Effects and Mechanism of Tanshinone II A in Proliferation, Apoptosis, and Migration of Human Colon Cancer Cells

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Background: The aim of this study was to explore the effect and mechanism of tanshinone II A on proliferation, apoptosis, and migration of human colon cancer cells.

Material/Methods: CCK-8 approach was carried out to evaluate proliferation after applying various levels of tanshinone II A to SW620 colon carcinoma cells. Flow cytometry (FC) was used to assess apoptosis. Transwell assay was performed to assess invasion in vitro, and the wound-healing assay was applied to assess migration. Western blot analysis was performed to evaluate translation of mTOR, while RT-PCR was carried out to assess transcription of VEGF.

Results: CCK-8 assay showed that tanshinone II A inhibited SW620 proliferation in comparison to the control group subsequent to 24 h, 48 h, and 72 h (P<0.001). FC revealed that tanshinone II A promoted SW620 apoptosis (P<0.001). The cell migration test revealed that the migration index of cells receiving tanshinone II A decreased. mTOR translation as well as VEGF transcription in cells receiving tanshinone II A was noticeably prohibited compared to control group (P<0.001).

Conclusions: Tanshinone II A is able to inhibit proliferation and migration of human colon cancer SW620 cells and promoted cell death. Its mechanism may be by downregulation of mTOR protein and VEGF mRNA.

MeSH Keywords: Arginase • Lynch Syndrome II • Sigmoid Neoplasms

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Background

Colon carcinoma (CC) is a common digestive tract malignancy. Changes in lifestyle and diet have led to increased CC incidence and mortality rates [1,2]. The current standard management of CC is surgery, and various comprehensive therapies are provided based on patients’ circumstances [3]. Nevertheless, since malignancies cause angiogenesis, metastasis and invasion are more frequent. Consequently, CC relapse is more frequent, even after surgery [4]. Angiogenesis is a crucial contributor to metastasis and growth of malignancies, since new vessel generation provides nutrition and oxygen to sustain malignant growth and eliminate metabolites generated via malignancies [5,6]. Studies have found that the risk of liver metastases in colon cancer patients is as high as 50% [7].

Tanshinone II A is a fat-soluble component extracted from the plant *Salvia miltiorrhiza*, with reported anticancer activity [8]. Tanshinone II A has primarily been used to treat cardiovascular diseases [9]. However, it is widely accepted that tanshinone II A is able to prohibit malignant proliferation and to reinforce malignant cell death, and significantly eliminates malignant cells [10,11]. Tanshinone II A treatment in murine gastric cancer (GC) models can inhibit GC cell proliferation during G0-G1 phase, which hampers DNA generation and inhibits proliferation of GC cells [12]. Some studies have found that mTOR signaling is the most closely related signal transduction pathway to cell proliferation and apoptosis, and plays a key regulatory role in many malignant tumors [13].

Despite the demonstrated impact of tanshinone II A on various types of malignancies, little research has been conducted on the contribution of tanshinone II A to biological activities of CC cells. It was hypothesized that tanshinone II A can exert an impact on biological activities of CC cells via influencing mTOR translation. Nevertheless, the understanding of how tanshinone II A modulates CC cells *in vitro* is insufficient. Consequently, our research investigated the impact and mechanism of tanshinone II A on proliferation, apoptosis, and migration of SW620 cells, aiming to generate further data and insights into CC treatment.

Material and Methods

Experimental materials and reagents

Human CC cells SW620 were bought from Shanghai Institute of Cell Biology, Chinese Academy of Sciences, and underwent freezing. We also used: Tanshinone II A (Shanghai Huzheng Biological Technology Co., HZ-21639); fetal bovine serum and DMEM culture media (GIBCO, USA); CCK-8 reagent kit (Dojindo Biological Technology Co., HZ-21639); fetal bovine serum and freezing. We also used: Tanshinone II A (Shanghai Huzheng Biological Technology Co., China). Our research was approved by the Ethics Committee of the Second Hospital of Jilin University.

Cell recovery, cultivation, passage, and cryopreservation

Frozen SW620 cells were acquired from liquid nitrogen and were thawed at 37°C. The thawed solution was subsequently moved to centrifuge tubes under sterile conditions. Cells underwent cultivation with DMEM media with 10% FSB in 5% CO₂ and 37°C. Growth was assessed with an inverted microscope, and the solution was renewed as needed. The medium was not renewed until confluency reached 80%. We used 3 mL of PBS in serum and for dead cell elimination. Pancreatic enzyme was supplemented for digestion, which was halted by supplementing DMEM complete medium including FBS when cells became rounded and transparent, as assessed by microscopy. If the cells adhered to the bottle wall, its bottom was tapped. The suspension underwent 5-min centrifugation at 1000 rpm at room temperature. Complete medium was used for resuspension. Cells were equally divided into 3 new cultivating bottles for passaging, and underwent subsequent cultivation. When cells reached logarithmic growth phase, they were acquired and underwent extra digestion, washing, and passaging. Cells were moved to 1.5-ml cryopreservation tubes and underwent overnight freezing at −80°C. Cryopreservation tubes were moved to liquid nitrogen the following day.

CCK-8 assay

SW620 cells underwent resuscitation as mentioned before as well as cultivation in plates containing 96 wells at a density of 3×10⁴/hole. Cells were classified into an intervention group and a control group. The latter received only cultivating medium. After cell adherence, the drug intervention group was treated with 2 ml tanshinone IIA at concentrations of 0.5 μg/ml, 1.0 μg/ml, 2.0 μg/ml, 5 μg/ml, and 10 μg/ml. CCK-8 solution (10 L/hole) was added at 24 h, 48 h, and 72 h after the treatment, and then cultured in an incubator for 2 h. Absorbance at 450 nanometers was assessed using a Spectra Max M5 enzyme-labeling instrument to assess proliferation. Procedures were carried out 3 times. Cell proliferation rates were determined in every group.

Annexin V-FITC assay

SW620 cells underwent cultivation at a steady temperature. Cells were placed into 6 bottles, with each containing 3×10⁵ cells. The first bottle only received cultivating medium and
was the control group. Bottles 2 to 6 received 2 milliliters of tanshinone IIA at concentrations of 0.5 ug/mL, 1.0 ug/mL, 2.0 ug/mL, 5 ug/mL, and 10 ug/mL. PBS was used to wash cells after 48 h, and then underwent 5-min centrifugation at 2000 rpm. Supernatant was eliminated and resuspension was achieved. Cells underwent 1-h incubation with 5 mL Annexin V-FITC and 5 mL PI at room temperature. FLEXLX flow cytometry (FC) was performed to assess cell death. Procedures were carried out 3 times.

**Transwell assay**

Cells were seeded into 24-well plates (3000/hole). The intervention group received supplementation as mentioned above. DMEM without serum was used to dilute the cells (3×10^4 cells/ml) 48 h after supplementation. The top chamber was supplemented with 25 mL diluted cells, while the bottom chamber received 600 mL DMEM medium including 20% FBS. PBS was used to wash the chamber after 24 h. Cells in chambers received 10-min fixation with 4% poly-formaldehyde solution, another PBS washing, 10-min drying with 0.5% crystal violet, and then PBS washing before clarification. Terminally, invasion was detected in 5 random fields under a microscope, with average value determined. Procedures were carried out 3 times.

**Wound-healing assay**

SW620 cells were obtained and prepared into single-cell suspension, which underwent dilution to 3×10^5 cells/ml as mentioned above. Plates containing 6 wells were used to plant the cells, which underwent cultivation in medium and with agents as mentioned above. We used 200-mL sterile pipette tips to divide cells into cell-free zones after 48 h. PBS was used to wash cells which were cultivated with renewed medium. We measured the width of the scratched cell-free zone at 3 sites under a microscope at 0 h (W0) and 24 h (W24) subsequent to cell division. Cell migration index (M1)=(W0–W24)/W0×100%.

**Western blot (WB) analysis**

The logarithmic phase SW620 cells were taken out, cracked, and collected in 6 culture bottles. Each bottle contained 3×10^5 cells. The first flask acted as the control group. Flasks 2 to 6 received supplementation with 2 mL of tanshinone II A at a concentration of 0.5 ug/mL, 1.0 ug/mL, 2.0 ug/mL, 5 ug/mL, and 10 ug/mL. Cells were cracked with mTOR proteins harvested after 48-h cultivation. We used 10% SDS-PAGE to isolate proteins, which were then moved to PVDF membranes. Five percent skimmed milk was added after 1-h sealing at room temperature. Primary anti-mTOR antibodies were added for overnight incubation at 4°C. Incubation was additionally carried out with the help of secondary antibodies. ECL developer was terminally colored.

**RT-PCR**

Melted cells underwent cultivation as mentioned before. Trizol reagent was used to isolate total RNA of VEGF transcripts. An ultraviolet spectrophotometer was used for purification and RNA quantification. Three micrograms of total RNA were obtained to reverse-transcribe into cDNA. Reactions were carried out at 37°C for 5 min, 42°C for 60 min, and 72°C for 10 min. cDNA was applied to PCR amplification with GAPDH acting as an internal control. Primers used were: VEGF-F: 5'-GTGCCCACTGAGGATCACA-3', VEGF-R: 5'-GCAAGGCCCCAGGAGGT-3', GAPDH-F: 5'-GTACGGCTGGGCTGCGATT-3', GAPDH-R: 5'-GCTCTTCTGCTGGGCTGTTG-3'. PCR circumstances of VEGF transcripts were: preliminary denatured at 95°C for 2 min, cycled at 95°C for 10 s, and 58°C for 60 s for 40 times, and terminally extended at 72°C for 90 s. Fluorescence qRT-PCR was performed 3 times.

**Statistical methods**

This study used SPSS20.0 software (Boyi Intelligence (Beijing) Information Technology Co.) for data analyses. Measurement outcomes are displayed as mean ±SD. Differences between groups were evaluated using the independent-samples t test, while differences among various groups were assessed by ANOVA. The paired t test was applied to assess differences at various time points. The level of significance was set as P<0.05.

**Results**

**Effect of tanshinone II A on SW620 proliferation**

Tanshinone II A prohibited SW620 proliferation in a dose-dependent manner. SW620 viability declined with elevated levels. Viability at 72 h was remarkably prohibited at 24 h and at 48 h (P<0.05) (Table 1, Figure 1).

**Effect of tanshinone II A on SW620 cell death**

SW620 death was reinforced via elevation of tanshinone II A level. The death rate was remarkably higher at 10 ug/mL in comparison to that at 2 ug/mL (P<0.05) (Table 2, Figures 2, 3).

**Effect of tanshinone II A on invasion and migration of SW620 cells**

The invasion ability of cells was inhibited via elevation of tanshinone II A level. The invasion index at tanshinone II A concentration of 10 ug/mL was significantly lower than that at 2.0 ug/mL (P<0.05) (Table 3, Figures 4, 5).
Effect of tanshinone II A on mTOR translation of SW620 cells

mTOR translation was inhibited in SW620 cells via elevation of tanshinone II A level, which was noticeably lower at 10 ug/mL than at 2.0 ug/mL (P<0.05) (Table 4, Figure 6).

Rt-PCR for investigation of VEGF transcription

VEGF transcription was inhibited via elevation of tanshinone II A level, which was noticeably lower with tanshinone II A at 10 ug/mL than at 2.0 ug/mL (P<0.05) (Table 5).

Discussion

CC is the third most common malignancy worldwide, and is a major cause of cancer-related morbidity and mortality [14]. Unregulated proliferation of normal cells contributes to malignancies. Invasion, metastasis, and basic features of malignancies also contribute to mortality [15,16]. It was revealed recently that several monomer compounds of herbal agents can trigger differentiation and death of malignant cells, consequently counteracting malignancies [17]. Multiple studies have investigated the ability of tanshinone II A to combat cancer. It was revealed that tanshinone II A plus cisplatin obviously increased death of prostate cancer cells [18]. Nevertheless,

Table 1. Survival rate of SW620 cells treated with tanshinone II A (%).

| Time | 0 ug/mL | 0.5 ug/mL | 1.0 ug/mL | 2.0 ug/mL | 5 ug/mL | 10 ug/mL | F   | P    |
|------|---------|-----------|-----------|-----------|---------|---------|------|------|
| 24 h | 96.21±3.87 | 87.14±1.77 | 79.45±2.61 | 61.44±2.21* | 50.54±1.31 | 40.19±2.57 | 227.2 | <0.001 |
| 48 h | 94.43±4.72 | 85.02±2.58 | 76.33±3.24 | 58.59±2.37* | 45.82±2.10 | 36.24±4.55 | 134.7 | <0.001 |
| 72 h | 92.55±5.63 | 83.41±3.26 | 69.18±4.45 | 51.18±3.66* | 41.49±4.36 | 21.81±8.05 | 80.66 | <0.001 |
| F    | 0.437    | 1.543     | 6.718     | 9.65      | 7.336    | 9.146   |       |      |
| P    | 0.665    | 0.288     | <0.050    | <0.050    | <0.050   | <0.050  |       |      |

* Compared with the concentration of 10 ug/mL, P<0.05.

Table 2. Effect of tanshinone II A on apoptosis rate of SW620 cells at 48 h (%).

| Factor | 0 ug/mL | 0.5 ug/mL | 1.0 ug/mL | 2.0 ug/mL | 5 ug/mL | 10 ug/mL | F   | P    |
|--------|---------|-----------|-----------|-----------|---------|---------|------|------|
| SW620  | 3.09±0.98 | 6.46±2.31 | 13.58±2.91 | 17.83±3.86* | 28.59±4.41 | 35.41±4.49 | 40.99 | <0.001 |

* Compared with the concentration of 10 ug/mL, P<0.05.
Figure 3. Flow cytometry charts for apoptosis rate at 48 h. (A) 0.0 ug/mL, (B) 0.5 ug/mL, (C) 1.0 ug/mL, (D) 2.0 ug/mL, (E) 5.0 ug/mL, (F) 10.0 ug/mL.
understanding of the mechanism of tanshinone II A during proliferation, migration, and death of CC cells is insufficient. It was demonstrated that mTOR is intimately linked with growth and proliferation of malignant cells and exerts an important effect on translation during G1 cell cycle [19]. One of the crucial circumstances of malignant growth and migration is generation of new vessels, during which VEGF plays an essential role [20,21]. Consequently, it was hypothesized that migration, death, and proliferation of CC SW620 cells are linked with mTOR and VEGF.

### Table 3. Effect of tanshinone II A on cell numbers and migration index of SW620 cells at 48 h.

| Factor                  | 0 ug/mL   | 0.5 ug/mL | 1.0 ug/mL | 2.0 ug/mL | 5 ug/mL | 10 ug/mL | F    | P     |
|-------------------------|-----------|-----------|-----------|-----------|---------|----------|------|-------|
| Cell-penetrating number | 68.66±5.53| 55.21±6.12| 47.82±6.93| 34.19±7.15*| 25.62±6.73| 11.35±2.76| 35.56| <0.001|
| Cell migration index    | 79.82±1.71| 66.36±1.22| 59.87±1.32| 52.82±1.42*| 39.67±2.53| 27.93±1.62| 364.1| <0.001|

* Compared with the concentration of 10 ug/mL, P<0.05.

### Figure 4. Effect of tanshinone II A on cell-penetrating quantity at 48 h. Invasion ability of SW620 was inhibited via elevation of tanshinone II A levels and was significantly decreased with tanshinone II A at 10 ug/mL in comparison to 2.0 ug/mL. It was also noticeably decreased with tanshinone II A at 2.0 ug/mL in comparison to 0 ug/mL (P<0.05). * Represents P<0.05.

### Figure 5. Effect of tanshinone II A on migration index of SW620 cells at 48 h. Migration index of SW620 was inhibited via elevation of the tanshinone II A level. It was noticeably lower with tanshinone II A at 10 ug/mL in comparison to 2.0 ug/mL. It was also noticeably lower with tanshinone II A at 2.0 ug/mL in comparison to 0 ug/mL (P<0.05). * Represents P<0.05.

### Table 4. Effect of tanshinone II A on the expression of mTOR protein in SW620 cells at 48 h.

| Factor | 0 ug/mL | 0.5 ug/mL | 1.0 ug/mL | 2.0 ug/mL | 5 ug/mL | 10 ug/mL | F    | P     |
|--------|---------|-----------|-----------|-----------|---------|----------|------|-------|
| mTOR   | 1.98±0.21| 1.87±0.23 | 1.61±0.17 | 1.32±0.14*| 1.15±0.13| 0.96±0.14| 16.30| <0.001|

* Compared with the concentration of 10 ug/mL, P<0.05.

### Figure 6. Western blot charts for mTOR expression levels at 48 h. Understanding of the mechanism of tanshinone II A during proliferation, migration, and death of CC cells is insufficient. It was demonstrated that mTOR is intimately linked with growth and proliferation of malignant cells and exerts an important effect on translation during G1 cell cycle [19]. One of the crucial circumstances of malignant growth and migration is generation of new vessels, during which VEGF plays an essential role [20,21]. Consequently, it was hypothesized that migration, death, and proliferation of CC SW620 cells are linked with mTOR and VEGF.
Our research investigated the effect of various levels of tanshinone II A on invasion, death, migration, and proliferation of CC cells. It was revealed via CCK-8 test that SW620 viability was inhibited in a dose-dependent manner by tanshinone II A level as well as by as extension of treatment time. Viability was inhibited the most with tanshinone II A at 10 ug/mL. Apoptosis assay revealed that apoptosis was inhibited via elevation of tanshinone II A level, showing that tanshinone II A inhibited proliferation and promoted death of SW620 cells. A study [22] used SW480, HCT116, and LOVO colorectal cancer cell lines to study the effects of tanshinone on cell viability and apoptosis, as well as its tumorigenicity. It was found that tanshinone significantly inhibits proliferation of colon cancer SW480, HCT116, and LOVO cell lines, and tanshinone can also inhibit the activation of signal transduction and transcriptional activator 3 (Stat3) pathways in colorectal cancer cells, and the results of the study are consistent with our conclusions. The number of invading cells and the migration index were decreased with elevation of tanshinone II A levels, showing that tanshinone II A was able to inhibit migration and invasion of SW620 cells. Studies [23] found that tanshinone II A showed an inhibitory effect on invasion and metastasis of human colon cancer cells, and it is believed that tanshinone II A may act through lowering urokinase plasminogen activator (uPA) and matrix metalloproteinase (MMP)-2 and MMP-9 levels, as well as by increasing the level of matrix metalloproteinase tissue inhibitor protein (TIMP) and inhibiting invasion and metastasis of human colon cancer cells in vitro and in vivo. This was confirmed by our present findings, and also suggests the possible mechanism of action of tanshinone II A.

Several studies [24] have discovered that tanshinone II A promotes death of lung cancer cells, which is in conformity with our results. Some studies [25] found that when mTOR is over-activated, it promotes the occurrence of tumors in many kinds of cancer tissues. mTOR inhibition can trigger cell death and inhibit proliferation. Our research revealed that mTOR translation of SW620 cells was inhibited in a dose-dependent manner and that tanshinone II A can prohibit proliferation and death of SW620 cells through downregulation of mTOR translation. We evaluated VEGF transcription of SW620 cells treated with various levels of tanshinone II A, showing that VEGF transcription was inhibited by tanshinone II A in a dose-dependent manner. It was previously discovered that tanshinone II A inhibits malignant growth and VEGF expression, which was in conformity with our outcomes [26]. It was also discovered in previous research that COX-2 can trigger VEGF expression, consequently reinforcing generation of malignant vessels [27].

### Conclusions

Tanshinone II can inhibit proliferation, migration, death, and invasion abilities of human CC SW620 cells, possibly by down-regulating mTOR and VEGF in order to influence biological activities of CC cells, offering an innovative strategy to treat CC. Nevertheless, the mechanism of VFGF and mTOR require further investigation.

### Conflict of Interests

None.

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### Table 5. Effect of tanshinone II A on VEGF mRNA expression in SW620 cells.

| Factor | 0 ug/mL | 0.5 ug/mL | 1.0 ug/mL | 2.0 ug/mL | 5 ug/mL | 10 ug/mL | F | P   |
|--------|---------|-----------|-----------|-----------|---------|---------|----|-----|
| VEGF mRNA | 5.31±1.31 | 4.22±0.98 | 3.01±0.79 | 2.35±0.75* | 1.79±0.66 | 1.01±0.21 | 10.49 | <0.001 |

* Compared with the concentration of 10 ug/mL, P<0.05.
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