of this study is to evaluate the effective delivery of anti-cancer drug such as curcumin for safe and affordable cancer treatment using nanotechnology and electrical pulses. Curcumin is the natural turmeric herbal extract and it is well known for anti-inflammatory, anti-oxidant, and anti-tumor properties. However, its use is limited due to the short biological half-life (13± 3.5 h). To enhance this non-toxic curcumin absorption at tumor tissue, we implemented dual technology a) first curcumin was encapsulated within the nanoparticles (CNP) to extend its bioavailability and b) apply mild electroporation (EP). To evaluate this study proposal, CNP was tested *in vitro* on live breast cancer cells of MDA-MB-231, (ATCC HTB-26). Two groups in triplicate (untreated control and CNP + EP treated) were tested. In each well breast cancer cells (1 x 10^6 cells) of >98% viability in 1%PBSA was treated with CNP (0, 200, 400 and 600µg equivalent of curcumin). After treatment electrical pulses were administered six, 1200V/cm, 100µs to deliver CNP into cells and study their anti-tumor response over a period of 72 hours by counting live and dead cells. The study results indicated that the synergistic effect of CNP +EP combined treatment may provide an important novel alternative for increasing cancer treatment efficacy. Further, studies are ongoing we anticipate these study results of combinatorial treatment modality could provide an effective cancer therapy.**

I. INTRODUCTION

Breast cancer is the most common cancer among the women prevailed all over the world, which is approximately 25% of all female malignancies [1]. Worldwide this is the second leading cause of cancer-related deaths among females, especially more deaths were reported in the developed countries. Many treatment modalities have been practiced for the effective treatment of breast cancer, however, toxicity associated with the current treatment modalities are a big challenge. This may be eliminated by using non-toxic drugs such as curcumin. Recent reports indicated that the curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) (Figure 1), is one of the well-known natural molecule exhibited for anti-tumor effect [2-3]. Curcumin is extracted from the plant

Curcuma longa, is known for antioxidant that exerts antiproliferative and apoptotic effects on live cells. Curcumin is known to induce inhibition of tumor cell growth via apoptosis. Curcumin inhibits the expression of following signals Ki-67, proliferating cell nuclear antigen (PCNA), and p53 mRNAs in breast cancer cells, and induced Bax mRNA expression with the down-regulation of p21 mRNA in the human mammary epithelial cell line (Figure 2) [4].

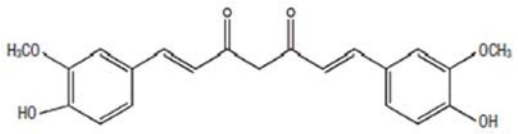

Figure 1. Chemical structure of Curcumin

Breast cancer is associated with activation of many pathways Wnt/β-catenin signaling pathway is one of the pathway. Curcumin plays key role to inhibit the expression of β-catenin, cyclin D1, and slug in both MCF-7 and MDA-MB-231 cells (Figure 2) [4-6].

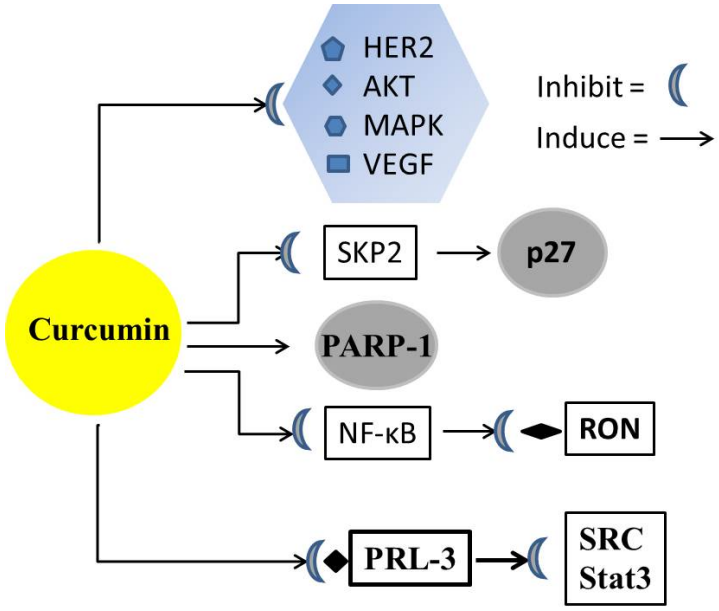

Figure 2. Curcumin inhibits the expression of receptor d'origine nantais (RON), human epidermal growth factor 2 (HER2), Akt, mitogen-activated protein kinase (MAPK), nuclear factor-κB (NF-κB), vascular endothelial growth factor (VEGF) and the phosphorylation of Src and stat3 through PRL-3 down-regulation, but induces the expression of p27 and poly (ADP-ribose) polymerase 1 (PARP-1) in cancer cells.

However, half-life of curcumin is short, and insoluble in aqueous medium hence suita-

ble delivery method is needed to enhance the therapeutic effect. For example, solubility of curcumin could be enhanced by using nanotechnology-based formulations. In this report we present the delivery of curcumin in cancer cells using nanotechnology and electroporation approaches.

II. Materials and Methods

Chemicals and reagents. Curcumin, Propidium iodide (PI), and other reagents were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The human breast cancer cell line (MDA-MB-231) was obtained from the American Type Culture Collection (ATCC) (ATCC HTB-26).

In vitro cell culture: The cells were placed into 75-cm^3 tissue culture flasks and grown at 37˚C in humidified air atmosphere (CO2 not required), in Dulbecco's Modified Eagle Medium (DMEM) (Life technologies, catalog number: 11965 -084) contained with 10% heat-inactivated FBS, 1% penicillin-streptomycin (10,000 U/ml penicillin; 10 mg/ml streptomycin).

The effects of EP and CNP on the viability of MDA-MB-231 cells: The MDA-MB-231 cells were harvested from the plate and evaluated for the cells quality and counted for the viable cells. Briefly, cells were aliquoted into the electroporation cuvette, (4 cm, $1x10^6$ cells/well, in 0.5mL of 1%PBSA) and treated with CNP at various concentrations (0, 200, 400 and 600µg equivalent of curcumin). After curcumin treatment electrical pulses were administered for six, 1200V/cm, 100 µs to deliver CNP into cells and transferred into the 6-well plates in a density of $1x10^6$cells/well and grown for 72 h. Control cells were received no drug treatment just EP alone. After 72 h drug treatment, anti-tumor response was evaluated by counting live and dead cells using FACS. FACS assay was performed using PI staining to measure the viable and non-viable cells. All data presented in this report are from at least two independent experiments.

III. Results and discussion

Figure 1-2 shows the chemical structure of the curcumin, and the molecular mechanism to inhibit various proteins of cancer cells [3-8]. This yellow color molecule insoluble in water hence, it was encapsulated as nano particles for better delivery into the cancer cells [9]. This curcumin encapsulated nanoparticles delivery performance was tested in various cancer cells and it was already reported [9]. Figure 3 exhibits the effect of CNP at various concentrations, with mild dose of EP on MDA-MB-231 breast cancer cells. Figure 3 also shows that MDA-MB-231 cells, after 72 h incubation, combination of CNP and EP effectively inhibited the proliferation when compared to control cells with EP alone. This result indicate that the number of viable cells decrease as the concentration of CNP delivery increases, suggesting that the cell death of MDA-MB-231 cells were curcumin dose dependent manner (figure 3). The viable cell percentages were (mean % ± SD) 93.8 ± 1.7, 60.4 ± 1.5, 52 ± 3.6, and 38 ± 4 for the concentration of 0, 200, 400, and 800 µg/well of curcumin, respectively. Thus, the present study shows, proliferations of MDA-MB-231 breast cancer cells were inhibited by curcumin based CNPs.

In comparison with previous report [10], our study agrees with the reported data, with respect to the curcumin properties such as inducing cytotoxicity and apoptosis in human breast cancer cells of MDA-MB-231[10]. A detailed study reports were available for the mechanism of the cytotoxic effect, this was due the increase of Bax, and decrease of Bcl-2 protein expression induces the apoptosis in cancer cells [4-8, 10-12]. Although, curcumin is known for anti-tumor effect, but the drug delivery is the challenging due to the insolubility in aqueous medium and short molecular half-life, in our study we attempted to circumvent this challenge by adopting nanotechnology and electroporation for effective drug delivery. Further studies are ongoing to tune this drug delivery approach.

In summary, our in vitro cell culture study results clearly indicated that the MDA-MB-231 breast cancer cells proliferation was reduced to 38%, after 72h incubation, by the CNP and EP combined dose treatment. This dual modality approach may be useful to augment the cancer treatment by way of synergistic effect. Further, this study indicated the combination of nanotechnology and electroporation could be an alternative method for effective delivery of the non-soluble cancer therapeutics.

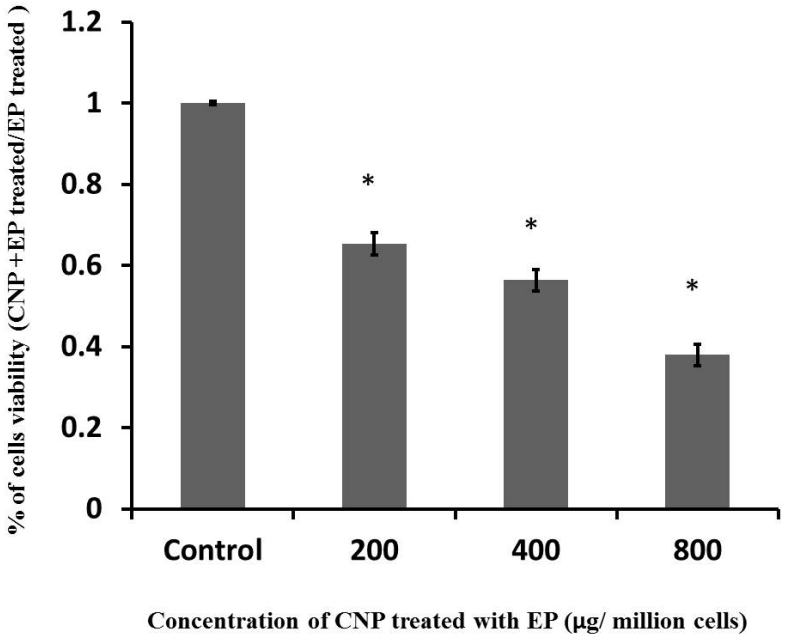

Figure 3: The MDA-MB-231 breast cancer cells ($1x10^6$cells/well) were treated with curcumin nanoparticles (CNP) of 0, 200, 400 and 800 µg equivalent of curcumin per well. After CNP treatment, these cells were immediately aliquoted in to the electroporation cuvettes of 4cm, and administered electroporation (EP) dose of six, 1200v/cm, 100 µs. After EP administration, cells were washed and cultured with DME medium for

72h. CNP, and EP treated cells were harvested, after 72 h incubation and evaluated for cells viability using PI and performed FACS assay to determine the dead and viable cells count. Control cells received EP only. Experiments were performed in triplicates, and repeated twice. *P<0.005.

IV. REFERENCES

[1] MT. Park, MJ. Kim, YH. Kang , et al., "Phytosphingosine in combination with ionizing radiation enhances apoptotic cell death in radiation-resistant cancer cells through ROS-dependent and -independent AIF release", *Blood 105*, 1724-33, 2005.

[2] J. Ravindran, S. Prasad, BB. Aggarwal, "Curcumin and cancer cells: how many ways can curry kill tumor cells selectively?", *The AAPS journal 11*, 495-510, 2009.

[3] AB .Kunnumakkara, P. Anand, BB. Aggarwal, "Curcumin inhibits proliferation, invasion, angiogenesis and metastasis of different cancers through interaction with multiple cell signaling proteins", *Cancer letters. 269*,199-225, 2008.

[4] C. Ramachandran, W. You, "Differential sensitivity of human mammary epithelial and breast carcinoma cell lines to curcumin", Breast cancer research and treatment 54 269-278, 1999.

[5] M. Banerjee, P. Singh, D. Panda, "Curcumin suppresses the dynamic instability of microtubules, activates the mitotic checkpoint and induces apoptosis in MCF-7 cells", *The FEBS journal 277*, 3437-48, 2010.

[6] JM. Adams, S. Cory, "The Bcl-2 protein family: arbiters of cell survival", *Science 281*, 1322-6, 1998.

[7] M. Nomura, S. Shimizu, T. Ito, et al., "Apoptotic cytosol facilitates Bax translocation to mitochondria that involves cytosolic factor regulated by Bcl-2", *Cancer research 59*, 5542-8 1999.

[8] KM. Murphy, V. Ranganathan, ML. Farnsworth et al., "Bcl-2 inhibits Bax translocation from cytosol to mitochondria during drug-induced apoptosis of human tumor cells", *Cell death and differentiation 7*, 102-11, 2000.

[9] W. Lin, C. Cooper, I. Camarillo, "The Effectiveness of Electroporation-based Nanocurcumin and Curcumin Treatments on Human Breast Cancer Cells" ESA, 2014.

[10] X. Wang, Y. Wei, S. Yuan S, et al., "Potential anticancer activity of tanshinone IIA against human breast cancer", *International journal of cancer Journal international du cancer 116*,799-807, 2005.

[11] TL. Chiu, CC. Su, "Curcumin inhibits proliferation and migration by increasing the Bax to Bcl-2 ratio and decreasing NF-kappaBp65 expression in breast cancer MDA-MB-231 cells", *International journal of molecular medicine 23*, 469-75, 2009.

[12] D. Liu, Z. Chen., "The effect of curcumin on breast cancer cells", *Journal of breast cancer 16*, 133-7, 2013.