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In vitro inhibition of invasion and metastasis in colon cancer cells by TanIIA

R.W. Zhang, Z.G. Liu, Y. Xie, L.X. Wang, M.C. Li and X. Sun

Jilin Provincial Key Laboratory of Molecular Geriatrics, Beihua University, Jilin, Jilin, China

Corresponding author: X. Sun
E-mail: sunxinbh@126.com

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ABSTRACT. The purpose of this study was to investigate the effect of the traditional Chinese medicine TanIIA on the viability, invasion, and metastasis of SW480 cells. SW480 cells were treated with TanIIA for 24 h, and MTT assays were performed to determine the effect of TanIIA on cell viability. Transwell transmembrane experiments were applied to test the effect of 1.0 mg/mL TanIIA on SW480 cell invasion and metastasis abilities. Western blotting was performed to determine the expression of the tumor cell metastasis proteins E-cadherin, vimentin, and MMP-9. The cell growth inhibition rates were 0%, 26 ± 4.3%, 43.47 ± 4.0%, 63.0 ± 5.5%, and 76.8 ± 7.8% for treatment with 0, 0.5, 1.0, 2.0, and 5.0 mg/L TanIIA, respectively. The differences in the cell viability inhibitory rates among all groups were statistically significant ($P < 0.05$). The Transwell assay results indicated that SW620 cell invasion and metastasis abilities were strongly inhibited by 1.0 mg/mL TanII. The western blotting results showed that the expression of E-cadherin was significantly increased and that the expression levels of vimentin and MMP-9 were significantly decreased after treatment with 1.0 mg/mL TanII for 24 h ($P < 0.05$). Tan

II can effectively inhibit the biological activity of colon cancer *in vitro* and prevent the invasion of colon cancer cells.

Key words: TanIIA; SW480 cells; Cell proliferation; Cell invasion

INTRODUCTION

The morbidity and mortality of colon cancer have increased with the gradual improvement in living standards in China. Colon cancer is currently ranked fourth among malignant tumors (Li and Zheng, 2011). Cell invasion or metastasis is regarded as one of the most important characteristics of malignant tumors, as metastasis is the main cause of death and disease recurrence in patients with colon cancer. The treatment options for late-stage colon cancer patients include surgery, radiotherapy, chemotherapy, and other comprehensive measures as standard care. However, the effects of these measures and the prognosis are still not ideal for patients. Therefore, it is important to explore the related mechanism of colon cancer metastasis in order to identify new treatment targets.

Tanshinone IIA (TanIIA) is a traditional Chinese medicine that is extracted from *Salvia miltiorrhiza* and used for the clinical treatment of cardiovascular diseases (Yang et al., 2008) TanIIA can be used to protect myocardial cells against oxidative stress and inflammation. It is also widely used in the treatment of coronary heart disease, angina pectoris, and other cardiovascular diseasesl (Liu et al., 2009; Ren et al., 2010). Recent studies have shown that TanIIA has potential as a cancer treatment (Lu et al., 2009) and can lead to the reversal of the malignant phenotype of cancers and reduce metastasis and invasion. Previous studies have shown that TanIIA can inhibit the proliferation of many tumor cells, such as breast cancer, lung cancer, osteosarcoma, liver cancer, leukemia, and ovarian cancer, indicating its excellent potential as an anti-tumor agent (Chien et al., 2012; Su, 2014a,b).

MATERIAL AND METHODS

Cell culture

Colon cancer SW480 cells were cultured in DMEM containing 10% fetal bovine serum in a 37°C cell incubator with 5% CO_2 saturated humidity. The cells grew in a single layer, and the medium was replaced every 2 to 3 days. After cells reached confluence at the bottom of the culture bottle, they were passaged at a ratio of 1:3.

Reagents

DMEM culture medium and fetal bovine serum were purchased from Gibco. TanIIA, MTT, and PI were purchased from Sigma (catalogue No). E-cadherin, vimentin, MMP-9, and GAPDH antibodies were purchased from Cell Signaling (catalogue No). Transwell Chambers were purchased from Corning (catalogue no). ECL kits were purchased from Pierce (catalogue no).

Cell viability assays

SW480 cells were seeded on 96-well plates at a density of 1.0 x 10^4 cells/L. Media

were changed after the cells attached to the surface. TanIIA was added to the complete culture media at different concentrations, and the cells were cultured for 24 h. A control group without the drug was established, and four wells were used for each group. The cell growth state was observed under an inverted microscope and photographed. For the MTT assay, 20 μL 5 mg/mL MTT was added to each well followed by incubation in the dark at 37°C. Then, the supernatant was removed, and 150 μL DMSO was added to each well, and the plate was oscillated for 10 min. The absorbance values were read on a microplate reader at 490 nm, and the results are recorded.

Cell invasion experiment

Transwell chambers, 24-well plates, Matrigel glue, and spearhead were pre-cooled in a refrigerator. The Matrigel glue and serum-free DMEM culture solution were diluted 1:3, and 50 μL/well was added to the upper well of the transwell chamber followed by incubation for 30~60 min at 37°C and 5% CO_2 in a constant temperature incubator. Pancreatin was used to harvest SW480 cells, which were re-suspended in a single-cell suspension in the upper well at 1 x 10^4 cells/well. Then, 500 μL DMEM culture solution without fetal bovine serum was added to the cells seeded in the upper well, and 500 μL DMEM complete culture solution containing 10% fetal bovine serum was added to the blank control group. Meanwhile, 500 μL DMEM culture solution containing 1.0 mg/L TanIIA but not fetal bovine serum was added to the upper well, and 500 μL DMEM complete culture solution without 1.0 mg/L TanIIA but containing 10% fetal bovine serum was added to the lower well in the experimental group. Three wells were used for each group. The plates were placed in a constant temperature incubator for 24 h, and then, the chambers were removed and fixed for 15 min with 95% ethanol at room temperature. Then, the chambers were stained with crystal violet for 30 min. The Matrigel glue and the remaining cells in the upper well of the transwell chamber were removed with a cotton swab. The inner film of the chamber was sliced with a blade and placed on a glass slide. The glass slide was inverted under an inverted phase contrast microscope to observe the cells that penetrated through semi-permeable membrane and attached to the lower layer of the chamber. Ten visual fields were randomly selected. The average cell number in each high power field was calculated, and each field was photographed.

Western blot

SW480 cells were seeded on 6-well plates. After the cells attached, 1.0 mg/L TanIIA was added to the wells. After incubation for 24 h, the wells were washed with pre-chilled PBS twice. The 6-well plates were then placed on ice and oscillated for 30 min and centrifuged at 12,000 *g* for 20 min. Total proteins were collected, and the Bradford method was used to quantify the protein level. Then, 40 μg protein was loaded on a gel for SDS-PAGE, after which the proteins were transferred to a PVDF membrane and blocked for 2 h in 5% skim milk. The membrane was incubated individually with E-cadherin, vimentin, MMP-9, and GAPDH primary antibodies overnight. Then, it was incubated with the secondary antibody conjugated with horseradish peroxidase for 2 h. ECL reagent was used for chromogenic exposure, and the X-ray film was exposed in a darkroom. The grey level ratio after target protein and GAPDH protein color development was used to represent the relative expression level of the target protein.

Statistical processing

The Student *t*-test has been used. All data are reported as means ± SD. *t*-test was used for comparisons among groups. A difference was considered statistically significant if $P < 0.05$.

RESULTS

Effect of TanIIA on SW480 cell proliferation

The MTT assay was used to measure the cell proliferation of each group after SW480 cells were incubated with different concentrations of TanIIA for 24 h. The inhibition rate of TanIIA on SW480 cell growth was calculated, and the results are shown in Table 1. The results indicate that the control group had the highest cell viability among all groups. In the treatment groups, as the concentration of TanIIA increased, proliferation decreased and the cell growth inhibition rate increased. These differences were significant compared with the control group ($P < 0.05$ in the 0.5-1.0 mg/L group and $P < 0.01$ in the 2.0-5.0 mg/L group). The results clearly indicate that TanIIA can inhibit the proliferation of colon cancer cells in a dose-dependent manner. The IC_{50} of TanIIA on SW480 cells was 1.5 mg/L as calculated using standard curve software.

Table 1. Effect of TanIIA with different concentrations on cell proliferation of SW480 cells (means ± SD).

Group (mg/L)	Cell proliferation (A value)	Cell growth inhibition rate (%)
0	0.92 ± 0.14	0
0.5	0.68 ± 0.15*	26 ± 4.3*
1.0	0.52 ± 0.11*	43.47 ± 4.0*
2.0	0.34 ± 0.09**	63.0 ± 5.5**
5.0	0.22 ± 0.04**	76.8 ± 7.8**

Effect of TanIIA on colon cancer cell invasion

Transwell assays were used to test the effect of 1.0 mg/L TanIIA on cell invasion *in vitro*. The results showed that TanIIA significantly inhibited the invasion ability of SW620 cells ($P < 0.05$) (Figure 1). These results indicated that TanIIA can inhibit colon cancer cell invasion *in vitro*.

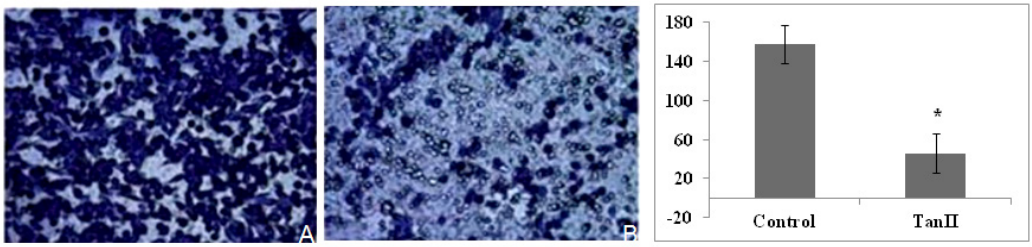

Figure 1. Effect of TanIIA on invasion of colon cancer cells (crystal violet staining, 400X). **A.** Blank control group; **B.** 1.0 mg/L TanIIA processing group.

Determination of invasion-related protein expression by western blotting

Cells were treated without (control group) or with 1.0 mg/L TanIIA for 24 h, and the effects of the treatment on the expression of E-cadherin, vimentin, and MMP-9 protein in SW480 cells were evaluated (Figure 2). The western blot results indicated that 1.0 mg/L TanIIA strongly increased the E-cadherin expression level and strongly decreased the expression levels of vimentin and MMP-9.

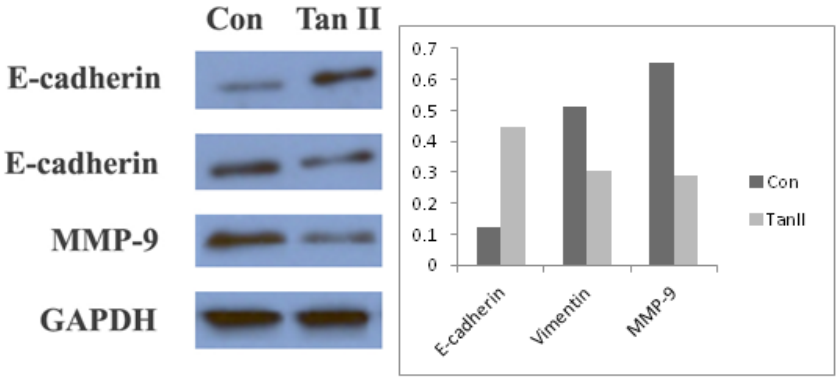

Figure 2. Effect of Tan IIA on cell invasion related protein expression.

DISCUSSION

Tan is derived from the ether and ethanol extracts of the root of *S. miltiorrhiza*. It is the main effective component of *S. miltiorrhiza*, and TanIIA has a natural antioxidant effect. The cardiovascular pharmacological effects of TanIIA include preventing atherosclerosis, shortening the myocardial infarction area, reducing myocardial oxygen consumption, and inhibiting thrombosis formation and platelet aggregation (Xu et al., 2006). Data indicate that Tan has better clinical prospects as an anti-tumor drug, as previous studies have shown that TanIIA can induce apoptosis in cervical cancer cells, HL-60 cells, and K562 cells, although its mechanism of action requires further study (Sung et al., 1999; Wang et al., 2003; Shan et al., 2009; Won et al., 2010; Chiu and Su, 2010; Yun et al., 2013).

The present study found that different concentrations of TanIIA could significantly inhibit the growth of colon cancer SW480 cells. The Transwell assay results also demonstrated that TanIIA could effectively inhibit the migration of colon cancer cells.

Studies have shown that insufficient expression of E-cadherin can reduce the adhesion force among tumor cells, causing them to become loose and infiltrate surrounding tissues, thus increasing the tumor malignancy grade (Zhang et al., 2015). Two another proteins, vimentin and MMP-9, are necessary for tumor cells to invade and expand into the surrounding stroma. In addition, higher gene expression levels of vimentin and MMP-9 are correlated with a higher tumor malignancy grade (Lampropoulos et al., 2012; Sipos and Galamb, 2012). The results of the present study showed that TanIIA strongly increased the expression of E-cadherin and decreased the expression of vimentin and MMP-9 in colon cancer cells, thereby inhibiting colon cancer cell invasion and metastasis. In addition, TanIIA clearly altered the expression of tumor metastasis-related proteins in colon cancer cells.

The results of this study show that TanIIA can inhibit the proliferation and invasion of colon cancer SW480 cells, and the possible mechanism of this action is by significantly altering the expression of colon cancer-related proteins. These findings provide a new approach to and theoretical basis for colon cancer drug therapy.

Conflicts of interest

The authors declare no conflict of interest.

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REFERENCES

Chien SY, Kuo SJ, Chen YL, Chen DR, et al. (2012). Tanshinone IIA inhibits human hepatocellular carcinoma J5 cell growth by increasing Bax and caspase 3 and decreasing CD31 expression *in vivo*. *Mol. Med. Rep.* 5: 282-286.

Chiu TL and Su CC (2010). Tanshinone IIA induces apoptosis in human lung cancer A549 cells through the induction of reactive oxygen species and decreasing the mitochondrial membrane potential. *Int. J. Mol. Med.* 25: 231-236.

Lampropoulos P, Zizi-Sermpetzoglou A, Papavassiliou AG, Kostakis A, et al. (2012). TGF-beta signalling in colon carcinogenesis. *Cancer Lett*. 314: 1-7.

Li GH and Zheng HJ (2011). Clinical analysis on 617 patients with colon cancer and rectal cancer. *Chin. Comm. Doctor* 30: 122-121.

Liu JJ, Zhang Y, Lin DJ and Xiao RZ (2009). Tanshinone IIA inhibits leukemia THP-1 cell growth by induction of apoptosis. *Oncol. Rep.* 21: 1075-1081.

Lu Q, Zhang P, Zhang X and Chen J (2009). Experimental study of the anti-cancer mechanism of tanshinone IIA against human breast cancer. *Int. J. Mol. Med.* 24: 773-780. http://dx.doi.org/10.3892/ijmm_00000291

Ren ZH, Tong YH, Xu W, Ma J, et al. (2010). Tanshinone II A attenuates inflammatory responses of rats with myocardial infarction by reducing MCP-1 expression. *Phytomedicine* 17: 212-218. http://dx.doi.org/10.1016/j.phymed.2009.08.010

Shan YF, Shen X, Xie YK, Chen JC, et al. (2009). Inhibitory effects of tanshinone II-A on invasion and metastasis of human colon carcinoma cells. *Acta Pharmacol. Sin.* 30: 1537-1542. http://dx.doi.org/10.1038/aps.2009.139

Sipos F and Galamb O (2012). Epithelial-to-mesenchymal and mesenchymal-to-epithelial transitions in the colon. *World J. Gastroenterol.* 18: 601-608. http://dx.doi.org/10.3748/wjg.v18.i7.601

Su CC (2014a). Tanshinone IIA inhibits gastric carcinoma AGS cells through increasing p-p38, p-JNK and p53 but reducing p-ERK, CDC2 and cyclin B1 expression. *Anticancer Res.* 34: 7097-7110.

Su CC (2014b). Tanshinone IIA inhibits human gastric carcinoma AGS cell growth by decreasing BiP, TCTP, Mcl-1 and Bcl-xL and increasing Bax and CHOP protein expression. *Int. J. Mol. Med.* 34: 1661-1668.

Sung HJ, Choi SM, Yoon Y and An KS (1999). Tanshinone IIA, an ingredient of Salvia miltiorrhiza BUNGE, induces apoptosis in human leukemia cell lines through the activation of caspase-3. *Exp. Mol. Med.* 31: 174-178. http://dx.doi.org/10.1038/emm.1999.28

Wang AM, Sha SH, Lesniak W and Schacht J (2003). Tanshinone (Salviae miltiorrhizae extract) preparations attenuate aminoglycoside-induced free radical formation *in vitro* and ototoxicity *in vivo*. *Antimicrobiol. Agents Chemother.* 47: 1836-1841. http://dx.doi.org/10.1128/AAC.47.6.1836-1841.2003

Won SH, Lee HJ, Jeong SJ, Lee HJ, et al. (2010). Tanshinone IIA induces mitochondria dependent apoptosis in prostate cancer cells in association with an inhibition of phosphoinositide 3-kinase/AKT pathway. *Biol. Pharm. Bull.* 33: 1828-1834. http://dx.doi.org/10.1248/bpb.33.1828

Xu W, Towers AD, Li P and Collet JP (2006). Traditional Chinese medicine in cancer care: perspectives and experiences of patients and professionals in China. *Eur. J. Cancer Care* 15: 397-403. http://dx.doi.org/10.1111/j.1365-2354.2006.00685.x

Yang R, Liu A, Ma X, Li L, et al. (2008). Sodium tanshinone IIA sulfonate protects cardiomyocytes against oxidative

stress-mediated apoptosis through inhibiting JNK activation. *J. Cardiovasc. Pharmacol.* 51: 396-401. http://dx.doi.org/10.1097/FJC.0b013e3181671439

Yun SM, Jeong SJ, Kim JH, Jung JH, et al. (2013). Activation of c-Jun N-terminal kinase mediates tanshinone IIA-induced apoptosis in KBM-5 chronic myeloid leukemia cells. *Biol. Pharm. Bull.* 36: 208-214. http://dx.doi.org/10.1248/bpb.b12-00537

Zhang Z, Yang C, Gao W, Chen T, et al. (2015). FOXA2 attenuates the epithelial to mesenchymal transition by regulating the transcription of E-cadherin and ZEB2 in human breast cancer. *Cancer Lett.* 361: 240-250. http://dx.doi.org/10.1016/j.canlet.2015.03.008