

Effects of oxymatrine on the adhesion of human hepatoma cell line QGY-7703 and the expression of integrin β 1.

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Abstract

Background: To explore the effect of Oxymatrine (OM) on cell-matrix adhesion of human hepatoma cell line QGY-7703 and the expression of integrin β 1.

Methods: The effect of OM on cell-matrix adhesion of human hepatoma cell line QGY-7703 was determined with cell adhesion assay and the effect on the mRNA and protein expression of integrin β 1 in QGY-7703 cells was detected by quantitative PCR and Western blot.

Results: Compared with the control group, QGY-7703 cell-matrix adhesion in the group treated with 1 mg.mL⁻¹ OM was not inhibited, while the groups treated with 2.5 mg.mL⁻¹ and 5 mg.mL⁻¹ OM treated cells (P<0.05) was significantly inhibited (p<0.05), accompanied by the decreased mRNA and protein expression of integrin β 1 (P <0.05).

Conclusion: *In vitro* OM can inhibit the cell-matrix adhesion and mRNA and protein expression of integrin β 1 of human hepatoma cell line QGY-7703.

Keywords: Oxymatrine (OM), Hepatocarcinoma cells, Cell-matrix adhesion, Integrin β 1.

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Introduction

Oxymatrine (OM) is the main component of alkaloids in *Sophora japonica*, *Sophora flavescens* and *Sophora alopecuroides* and its molecular formula is C₁₅H₂₄N₂O. Some studies have shown that oxymatrine has anti-virus, anti-liver fibrosis, antiarrhythmic, anti-inflammatory effect [1-4]. Not only that, OM was also found to have an effect on anti-tumor. For example, OM can inhibit proliferation and migration of human glioblastoma cells by induction of apoptosis [5]. It is now demonstrated that oxymatrine has a significant inhibitory effect on hepatocellular carcinoma cells, which may be achieved by inhibiting cancer cell division and reducing cytotoxicity [6,7]. However, it has also been found that OM, at the dose of 320 mg/kg, has a damaging effect on liver [8]. Integrin β 1, a member of the β sub-family, forms dimers with eight different α subunits in vertebrates [9]. Some researchers suggested that β 1-null cells from various types of cancer became sensitive to chemotherapy and radiotherapy, because integrin β 1 regulates viability and proliferation [10-12]. Cell-extracellular matrix adhesion is a fundamental process that is important in multiple hallmarks of breast cancer, including conferring a proliferative advantage, inducing pro-survival pathways and driving cell migration and invasion [13,14]. Both integrin β 1 and cell-extracellular matrix adhesion are related to the onset of human cancers [12,14]. It has not yet been clearly reported that OM plays a role in the migration and invasion of liver cancer cells. Therefore, the aim of this study is to

investigate the effect of different concentration OM on the cell adhesion of human hepatocellular carcinoma cell line QGY-7703 and integrin β 1 expression, and related possible molecular mechanism.

Materials and Methods

Cells and reagent

Oxymatrine was purchased from Shaanxi Huike Plant Development Co., Ltd. (China), purity of 98%. Human liver cancer cell line QGY-7703 was donated by Tianjin Life Science Center (China). RPMI1640 cell culture mediums were purchased from Gibco Co., Ltd. (USA). Trizol, RT-PCR kit, SYBR Green ERTM qPCR SuperMix kit was purchased from Dalian Baosheng Biotech Co., Ltd. (China). Matrigel was purchased from Becton, Dickinson and Company (New Jersey, USA). The antibody was purchased from Tianjin Purcell Biotechnology Company (China).

Cell culture

The human hepatocellular carcinoma cell line QGY-7703 was cultured in RPMI1640 containing 10% calf serum, 100 IU.mL⁻¹ penicillin and streptomycin at 37°C in 5% CO₂ incubator. The cells were digested with 0.1% trypsin (0.02% EDTA) and passaged once every 2-3 d.

Administration and grouping

Each individual experiment was provided with blank control group, cell control group and experimental group. The cells in the logarithmic growth phase were inoculated into 24-well cell culture plates at $4 \times 10^5 \text{ mL}^{-1}$. The blank control group only added the cell culture medium (2 mL/well) without cells. The cell control group only added cells (2 mL/well) without OM. The cells and OM (2 mL/well) were added in the experimental group, and divided into 1, 2.5 and 5 $\text{mg}\cdot\text{mL}^{-1}$ concentration groups based on the OM concentration (OM 1, OM 2.5, OM 5 group).

Cell adhesion assay for cell-matrix adhesion detection

The Matrigel gel was coated on each well of a 96-well plate and the extracellular matrix was imitated with Matrigel. The cells were then digested with trypsin for 48 h to adjust the cell density to $2.5 \times 10^4 \text{ mL}^{-1}$, and the 100 μL cell suspension was added to the 96-well plate. The plates were incubated for 3 h at 37°C , the supernatant was removed, washed twice with $1 \times \text{PBS}$ and 100 μL bovine serum albumin (0.5%) was added to each well at 37°C to block for 2 h, again remove the supernatant, wash with $1 \times \text{PBS}$ twice. Five wells were set in each group, and placed in 37°C incubator for continue culture. The supernatant were removed at 30, 60 and 90 min after culturing, and the cells were washed three times with PBS to wash off cells that did not adhere. Then, 100 μL RPMI 1640 complete culture medium and 100 μL MTT were added to each well, and placed in 37°C incubator to continue culture for 4 h. Then, take the culture plate and, centrifuge at room temperature for 5 min at the speed of $3000 \text{ r}\cdot\text{min}^{-1}$. Afterwards, the supernatant was discarded and 100 μL DMSO was added to dissolve the granules. The plates were placed on a shaker and shaken at room temperature for 20 min. The absorbance values (A570 nm) at wavelength of 570 nm were measured with a microplate spectrophotometer.

Real-time fluorescence quantification

PCR was used to detect the expression of integrin $\beta 1$ mRNA. The cells in logarithmic growth phase were inoculated into 25 mL cell culture flasks (2.5×10^5 cells/bottle) and 3 mL per bottle. After the cells were adhered, OM was added for 48 h culture and the total RNA of each group was extracted (Trizol method). RT kit was used to synthesize cDNA. Real-time quantitative PCR kit was utilized for PCR reaction. The primer sequence used was as following: integrin $\beta 1$ primer: Upstream 5'-ATG AAT TTA CAA CCA ATT TTT C-3'; Downstream 5'-TCA TTT TCC CTC ATA CTT CG-3'; β -actin primer: Upstream 5'-CCT CAT GCC ATC CTG CGT CTG- 3'; Downstream 5'-TTG CTC GAA GTC TAG GGC AAC ATA G-3'. The reaction system was as following: template cDNA, 1 μL ; upstream primer (5 μM), 1 μL ; downstream primer (5 μM), 1 μL ; $2 \times \text{SYBR GreenERTM qPCR SuperMix}$, 10 μL ; sterilized double distilled water, 7 μL ; total volume 20 μL . PCR reaction conditions: (1) 95°C 3 min (pre-denaturation); (2) 94°C 30 s (denaturation); (3) 57°C 30 s (annealing); (4) 72°C 30 s (extended, extended terminal SYBR fluorescence

signal acquisition); 40 cycles for steps (2)-(4). The relative content of integrin $\beta 1$ in the sample was calculated (compared with the reference gene β -actin), calculated as: $2^{-\Delta\Delta\text{Ct}}$, where $\text{Ct}=(\text{ct}(\text{integrin } \beta 1 \text{ in experimental group})-\text{Ct}(\beta\text{-actin in experimental group}))-(\text{Ct}(\text{integrin } \beta 1 \text{ in control group})-\text{Ct}(\beta\text{-actin in control group}))$.

Western blot for integrin $\beta 1$ expression

The cells in logarithmic growth phase were seeded in 24-well plate (2×10^4 /well), 1 mL per well. After cell adherence, OM was added and the cells were incubated for 48 hours. Then RIPA lysis buffer was added to prepare cell protein samples. 6% SDS-PAGE was used for electrophoresis, which was then electro transferred to the nitrocellulose membrane, and placed in blotto buffer at room temperature for 2 h. After that, the membrane placed in the corresponding antibody (anti-integrin $\beta 1$ antibody or rabbit anti-actin antibody, 1:100 or 1:500 dilution) blotto, shaken overnight at 4°C , washed with TBST for 3 times (5 min/time). The membrane was placed in blotto containing the corresponding secondary antibody (HRP-labelled goat anti-mouse or goat anti-rabbit IgG antibody), shaken at room temperature for 1.5 h at room temperature, washed with TBST for 5 times (5 min/time). ECL was used for luminescence and the relative density of integrin $\beta 1$ was analyzed by using image analysis and processing system software for exposure, development and image acquisition. The optical density of each protein band was scanned. In order to calculate the ratio of the optical density of the integrin $\beta 1$ band to the optical density of the corresponding GAPDH (internal reference) band, the corrected integrin $\beta 1$ expression intensity value was calculated. The expression of integrin $\beta 1$ in the experimental groups was compared. The expression of the control group was considered as 1, the expression of integrin $\beta 1$ in the experimental groups was calculated and the histogram was obtained.

Statistical method

Data were statistically analyzed with SPSS 16.0. The data were expressed as mean \pm standard deviation ($\bar{x} \pm s$). The single factor analysis of variance was applied in multiple samples comparison. $P \leq 0.05$ means statistically significant differences between the two samples.

Results

Effect of OM on matrix adhesion of QGY-7703 cell

The OD (A570 nm) of the three does OM groups and the control group were not the same. Compared with the control group, the OD values of OM 2.5 and OM 5 group were significantly decreased ($P < 0.01$). Compared with OM 1 group, the OD value of OM 5 group was significantly decreased ($P < 0.01$) (Figure 1).

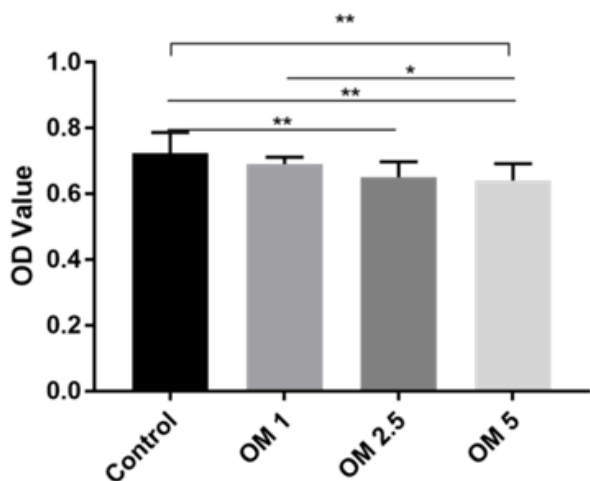


Figure 1. Effect of OM on matrix-adhesion of QGY-7703 cells.

Effect of OM on expression of integrin $\beta 1$ mRNA in QGY-7703 cells

The integrin $\beta 1$ mRNA expression in the OM groups of three doses and the control group of were not the same. Compared with the control group, the levels of $\beta 1$ mRNA in OM 2.5 and OM 5 groups were significantly decreased ($P < 0.01$). Compared with OM 1 group, $\beta 1$ mRNA levels in OM 2.5 and OM 5 groups were significantly lower ($P < 0.01$) (Figure 2).

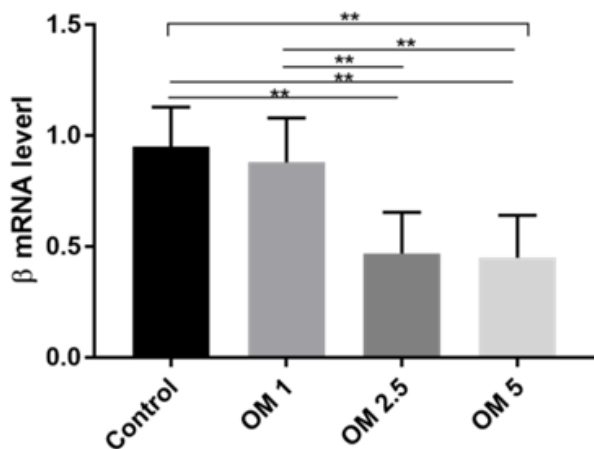


Figure 2. Effect of OM on integrin $\beta 1$ in QGY-7703 cells.

Effect of OM on expression of integrin $\beta 1$ protein in QGY-7703 cells

The contents of integrin $\beta 1$ protein in OM groups of three does and control group were not the same. Compared with the control group, the levels of $\beta 1$ protein in OM 2.5 and OM 5 groups were significantly decreased ($P < 0.01$). Compared with OM 1 group, the levels of $\beta 1$ protein in OM 2.5 and OM 5 groups were significantly decreased ($P < 0.01$). Electrophoresis bands showed that the bands of OM 1, OM 2.5 and OM 5 were also more and more thin with the increased concentrations (Figure 3).

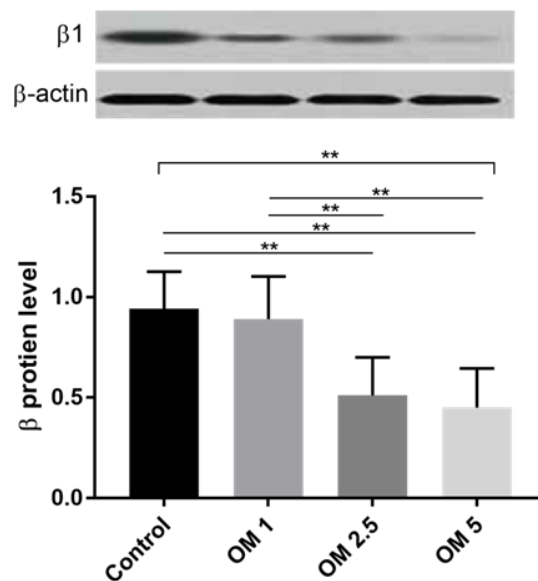


Figure 3. Effect of OM on integrin $\beta 1$ protein expression in QGY-7703 cells.

Discussion

Hepatocellular carcinoma is the fifth most common type of cancer in the world and is the third leading cause of cancer-related mortality worldwide [15]. The high mortality of liver cancer is mainly due to high recurrence and metastasis. Liver cancer metastasis is an extremely complex “multi-step” process, liver cancer cells leave the primary tumor and invaded into blood vessels and lymphatic vessels through the extracellular matrix (extracellular matrix, ECM), then the metastases was formed [16]. In this process, the combination of hepatoma cells and ECM is the initial step of invasive growth and distant metastasis of hepatocarcinoma cells [17]. A recent study showed that OM inhibits hepatocyte apoptosis by suppressing the TLR4/PI3K/Akt/GSK-3 β signalling pathway [18]. OM has a variety of pharmacological actions. It has a protective effect on ischemic and reperfusion injury in liver, and the intestines and heart *via* its anti-apoptotic and anti-inflammatory activity [19-21]. As a result, this study first used the cell adhesion test to detect the effect of OM on matrix adhesion ability of liver cancer cells. The results showed that OM at higher concentrations (2.5 and 5 mg.mL⁻¹) could inhibit the adhesion of hepatocarcinoma cells to matrix. Cell adhesion molecules are one of the important factors of tumor metastasis, tumor metastasis in the various steps involved in the number of cell adhesion molecules or functional changes. Cell adhesion molecules (CAMs) are a class of transmembrane glycoproteins present on the cell surface or ECM that mediate cell-cell or cell-ECM contact with each other by binding to the corresponding ligand on the cell or ECM surface combination. The results suggested that OM at higher concentrations (2.5 and 5 mg.mL⁻¹) can inhibit the expression of integrin $\beta 1$ mRNA and protein. The integrin family is a large group of cell adhesion molecules, widely expressed in a variety of tissue cells, which is a heterologous dimer composing of the α and β

subunits of transmembrane protein through non-covalent bond and plays an important role in mediating cell ECM adhesion. Some studies have shown that in tumor cells, expression and function of integrin often have significant changes that have promoting effect on the adherence, migration and invasion and angiogenesis of tumor cells. In hepatocarcinoma cells, the expression of integrin $\beta 1$ was significantly enhanced, playing a key role in the invasion and metastasis. Therefore, after confirmation the inhibitory effect of OM on matrix adhesion in QGY-7703 cells, the integrin $\beta 1$ expression after OM treatment was determined.

Conclusion

The results demonstrated that the mRNA and protein expression of integrin $\beta 1$ in QGY-7703 cells in OM treatment groups were significantly lower than that in the control group ($P < 0.05$). However, whether there is a direct causal relationship between the two still need to be further verified in subsequent studies.

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