Sericin Regulates the Proliferation of Human Gastric Cancer MKN45 Cells via the Autophagy Pathway Postprint

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Abstract

Objective: To investigate the effect of sericin on the proliferative activity of gastric cancer MKN45 cells and its underlying mechanism. Methods: MKN45 cells were transfected with LC3 dual-fluorescence autophagy virus, and stable MKN45 cell lines expressing the LC3 dual-fluorescence autophagy virus were selected using puromycin. The experiment was divided into three groups: blank control group (Blank), sericin group (Sericin), and sericin plus autophagy inhibitor group (Sericin+3-MA). After 48 h of culture, cell proliferative activity was measured using CCK-8 reagent, and the half-maximal inhibitory concentration (IC50) of sericin was determined from the data. Cells were then treated with this concentration for 48 h, and apoptosis and cell cycle were detected by flow cytometry, autophagy was examined by electron microscopy, and protein expression of LC3, p62, and Beclin-1 was detected by Western blotting. Gastric cancer xenograft nude mice were divided into two groups: control group (Saline) and sericin group (Sericin), with 5 mice in each group. Saline and sericin were injected respectively, and tumor volume and weight were measured. Results: Compared with the blank control group (Blank), the sericin group (Sericin) showed significantly inhibited proliferative activity of MKN45 cells, increased apoptosis ($P<0.01$), and cell cycle arrest at G2/M phase ($P<0.01$). Compared with the blank group, cells treated with sericin showed significantly increased autophagosomes under electron microscopy; Western blotting revealed upregulated LC3-II expression, increased Beclin-1 expression, and gradually downregulated p62 expression; the experimental results of the sericin plus autophagy inhibitor group (Sericin+3-MA) were intermediate between the two. In the animal experiment, compared with the control group (Saline), the sericin group (Sericin) showed significant decreases in tumor volume and weight. Conclusion: Sericin can affect the proliferative activity of gastric cancer MKN45 cells by regulating autophagy.

Full Text

Preamble

Sericin Regulates Proliferation of Human Gastric Cancer MKN45 Cells Through Autophagic Pathway

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Abstract

Objective: To investigate the effect of sericin on the proliferation of human gastric cancer MKN45 cells and explore the underlying molecular mechanism. **Methods:** MKN45 cells were transfected with an LC3 double-fluorescent autophagy virus, and stable transfectants were selected using puromycin. Cells were divided into three groups: blank control (Blank), sericin treatment (Sericin), and sericin plus autophagy inhibitor (Sericin+3-MA). After 48 hours of incubation, cell proliferation was measured using CCK-8 assay to determine the half-maximal inhibitory concentration (IC50). Cells were then treated at this concentration for 48 hours and analyzed for apoptosis and cell cycle distribution by flow cytometry, autophagy by electron microscopy, and expression of LC3, p62, and Beclin proteins by Western blotting. In a xenograft model, tumor-bearing nude mice were divided into two groups (n=5 each) receiving either saline or sericin injections, and tumor volume and weight were measured. **Results:** Compared with the blank group, sericin treatment significantly inhibited MKN45 cell proliferation and increased apoptosis ($P<0.01$) while inducing G2/M phase arrest ($P<0.01$). Electron microscopy revealed markedly increased autophagosomes in sericin-treated cells. Western blotting showed upregulated LC3-II expression, increased Beclin levels, and gradually decreased p62 expression. The Sericin+3-MA group showed intermediate results. In animal experiments, sericin significantly reduced tumor volume and weight compared with the control group. **Conclusion:** Sericin can inhibit the proliferation of gastric cancer MKN45 cells by regulating autophagy.

Keywords: sericin; autophagy; cell proliferation; gastric cancer

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Introduction

Gastric cancer is the second most common malignant tumor worldwide and the third leading cause of cancer-related mortality, posing a serious threat to human health. Current treatment primarily involves surgery and chemotherapy,

but the side effects of chemotherapy remain a critical challenge, leading to reduced quality of life and poor patient compliance. In recent years, researchers have explored optimized chemotherapy regimens for gastric cancer, including the development of novel nanomaterials to achieve low-toxicity, high-efficiency chemotherapy. Nanoscale material design has attracted increasing attention for solving problems in tumor chemotherapy, particularly in drug delivery. Nanomaterials primarily function by encapsulating drugs to reduce delivery losses and side effects while enabling targeted delivery, making them a viable strategy for addressing current chemotherapy challenges.

Nanodrug carriers are typically fabricated from natural or synthetic polymer materials. Sericin, a macromolecular material extracted from natural silk, offers advantages of low cost, good biocompatibility, and proven safety. Although sericin has been incorporated as a component in some synthetic nanocarriers, its medical applications have primarily focused on tissue engineering scaffolds, with limited exploration in chemotherapy. Moreover, while sericin has been shown to affect skin and colon tumors in animal models, the underlying mechanisms remain unclear. Therefore, this study aimed to investigate the effects of sericin on the viability of MKN45 gastric cancer cells and elucidate the associated mechanisms.

Materials and Methods

1.1 Materials

The CCK-8 cell proliferation assay kit was purchased from Beyotime Biotechnology (Shanghai). LC3 double-fluorescent autophagy virus, apoptosis detection kit, and cell cycle analysis kit were obtained from Genechem (Shanghai). Sericin was purchased from Buk Pharmaceutical Technology (Shanghai). Monoclonal antibodies against LC3, p62, Beclin, and GAPDH were acquired from Cell Signaling Technology (USA).

1.2.1 Cell Culture and Transfection

MKN45 cells were routinely cultured in DMEM medium supplemented with 10% fetal bovine serum at 37°C in a 5% CO incubator. Logarithmic-phase cells were seeded in 6-well plates at a density of approximately 4×10 cells per well. After 24 hours, cells were transfected with LC3 double-fluorescent autophagy virus according to the manufacturer' s instructions, and stable transfectants were selected using puromycin. Experimental groups were established as follows: blank control (Blank), sericin treatment (Sericin), and sericin plus autophagy inhibitor (Sericin+3-MA).

1.2.2 Detection of Antitumor Effects In Vitro and In Vivo

For in vitro studies, cells were collected and seeded in 96-well plates at approximately 1,000 cells per well in a 100 L culture system. After 24 hours of routine culture, various concentrations of sericin were added. At the 48-hour time point, 10 L of CCK-8 reagent was added and incubated for 2 hours, followed by absorbance measurement at 450 nm to assess cell proliferation and generate growth curves. For in vivo studies, nude mice bearing gastric cancer xenografts were divided into two groups (n=5 each): a control group receiving saline injections and a sericin group receiving sericin injections. Tumor volume and weight were measured on days 7, 9, 11, 13, and 15 post-injection to generate tumor growth curves.

1.2.3 Cell Viability Assay with Autophagy Inhibition

Cells were seeded in 96-well plates at approximately 1,000 cells per well in a 100 L culture system. After 24 hours, the Sericin group received three concentrations of sericin (500, 600, and 700 μg/mL), while the Sericin+3-MA group received the same sericin concentrations plus the autophagy inhibitor 3-MA. At the 48-hour time point, 10 L of CCK-8 reagent was added, followed by 2-hour incubation and absorbance measurement at 450 nm to assess cell viability and generate survival rate comparisons.

1.2.4 Flow Cytometric Analysis of Apoptosis

Cells were treated according to method 1.2.2. After 48 hours, cells were harvested and processed according to the apoptosis kit instructions. The proportion of apoptotic cells in each group was detected by flow cytometry.

1.2.5 Flow Cytometric Analysis of Cell Cycle

Cells were treated according to method 1.2.2. After 48 hours, cells were harvested and processed according to the cell cycle kit instructions. The distribution of cells across different cycle phases was analyzed by flow cytometry.

1.2.6 Fluorescence Microscopy Detection of Autophagy

Stable transfectants expressing the autophagy reporter were treated according to method 1.2.2. After 48 hours, red and green fluorescence expression was observed under an inverted fluorescence microscope using excitation wavelengths of 560 nm and 480 nm, respectively.

1.2.7 Transmission Electron Microscopy Analysis of Autophagy

Cells were treated according to method 1.2.2. After 48 hours, cells were harvested and fixed by slowly adding electron microscopy fixative (1.5-2.5% neutral glutaraldehyde) along the tube wall. Following fixation at 4°C for 15 minutes,

samples were embedded in resin, sectioned, and observed under a transmission electron microscope (HITACHI HT7700, Japan).

1.2.8 Western Blotting Analysis of Autophagy-Related Proteins

Cells were treated according to method 1.2.2. After 48 hours, cells were collected and total protein was extracted using cell lysis buffer. Protein concentration was determined by BCA assay, and 20 μg of protein per lane was separated by 10% SDS-PAGE and transferred to nitrocellulose membranes. After blocking with 5% skim milk, membranes were incubated overnight at 4°C with primary antibodies against LC3 (1:1,500), GAPDH (1:3,000), p62 (1:1,000), and Beclin (1:1,000). The next day, membranes were washed three times with PBST and incubated with rabbit secondary antibody (1:5,000) for 1 hour at room temperature. After three additional PBST washes, protein bands were visualized using ECL chemiluminescence.

Statistical Analysis

Experimental data are presented as mean ± standard deviation. Comparisons between two groups were performed using Student' s t-test for normally distributed data or Mann-Whitney U test for non-normally distributed data. $P<0.05$ was considered statistically significant.

Results

2.1 Sericin Inhibits Proliferation of MKN45 Gastric Cancer Cells

CCK-8 assays showed that after 48 hours of co-incubation with various concentrations of sericin, the viability of MKN45 gastric cancer cells decreased significantly in a dose-dependent manner (Figure 1A), demonstrating that sericin inhibits gastric cancer cell proliferation in vitro. The half-maximal inhibitory concentration (IC50) was calculated to be 543 μg/mL. In the xenograft model, tumor size increased over time, but both tumor volume and weight were significantly reduced in the sericin group compared with the control group (Figures 1B and 1D), confirming the antitumor effect of sericin in vivo. Notably, there was no significant difference in body weight gain between the two groups, indicating favorable biosafety of sericin treatment (Figure 1C).

2.2 Sericin Induces Autophagy in MKN45 Gastric Cancer Cells

Fluorescence microscopy and transmission electron microscopy observations (Figure 2) revealed that compared with the blank control group, sericin treatment caused a significant increase in autophagosomes in gastric cancer cells. This was evidenced by the appearance of green puncta representing autophagosomes in the GFP-LC3 fluorescence system and the presence of

double-membrane structures enclosing cytoplasmic components under electron microscopy.

2.3 3-MA Reverses Sericin-Induced Inhibition of MKN45 Cell Proliferation

MKN45 cells were co-incubated for 48 hours with three concentrations of sericin (500, 600, and 700 μg/mL) either alone or in combination with the autophagy inhibitor 3-MA. CCK-8 assays (Figure 3) showed that cell viability in the Sericin+3-MA group was significantly higher than in the Sericin group (t=2.788, 6.595, and 8.227 for the three concentrations, respectively), demonstrating that 3-MA antagonizes the proliferation-inhibiting effect of sericin on MKN45 cells.

2.4 Sericin Induces Apoptosis in MKN45 Gastric Cancer Cells

Flow cytometric analysis was performed after treating MKN45 cells with sericin at the IC50 concentration for 48 hours. The results demonstrated that sericin significantly inhibited the anti-apoptotic capacity of gastric cancer cells, with marked increases in early apoptosis (P=0.0068), late apoptosis (P=0.0066), and total apoptosis (P=0.0005) compared with the control group (Figure 4). Furthermore, the Sericin+3-MA group showed significant reductions in early apoptosis (P=0.0173), late apoptosis (P=0.0339), and total apoptosis (P=0.0039) compared with the Sericin group.

2.5 Sericin Arrests MKN45 Gastric Cancer Cells at the G2/M Phase

Flow cytometric analysis after 48 hours of treatment with sericin at the IC50 concentration revealed that the sericin group had a significant increase in cells arrested at the G2/M phase compared with the blank control group (P=0.001, Figure 5). This effect was significantly attenuated in the Sericin+3-MA group (P=0.0059, Figure 5).

2.6 Sericin Activates the Autophagy Pathway in MKN45 Gastric Cancer Cells

Western blotting analysis showed that compared with the blank control group, sericin treatment resulted in upregulated LC3-II expression, increased Beclin levels, and gradually downregulated p62 expression (Figure 6). The Sericin+3-MA group exhibited intermediate protein expression levels between the other two groups, confirming that sericin induces autophagy in gastric cancer cells.

Discussion

In addition to demonstrating antioxidant activity, inhibiting lipid peroxidation, and suppressing tyrosinase activity in vitro, sericin has been shown to affect

skin and colon tumors in animal models. However, the mechanisms underlying these effects remain inadequately elucidated. Therefore, this study investigated the impact of sericin on MKN45 gastric cancer cell viability and the associated mechanisms.

Common chemopreventive mechanisms include inhibition of cell proliferation, induction of apoptosis, and regulation of the cell cycle. In this study, increasing sericin concentrations led to a significant decrease in MKN45 gastric cancer cell viability, indicating that sericin can inhibit gastric cancer cell proliferation. The xenograft experiments further confirmed the tumor growth-inhibiting effect of sericin in vivo. Notably, previous studies using sericin as a nanocarrier component did not demonstrate antitumor effects, likely due to lower concentrations used. This aligns with our finding that sericin does not inhibit cell proliferation at low concentrations, suggesting a concentration-dependent effect.

Apoptosis represents a crucial process in gastric cancer chemotherapy. Our results showed that sericin treatment significantly increased both early and late apoptosis rates in gastric cancer cells. Early apoptosis is characterized by plasma membrane changes, including phosphatidylserine externalization and mitochondrial membrane potential decline, while late apoptosis involves chromatin fragmentation. These stages depend on various apoptotic stimuli and the balance between anti-apoptotic and pro-apoptotic proteins. Thus, sericin may affect yet-unidentified proteins associated with gastric cancer cell apoptosis.

Furthermore, sericin demonstrated cell cycle arrest properties. The cell cycle is a complex process involving cell proliferation, and defects in its normal regulation can lead to uncontrolled proliferation and cancer development. In gastric cancer research, arresting cells at the G2/M phase represents an important chemopreventive target. Our study showed that sericin treatment significantly increased the proportion of cells arrested at G2/M compared with the control group, indicating that sericin can inhibit cell proliferation by blocking the cell cycle. This mechanism is similar to that of conventional clinical agents such as cytarabine and fluorouracil, which also kill cancer cells by arresting the cell cycle.

Autophagy is a highly conserved lysosome-dependent catabolic pathway in eukaryotes that plays important roles in various physiological and pathological processes, including tumor development. For instance, starvation-induced autophagy can arrest tumor cells at the G2/M phase. Consequently, many studies have targeted autophagy as a key strategy for cancer therapy. While autophagy in the tumor microenvironment may promote tumor growth, the role of autophagy within tumor cells themselves remains controversial. Nanomaterials such as tantalum nanoparticles, quercetin nanoparticles, and silica nanoparticles have been shown to induce autophagy. We hypothesized that sericin might inhibit gastric cancer cell proliferation by inducing autophagy.

Using a GFP-LC3 fluorescence system, we observed green puncta representing autophagosomes under an inverted fluorescence microscope. Transmission electron microscopy further confirmed the presence of double-membrane structures

enclosing cytoplasmic components in sericin-treated cells, providing initial evidence that sericin induces autophagy in gastric cancer cells. To further validate this mechanism, we employed 3-methyladenine (3-MA), a commonly used autophagy inhibitor. Compared with the sericin-only group, the Sericin+3-MA group showed significantly increased cell viability and decreased apoptosis and cell cycle arrest, demonstrating that 3-MA antagonizes the effects of sericin and confirming that sericin' s antiproliferative mechanism is autophagy-dependent.

Western blotting analysis of autophagy-related proteins revealed that sericin treatment upregulated LC3-II expression, downregulated p62 expression, and increased Beclin levels—all consistent with enhanced autophagy. Moreover, the Sericin+3-MA group showed reduced expression of these autophagy markers. These results collectively indicate that sericin inhibits MKN45 gastric cancer cell proliferation by inducing autophagy.

A limitation of this study is that we have not fully elucidated the molecular mechanism by which sericin regulates autophagy in gastric cancer cells. The PI3K/AKT/mTOR signaling pathway is a common regulator that suppresses tumor autophagy, and we speculate that sericin may increase tumor cell autophagy by blocking this pathway. Our research group will conduct further investigations into the specific mechanisms underlying sericin-induced autophagy in tumor cells.

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Note: Figure translations are in progress. See original paper for figures.