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Asia Pharma 2017 : Thymoquinone Inhibits Bone Metastasis of Breast Cancer Cells Through Abrogation of the CXCR4 Signaling Axis - Muthu K Shanmugam - National University of Singapore

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Breast cancer is the second most common cancer that afflicts women, with an estimated 1.67 million women diagnosed with breast cancer in 2012 . Breast cancer ranked fifth in cancer-associated deaths among all cancers globally in 2012 . While incredible development has been made for the treatment of breast cancers, the treatment of triple negative breast cancer (TNBC) is quite a challenge owing to its destructive features and limited treatment options . Often, chemotherapy for breast cancer is ridden with side effects and drug resistance, leading to failure in therapy. Compelling evidence suggests that if breast cancer metastasis to distant site organs can be prevented, then it is an indicator for good prognostic outcome . Interactions between chemokines and their cognate receptors play important roles in tumor metastasis. The interaction between chemokine receptor type 4 (CXCR4) and its cognate ligand stromal-derived factor-1 (SDF1 or CXCL12) plays a crucial role in the regulation of migration and metastasis in a variety of solid tumors including breast cancer. The master transcription factor nuclear factor kappa-light-chain-enhancer of activated B cells (NF-□B) plays a pivotal role in the development and progression of inflammation-driven diseases including cancer, TQ was reported to abrogate NF-□B activation and augment cellular apoptosis . Several other studies have shown that TQ can also down-regulate protein kinase B and extracellular receptor kinase signaling pathways . Woo et al., 2011 reported that TQ can exert a strong anti-proliferative effects in TNBC cells by activating peroxisome proliferator-activated receptor gamma (PPAR□). Breast cancer has a greater tendency to spread to the bone marrow of the femora, tibiae, and the mandibular bones. Therefore, intracardiac injection method has been employed to develop osteolytic bone metastasis model. On the basis of the crucial role of SDF1/CXCR4 in breast cancer metastasis to femora, tibiae, and mandibles, we systemically injected via an intracardiac route MDA-MB-231-luc+ cells to test the ability of TQ to modulate CXCR4 expression and thereby inhibit metastasis to the bones and other organs.

Human breast cancer MCF7, MDA-MB-231, and BT-549 were purchased from ATCC. The MDA-MB-231-luciferase expressing cell line was purchased from Cell Biolabs, United States. The cells were cultured in DMEM supplemented with 10% fetal bovine serum (FBS) and antibiotics penicillin/streptomycin. Thymoquinone (TQ), N-Acetyl-L-leucyl-L-leucyl-L-norleucinal (ALLN), chloroquine, formic acid, Tris, glycine, NaCl, sodium dodecyl sulfate (SDS), crystal violet, and bovine serum albumin were purchased from Sigma-Aldrich .A 50 mM solution of TQ was prepared with dimethyl sulfoxide (DMSO) and stored as small aliquots at -20°C. RPMI 1640, FBS, antibiotic-antimycotic mixture, and lipofectamine were obtained from Invitrogen). Antibody against CXCR4 (polyclonal) was obtained both from Abcam and Santa Cruz. Vehicle or TQ-treated breast cancer cell lines and tumor tissues obtained from the mouse study were lysed in RIPA lysis buffer .Nuclear extracts of TQ treated or control cells were prepared using TransAM nuclear extract kit according to the manufacturer's instructions. Whole cell lysates was resolved on a 10% SDS polyacrylamide gel electrophoresis (SDS–PAGE) gel. The proteins were electro-transferred to a nitrocellulose membrane (Bio-Rad), blocked with Blocking One ,and probed with antibodies of interest overnight at 4°C. The blot was washed with tris-buffered saline with 0.1% Tween-20, exposed to HRP-conjugated secondary antibodies for 1 h, and finally examined by chemiluminescence using Western Bright Sirius HRP substrate (Advansta). Images were captured using a ChemiDoc XRS+ imaging system and analyzed using Image LabTM software as described previously Densitometric analysis was done using ImageJ software. To determine NF-□B activation, nuclear extracts were prepared using a nuclear extraction kit according to the manufacturer's instructions. NF-□B DNA-binding activity was analyzed using the TransAM NF-□B p65 transcription factor assay kit, following the manufacturer's instructions. The enzymatic product was measured at 450 nm with a microplate reader as previously described. Human MDA-MB 231 and BT-549 TNBC cells were seeded in 15-cm culture dishes and treated with TQ (50 μM) for 0, 2, 4, 6, and 8 h. At the end of the treatment period the cells were fixed with 1% formaldehyde at room temperature for 10 min and neutralized with glycine. The cells were collected, resuspended in CHIP lysis buffer, and sonicated (Vibra-Cell,TM Newtown, CT, United States). Samples were incubated with protein-G beads that had been pre-incubated with 4–10 μg of anti-NF-□B antibody (Cell Signaling Technology, MA, United States) or negative control IgG .

Numerous reports have previously suggested that CXCR4 is over-expressed in a variety of tumors including gastric, ovarian, pancreatic, melanoma, renal, cervical, colon, and hematological malignancies. However, there are no substantial studies elaborating upon the detailed mechanism(s) of CXCR4 over-expression in tumor cells. umor bearing chick embryos have been used to study anti-cancer effects of drugs . In our study, we implanted breast cancer cells onto the CAM and evaluated the effects of TQ on tumor growth and vascularity. We found that TQ dose-dependently inhibited tumor growth and potently reduced the tumor vascular volume. Furthermore, we found that intraperitoneal administration of TQ at doses of 2 or 4 mg/kg b.w for 4 weeks could inhibit the growth and metastasis of breast cancer tumors.

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Biography :

Muthu K Shanmugam is a senior research fellow in the Department of Pharmacology, National University of Singapore, Yong Loo Lin School of Medicine. He got his PhD in cancer pharmacology and he is currently working as a senior research fellow. He has twelve years of experience in experimental laboratory research and have published in journal papers and presented at international conferences. Muthu K Shanmugam has vast experience in cancer biology, inflammatory diseases, orthotopic, xenograft and transgenic mice models, in molecular biology, cell and tissue culture experiments. In addition, he is trained in high-throughput technology such as cDNA microarray technology, antigen and antibody array technology, two dimensional gel electrophoresis, mass spectrometry, pharmacokinetics and in the development of array based clinical diagnostic tools.

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