

Original Research Article

Wortmannin as Targeted Therapeutic Agent for the Treatment of Triple-Negative Breast Cancer

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Abstract

Purpose: To investigate the inhibitory effect of wortmannin on inhibition of BT-20 and BT-474 breast cancer cells in Athymic nu/nu mice model.

Methods: Forty Athymic nu/nu mice were randomly assigned to 4 groups of 10 each, namely, BT-20 control, BT-20 treatment, BT-474 control and BT-474 treatment groups. The mice were injected with 2.5×10^5 BT-20 and BT-474 cells under anesthesia. Those in the treatment groups were given wortmannin (7 mg/kg) in DMSO daily intraperitoneally whereas the animals in control groups received an equal volume of DMSO for 21 days after the cancer attained palpable stage. Western blot analysis was carried out using enhanced chemiluminescence reagent while Protean IEF cell unit was used for 1-D electrophoresis.

Results: The results showed a significant decrease in the growth of the tested cancer cell lines on treatment with wortmannin at 7 mg/kg daily for 21 days. The volume of tumor in the treatment group was reduced to 42.72 ± 9.45 compared to 79.43 ± 17.11 mm³ in the control group after 21 days treatment ($p < 0.005$). It also changed the expression of microRNA in BT-20 cells. Exposure of BT-20 cells to wortmannin for 24 h resulted in the altered expression of proteomes. Wortmannin treatment increased the expression of miRs including miR 19a/b, 22, 29b/c, 181c/d, 195 and 663. Western blot data showed increase in the expression of NME1 (NM23 H1) and reduction in the expression of vimentin after treatment with wortmannin.

Conclusion: Wortmannin can be of benefit in the treatment of human breast cancer.

Keywords: Wortmannin, Vimentin, Tumor growth, Expression of microRNA, Proteomes

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INTRODUCTION

Breast carcinoma is a challenging health issue at present affecting one out of every eight women, especially in US [1]. It is estimated that about 20 % of the patients with primary breast cancer rapidly develop distant metastases leading to higher mortality rates [2]. The median survival period for breast cancer patients is around three years [2]. Breast cancer has been found commonly in women and its proportion in men is very low [3]. Triple negative breast cancer

(TNBC) comprises a group of heterogeneous diseases which are prevalent among African and American women and is more aggressive compared to other cancers [4-6]. It has been reported that TNBC spreads rapidly to distant tissues and leads to mortality within very short time span of the disease [4]. Therefore, the discovery of molecules which target the TNBC is desired for its treatment.

The commonly used chemotherapeutic agents for cancer treatment are antibiotics belonging to

the anthracycline, bleomycin, actinomycin, mitomycin and aureolic acid families [7]. Among these families, anthracyclines exhibit promising anti-tumor activities through the suppression of topoisomerase II [8,9]. Another important natural isolate wortmannin from the fungus *Talaromyces wortmanni* has been shown to act as inhibitor of signal transduction pathways targeting 3 kinase (PI3K) activity [10]. Targeting signal transduction cascades by involvement of bioactive compounds is believed to be a promising strategy for carcinoma therapy [11]. The present study was designed to investigate the effect of wortmannin on breast carcinoma inhibition, miR expression and proteome expression.

EXPERIMENTAL

Materials

Cell lines and culture

Human breast carcinoma cell lines, BT-20 and BT-474 were purchased from the Riken Bioresource Center (Tsukuba, Japan). The cells were cultured in cultured Dulbecco's modified Eagle's medium/F-12 (DMEM/F-12) (Wako) supplemented with 10 % fetal bovine serum (FBS) (Cambrex, Walkersville, MD, USA).

Drug

Wortmannin was isolated from the fungus, *Talaromyces wortmanni*, using solvent extraction followed by column chromatography in 10 % methanol and chloroform.

Xenograft model of tumorigenesis

Athymic nu/nu 6-week-old female mice (Harlan Sprague-Dawley) were fed irradiated sterile food and water. The mice were acclimatized to the laboratory environment one week before the experiment was started. The animals were randomly assigned to 4 groups with 10 each, BT-20 control, BT-20 treatment, BT-474 control and BT-474 treatment groups. The animals were injected with BT-20 or BT-474 cells (2.5×10^5) in the mammary fat pad under anesthesia. The animals in the treatment groups were given wortmannin (7 mg/kg) in DMSO daily intraperitoneally whereas the animals in control groups received equal volume of DMSO for 21 days after the cancer attained palpable stage. During the treatment period volume of cancerous mass was calculated after every 2 days by measuring the tumor size with digital calipers. After the completion of the treatment, the animals were sacrificed to extract the tumors, uterus, liver, and lungs. The tissues were immediately

fixed in formalin and stored under cold conditions using liquid nitrogen. The liquid nitrogen frozen samples were then used for other analyses.

The animal experiments were performed according to the rules of the ethical committee of the Weifang People's Hospital, No. 207 Guangwen Street, Kuiwen District, Weifang, Shandong. All measures were taken to minimize suffering in the animal.

Western blot analysis

The cells after treatment with wortmannin were washed with phosphate-buffered saline (PBS) and lysed in lysis buffer containing aprotinin (10 mg/mL), leupeptin (10 mg/mL) (ICN Biomedicals, Inc., Asse Relegem, Belgium) and phenylmethylsulfonyl fluoride (Sigma-Aldrich). The proteins were separated on SDS-PAGE, loaded on PVDF and treated with primary antibodies. After incubation the membrane was incubated with secondary antibodies conjugated to horseradish peroxidase. The blots were examined using enhanced chemiluminescence reagent (ELPS, Seoul, Korea).

Proteomics analysis

The cells, after treatment with wortmannin or normal saline for 24 h, were subjected to 2D electrophoresis. Protean IEF cell unit (BioRad, Hercules, CA, USA) was used for 1-D electrophoresis and the proteins were separated using Precast 11 cm IPG strips. BioRad Criterion electrophoresis cell system was employed for 2-D electrophoresis and Gel Doc XR image system (BioRad) was used for analysis of stained gels. The scanned gels were analyzed with PDQuest software (version 8.01).

Statistical analysis

All the experiments were performed in triplicate. Student t-test or analysis of variance (ANOVA) was used for statistical analysis. Differences were considered statistically significant at $p < 0.05$.

RESULTS

Inhibition of breast carcinoma growth

The animals in both treatment and control groups were administered carcinoma cells, BT-20 or BT-474 along with reduced growth factor matrigel (Fig 1A,B). When the tumor attained palpable stage, animals in the treatment group were given wortmannin (7 mg/kg/day) and those of control group received an equal volume of normal saline.

It was observed that the volume of tumor in treatment group was markedly small compared to control group. The tumor volume in the treatment and control groups were 42.72 ± 9.45 and $79.43 \pm 17.11 \text{ mm}^3$, respectively after the completion of the treatment period.

Effect of wortmannin on the miRnome

The BT-20 cells on exposure to wortmannin for 24 h showed prominent changes in the

expression of miRNA whereas no such changes were observed in untreated control cells. Wortmannin treatment significantly enhanced the expression of some of the miRs including miR 19a/b, 22, 29b/c, 181c/d, 195 and 663 (Figure 2A). In addition wortmannin treatment inhibited the expression of some of the miRs including, 193a 5p, 197, 224, 486 5p, and 542 5p (Figure 2B).

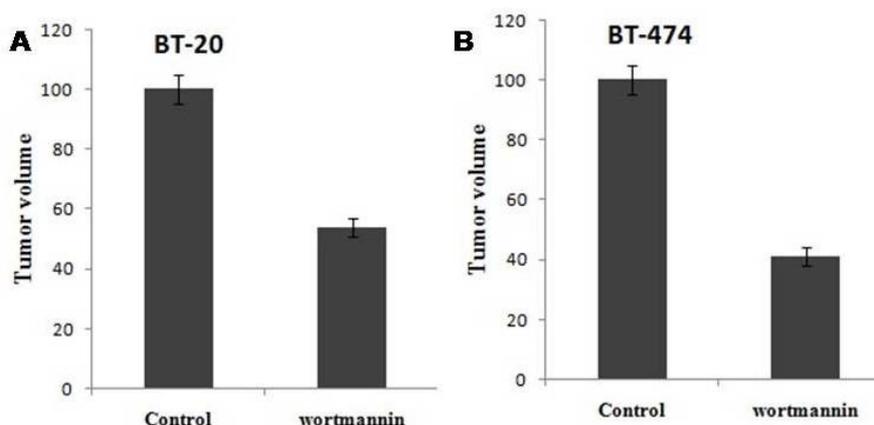


Figure 1: Suppression of tumor growth by wortmannin in breast carcinoma. The mice were administered (A) BT-20 or (B) BT-474 and after palpable tumor growth injections of wortmannin (7 mg/kg) or saline were given to animals

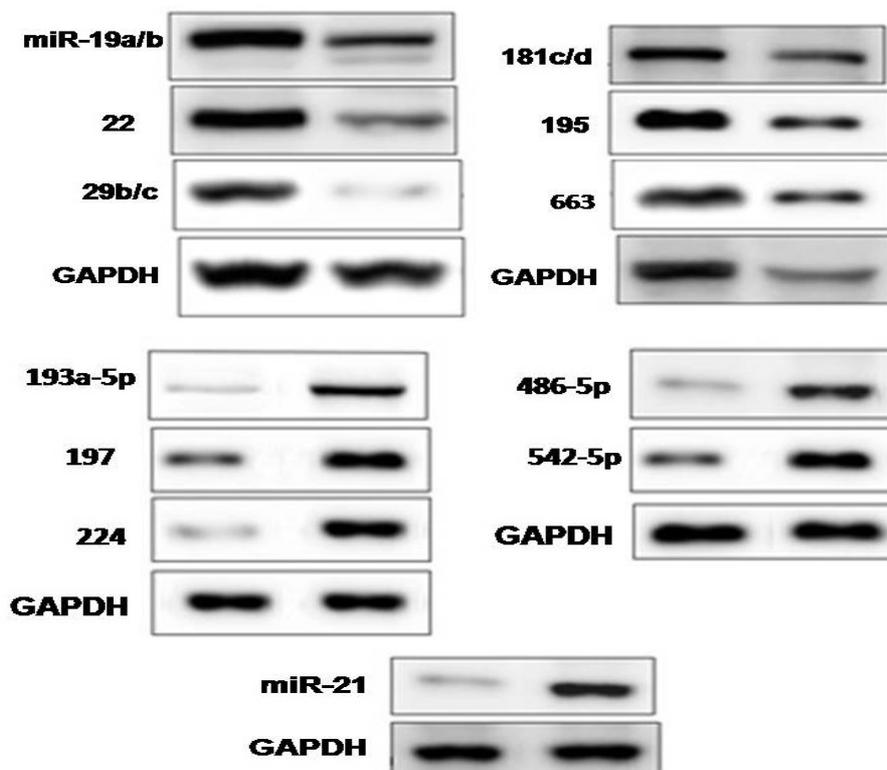


Figure 2: Effect of wortmannin on microRNA expression in BT-20 cells after 24 h

Wortmannin changes the proteome of BT-20 breast carcinoma cells

Exposure of BT-20 cells to wortmannin for 24 h induced changes in the expression of proteomes. The results from Western blot analysis showed increase in the expression of NME1 (NM23 H1) by wortmannin treatment (Figure 3). The expression of vimentin was significantly decreased by treatment with wortmannin (Figure 3).

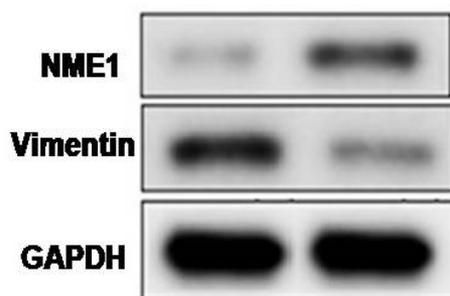


Figure 3: Effect of wortmannin on the expression of tumor genes

DISCUSSION

In cancerous tissues miR expression shows changes which are known to be associated with increase in tumor growth, resistance to drugs and spread of cancer cells [16-18]. The results obtained in the present study showed that wortmannin induces expression of miRs and thus acts as suppressor of tumor growth. It has been reported that miRs exhibits anti-carcinoma effect on tumor growth by interfering with the expression of Jun B and Jun D [19]. miR 19 suppresses the expression of tissue factor which is involved in the progress of tumors [20]. The results from the present study revealed increase in the expression of tumor suppressor genes on exposure to wortmannin. Studies have demonstrated that miR 193a 5p genes play an important role in the progression of tumor growth [21]. In the present study it was observed that the expression of the genes was inhibited by exposure to wortmannin.

It is reported that miR 21 gene is responsible for inducing resistance to drugs resulting in poor tumor prognosis rate [22,23]. Wortmannin induced inhibition of these genes and thus prevented the development of resistance to drugs. A surface marker found during change of epithelial cells to mesenchymal cells is over-expressed in breast cancer cells [15]. The expression of vimentin is significantly enhanced following treatment with wortmannin. Therefore,

wortmannin suppresses the growth of tumors in breast cancer cells.

CONCLUSION

Wortmannin inhibits the growth of breast carcinoma cells via change in expression of miR. Thus, wortmannin can be used for the treatment of breast cancer.

REFERENCES

1. American Cancer Society, 2010. *Breast Cancer Statistics: Breast cancer overview: how many women get breast cancer, 2010.*
2. Stevanovic A, Lee P, Wilcken N. *Metastatic breast cancer. Aust Fam Physician* 2006; 35: 309-312.
3. Anderson WF, Jatoi I, Tse J, Rosenberg PS. *Male breast cancer: a population based comparison with female breast cancer. J Clin Oncol* 2010; 28: 232-239.
4. Reis Filho JS, Tutt AN. *Triple negative tumours: a critical review. Histopathology* 2008; 52: 108-118.
5. Bauer KR, Brown M, Cress RD, Parise CA, Caggiano V. *Descriptive analysis of estrogen receptor (ER) negative, progesterone receptor (PR) negative, and HER2 negative invasive breast cancer, the so called triple negative phenotype: a population based study from the California cancer registry. Cancer* 2007; 109: 1721-1728.
6. Dent R, Trudeau M, Pritchard KI, Hanna WM, Kahn HK, Sawka CA, Lickley LA, Rawlinson E, Sun P, Narod SA. *Triple negative breast cancer: clinical features and patterns of recurrence. Clin Cancer Res* 2007; 13: 4429-4434.
7. Cragg GM, Newman D J, Weiss RB. *Coral reefs, forests, and thermal vents: the worldwide exploration of nature for novel antitumor agents. Semin Oncol* 1997; 24: 156-163.
8. Monica B, Rosalba F, M. Evelina B, Giovanni C. *In vivo site specificity and human isoenzyme selectivity of two topoisomerase II poisoning anthracyclines. Cancer Res* 2000; 60: 3770-3776.
9. Patrick Y. *Major microbial diversity initiative recommended. Am Soc Microbiol News* 1997; 63: 417-421.
10. Cadenas ME, Sandfrison A, Cutler NS. *Signal transduction cascades as targets for therapeutic intervention by natural products. Trends Biotechnol* 1998; 16: 427-433.
11. Adjei AA. *Signal transduction pathway targets for anticancer drug discovery. Curr Pharmaceut Design* 2000; 6: 361-378.
12. Patel JB, Appaiah HN, Burnett RM, Bhat-Nakshatri P, Wang G, Mehta R, Badve S, Thomson MJ, Hammond S, Steeg P, Liu Y, Nakshatri H. *Control of EVI 1 oncogene expression in metastatic breast cancer cells through microRNA miR 22. Oncogene* 2011; 30: 1290-1301.

13. Bonci D. Micro RNA 21 as therapeutic target in cancer and cardiovascular disease. *Recent Pat Cardiovasc Drug Discov* 2011; 5: 156 161.
14. Russell RL, Pedersen AN, Kantor J. Relationship of nm23 to proteolytic factors, proliferation and motility in breast cancer tissues and cell lines. *Br J Cancer* 1998; 78: 710 717.
15. Kokkinos MI, Wafai R, Wong MK, Newgreen DF, Thompson EW, Waltham M. Vimentin and epithelial mesenchymal transition in human breast cancer – observations in vitro and in vivo. *Cells Tissues Organs* 2007; 185: 191 203.
16. Farazi TA, Spitzer JI, Morozov P, Tuschl T. miRNAs in human cancer. *J Pathol* 2011; 223: 102 115.
17. Winter J, Diederichs S. MicroRNA biogenesis and cancer. *Methods Mol Biol* 2011; 676: 3 22.
18. Allen KE, Weiss GJ. Resistance may not be futile: microRNA biomarkers for chemoresistance and potential therapeutics. *Mol Cancer Ther* 2011; 9: 3126 3136.
19. TiliE, Michaille JJ, Adair B. Resveratrol decreases the levels of miR 155 by upregulating miR 663, a microRNA targeting JunB and JunD. *Carcinogenesis* 2011; 31: 1561 1566.
20. Zhang X, Yu H, Lou JR. MicroRNA 19 (miR 19) regulates tissue factor expression in breast cancer cells. *J Biol Chem* 2011; 286: 1429 1435.
21. Ory B, Ramsey MR, Wilson C. A microRNA dependent program controls p53 independent survival and chemosensitivity in human and murine squamous cell carcinoma. *J Clin Invest* 2011; 121: 809 820.
22. Mei M, Ren Y, Zhou X. Downregulation of miR 21 enhances chemotherapeutic effect of taxol in breast carcinoma cells. *Technol Cancer Res Treat* 2011; 9: 77 86.
23. Gong C, Yao Y, Wang Y, Liu B, Wu W, Chen J, Su F, Yao H, Song E. Up regulation of miR 21 mediates resistance to trastuzumab therapy for breast cancer. *J Biol Chem* 2011; 286: 19127 19137.