Ginsenoside Rb1 inhibits proliferation and promotes apoptosis by regulating HMGB1 in uterine fibroid cells

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Introduction

Uterine fibroids are common benign tumors of the genital system in women of childbearing age. According to statistics, about 80% of women in childbearing age have uterine fibroids, which seriously endanger women's physical and mental health [1]. Ginsenoside is an effective chemical component of ginseng, with extensive pharmacological effects. Studies have shown that ginsenoside Rb1 can inhibit the proliferation of human acute myeloid leukemia cell line KG1α in vitro, which exhibits a dose–effect relationship [2]. Rb1 can inhibit proliferation and induce apoptosis in SW480 cells [3]. The expression of HMGB1 in pancreatic cancer, rectal cancer, gastric cancer and other tumors is associated with tumor development, tumor size, invasion and lymphatic metastasis [4,5]. The down-regulated expression of HMGB1 can significantly inhibit ovarian cancer cells proliferation and invasion in vitro [6]. The expression of HMGB1 in uterine fibroids was significantly higher than that in the paraneoplastic tissues, suggesting that HMGB1 may be closely related to the occurrence and development of uterine fibroids in proliferative diseases [7]. However, the relationship between ginsenoside Rb1 and HMGB1 in uterine fibroids is not fully understood. In this study, the primary uterine fibroid cells were studied, and the effects of ginsenosides, HMGB1 knockdown or overexpression on proliferation and apoptosis of uterine fibroid cells were observed. It was revealed that ginsenoside Rb1 could inhibit uterine fibroid cells proliferation and promote apoptosis by down-regulating HMGB1, supporting the clinical application of ginsenosides.

Materials and methods

Materials

Ginsenoside Rb1 was purchased from the natural pharmacology laboratory of Bethune Medical University, China; DMEM medium, fetal bovine serum and MTT were purchased from Gibco, USA; LipofectamineTM2000, reverse transcription kit, Annexin V-FITC/PI apoptosis detection kit, were purchased from AMRESCO, USA.

Cell isolation, culture and grouping

Isolation and culture of uterine fibroids were carried out according to the method of manufacturer [8,9]. Then, uterine fibroid cells were treated with ginsenoside Rb1 (0, 20, 40, 80, 160 μmol/L), and labeled as 0 μmol/L group, 20 μmol/L group, 40 μmol/L group, 80 μmol/L group and 160 μmol/L group, respectively. According to the instructions of Lipofectamine™2000, pcDNA3.1 and pcDNA3.1-HMGB1 were transfected into uterine fibroid cells and treated with Rb1 (80 μmol/L), si-NC and si-HMGB1 which were transfected into uterine fibroid cells, respectively, named as Rb1 + pcDNA3.1 group, Rb1 + pcDNA3.1-HMGB1 group, si-NC group and si-HMGB1 group. After...
successful transfection, they were all used for the following experiments.

**MTT experiment**
Appropriate amount of 1.2.1 cells were taken, 20 μL 5 g/L of MTT solution was added and cultured for 4 h, then the supernatant was discarded, 150 μL of DMSO per well was added, shaken to dissolve the crystal, and the cells at 490 nm absorbance (A) were detected.

**Western blot**
An appropriate amount of 1.2.1 cells were taken, RIPA lysed and quantified with BCA Protein Assay Kit, denatured and centrifuged, and the supernatant for protein loading was taken. Electrophoresis–transfer–block–incubation with I-antibody–incubation with II antibody–image formation were all carried out according to the routine procedures of Western blot experiments. The target strip of gray value was analyzed by Image J, and the target protein was expressed by the ratio of the gray value of the target strip to the gray value of GADPH.

**Annexin V-FITC/PI flow cytometry experiment**
Firstly, the cells in each transfected group were suspended in 500 μL of binding buffer. Then, 5 μL of Annexin V-FITC and PI were added into the cells and allowed to stand in the dark for 15 min at room temperature. The apoptotic rate was evaluated by flow cytometry. Apoptotic rate of cells (%) is the sum of early apoptotic rate and late apoptotic rate. Process was repeated 3 times for each sample.

**Statistical processing**
All experiments in this study were repeated at three times and the data were analyzed using SPSS 21.0 software (Chicago, IL). Measurement data were expressed as mean ± standard deviation (x ± s). One-way analysis of variance was performed to compare differences among multiple groups. Pairwise comparisons were performed using SNK-q test. p < .05 was considered statistically significant.

**Results**

**Effects of different concentrations of ginsenoside Rb1 on the inhibition rate of human uterine fibroid cells**
MTT assay was applied to detect the inhibition rate of ginseng saponins (0, 20, 40, 80, 160 μmol/L) in uterine fibroid cells. Compared with 0 μmol/L group, the inhibitory rates in 20, 40, 80 and 160 μmol/L groups were apparently elevated. Compared with 20 μmol/L group, the inhibitory rates were significantly increased in 40, 80 and 160 μmol/L groups. Compared with 40 μmol/L group, the inhibitory rates were significantly increased in 80 and 160 μmol/L groups (Figure 1), both of which were statistically significant (p < .05). However, there was no significant difference in the inhibitory rate of human uterine fibroid cells between 80 μmol/L and 160 μmol/L groups. Therefore, Rb1 at the concentration of 80 μmol/L was used for follow-up experiments.

**Effect of ginsenoside Rb1 on apoptosis of human uterine fibroid cells**
The apoptosis of human uterine fibroid cells which were treated with ginsenoside Rb1 (80 μmol/L) for 48 h were measured by flow cytometry (Figure 2(A)). Compared with the Con group, the apoptosis rate of Rb1 cells in Rb1-treated group was remarkably increased (Figure 2(B)), statistically significant at p < .05.

**Effect of ginsenoside Rb1 on the expression of HMGB1 in human uterine fibroid cells**
HMGB1 expression in human uterine fibroid cells treated with ginsenoside Rb1 was detected by qRT-PCR and Western blot assay. Compared with the Con group, the mRNA level of HMGB1 was drastically downregulated in the Rb1 treatment group (Figure 3(A)). Moreover, the protein expression of HMGB1 was also drastically downregulated (Figure 3(B,C)), both were statistically significant at p < .05.

**Knockdown of HMGB1 repressed HMGB1 expression in uterine fibroid cells**
Si-NC and si-HMGB1 were transfected into uterine fibroid cells. Compared with the si-NC group, the mRNA expression level of HMGB1 was significantly downregulated in the si-HMGB1 group (Figure 4(A)). Likewise, the protein expression level of HMGB1 was also significantly downregulated after si-HMGB1 transfection (Figure 4(B,C)), both were statistically significant at p < .05.
Effects of HMGB1 knockdown on proliferation and apoptosis of uterine fibroid cells

Cell viability was detected by MTT assay and we found that the cell viability in si-HMGB1 group was significantly decreased compared with that in si-NC group (Figure 5(A)). Then, flow cytometry analysis indicated that the apoptosis rate of uterine fibroid cells was significantly increased in the si-HMGB1 transfection group (Figure 5(B)), significant at p < .05.

**Effects of HMGB1 knockdown on proliferation and apoptosis of uterine fibroid cells**
Overexpression of HMGB1 reversed the inhibition of ginsenoside Rb1 on proliferation and apoptosis of human uterine fibroid cells

Compared with the Con group, the protein expression level of HMGB1 was significantly decreased in the Rb1 group, while the protein expression level of HMGB1 was significantly increased in Rb1 + pc DNA3.1-HMGB1 group compared with that in Rb1 + pc DNA3.1 group (Figure 6(A,B)). In contrast to the Con group, the cell viability of Rb1 group was distinctly suppressed, and the cell viability of Rb1 + pc DNA3.1-HMGB1 group was significantly higher than that of the Rb1 + pc DNA3.1 group (Figure 6(C)). Furthermore, the apoptosis rate of Rb1-treated group was significantly promoted when compared to the Con group, and the apoptosis rate of Rb1 + pc DNA3.1-HMGB1 group was significantly lower than that of Rb1 + pc DNA3.1 group (Figure 6(D)). It can be seen that the overexpression of HMGB1 rescues the inhibition of ginsenoside Rb1 on proliferation and the promotion of ginsenoside Rb1 on apoptosis of human uterine fibroid cells.

Discussion

Tumor is the world’s malignant disease with the highest fatality rate, which is a serious threat to the safety of all human beings. Although many efforts have been made and many therapies have been used for the treatment of cancers, the end result is not always very satisfactory [10]. Currently, the most common treatment is drugs chemotherapy; however, the therapeutic efficacy would be suppressed due to serious side effects and drug resistance [11]. Chinese herbal medicine has a long history of application in Chinese history, including anti-cancer treatment [12,13]. Paclitaxel, tripterygium, gallnut, and sage mushroom have anti-cancer effects [14,15]. There is a growing evidence indicating that ginsenosides can accelerate apoptosis, restrain proliferation, metastasis and angiogenesis, and promote immunity in various cancer cells. Zhu et al. [16] in vivo and in vitro study of uterine fibroids found that ginsenoside Rh2 could inhibit the growth of rat uterine fibroids, and reduce serum hormone levels in a dose-dependent manner. With ELISA, qRT-PCR and Western blot detection of uterine fibroid cells confirmed that Rh2 decreased estrogen receptor alpha (ERα) and c-Src activity, increased p38 MAPK activity, reversed the ERα activity induced by PP2 and SB203580 (specific inhibitors of c-Src and p38 MAPK), which revealed the anti-proliferative effect of Rh2 and might be involved in the regulation of ERα/ Src/p38 MAPK activity. In this study, MTT method was used to detect cell viability of uterine fibroid cells treated with ginsenoside Rb1, and it was found that ginsenoside Rb1 could inhibit uterine fibroid cells proliferation and the inhibitory effect was negatively correlated with the concentration of Rb1, which was consistent with the effect of Zhu’s study on ginsenoside Rh2 in uterine fibroids. Further studies found that ginsenoside Rb1 could promote the apoptosis of uterine fibroid cells and down-regulate the expression of HMGB1 using flow cytometry, Western blot and qRT-PCR detection.
High-mobility protein (HMG) consists of three members: HMGA, HMGB and HMGN, of which HMGB includes HMGB1, HMGB2 and HMGB3 [17]. HMGB1 is a ubiquitous and highly conserved nuclear protein involved in nucleosome maintenance and gene transcription. In addition, HMGB1 is involved in DNA recombination, repair and replication [18]. As early as the end of the twentieth century, a large number of studies reported that HMGB1 played an important role in uterine fibroids [19,20]. As early as 1999, German research team by Michael et al. [21] found that HMGI is highly expressed in uterine leiomyoma, and immunohistochemistry is used to verify that HMGI-C is only expressed in leiomyoma smooth muscle cells. These findings suggest that overexpression of HMGI protein is critical for the pathogenesis of uterine leiomyoma. A year later, American team by Tallini et al. [22] used probes for in-situ-hybridization analysis in benign tumors (uterine leiomyoma, lipoma, endometrial polyps) which found that HMGI-C or HMGI(Y) was highly expressed, and associated with 12q15 and 6p21 chromosomal mutations, revealing that in a biphasic lesion composed of a mixture of stromal cells and epithelial cells, the mesenchymal component is the site of HMGI gene variation. In this study, gene intervention (knockdown HMGB1 and overexpressing HMGB1) was used to detect the proliferation and apoptosis abilities of uterine fibroid cells. Knockdown of HMGB1 inhibited proliferation and promoted apoptosis of uterine fibroid cells. Overexpression of HMGB1 rescued the effects of ginsenoside Rb1 inhibition on proliferation and apoptosis of uterine fibroid cells.

In summary, ginsenoside Rb1 can inhibit proliferation and promote apoptosis of uterine fibroid cells, which may be related to the direct negative regulation of HMGB1, and provide a basis for clinical treatment of uterine fibroids.

Disclosure statement
No potential conflict of interest was reported by the authors.

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