Abstract

Breast cancer is the leading cause of cancer deaths among women worldwide. Despite the progress made in the development of potent chemotherapy drugs, their toxicity to normal tissues and adverse side effects in multiple organ systems as well as drug resistance have remained the major obstacles for the successful clinical use. The *in vitro* and *in vivo* studies in the last few decades have demonstrated that some phytochemicals derived from "natural products" such as fruits, vegetables and certain spices, referred as chemopreventive agents include capsaicin, trans-anethole, thymoquinone, diosgenin and allicin, which not only reduces the adverse side effects but improve also the effectiveness of chemotherapeutics to patients undergoing therapy. With this background in this piece of work we focused on elucidating the thymoquinone (*Nigella sativa*, a dietary phytochemical) potential in breast cancer therapeutics. Our study systematically explained the anti-apoptotic effects of thymoquinone (TQ) on Akt mediated mTOR signaling and its inhibitory effect on translational machinery involved in Cyclin D1 expression thus mediating G_1 phase arrest in breast cancer cells. We elucidated the possible role of TQ in overcoming the complications of the existing tamoxifen (TAM) chemotherapy. TQ in combination with TAM inhibited tumor cell growth, proliferation and angiogenesis, while at the same time potentially preventing the development of TAM-insensitivity. TQ treatment synergizes with a low dose of TAM to induce apoptosis through XIAP mediated Akt downregulation *in vivo* and *in vitro*. Further, we employed TQ as radiosensitizer to investigate its metastasis reversal abilities in irradiated breast cancer cell lines by assessing morphology, migratory and invasive attributes. Radiation induces TGF-β mediated metastatic progression through EMT in cancer cells. We affirmed the TGF-β restoring ability of TQ in radiation driven metastasis thus reinstating EMT and radio-resistance. Here, paclitaxel was chosen as an apoptosis inducer in TGF-β restored cells and confirmed its cytotoxic effects in radiation alone and TQ sensitized irradiated cells. Akt overexpression in cancer causes resistance to traditional chemotherapeutics. Further, to mimic the chemo-resistance, we developed Akt-overexpressed and TAM-resistant MCF 7 breast cancer cell line. Silencing Akt through siRNA provides new therapeutic options. We developed, designed and characterized the nanoparticulate that can uphold siRNA-Akt without any alteration along with TQ in overcoming the Akt mediated drug resistance in breast cancers. We describe novel multilayered gold niosomes displaying enhanced Akt-siRNA delivery that result in efficient silencing of Akt and its downstream targets involved in the survival of breast cancers *in vivo* and *in vitro*. We propose that TQ can be employed as an endogenous Akt suppressor which inhibits breast cancer cell survival, and prevent Akt induced therapeutic resistance. Our pre-clinical studies showed effective approach with promising results in synergistic combination of TQ with TAM, paclitaxel and radiation as chemo-/radio-sensitizer. This study will provide the groundwork for future clinical studies and aid in developing TQ as potent adjuvant for chemo/radiation therapy.

Key words: Thymoquinone, Tamoxifen, Paclitaxel, Apoptosis, Breast Cancer, Radiation, Gold Niosome Nanocomposite, Akt, TGF-β, XIAP, siRNA Akt, Metastasis, Epithelial to mesenchymal transdifferentiation, Xenograft