Effects of Ginsenoside Biopolymer Nanoparticles on the Malignant Behavior of Non-Small-Cell Lung Cancer

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1. Introduction

With the aging of the population and the deterioration of the living environment, lung cancer has become one of the most common malignant tumors worldwide, and both morbidity and mortality are on the rise. It has become a malignant tumor with the highest morbidity and mortality in China with about 80% to 85% of them being non-small-cell lung cancer [1]. Ginsenoside Rg3 is one of the active ingredients in the ginseng extract. According to clinical evidence, ginsenoside Rg3 can inhibit malignant behaviors of tumors such as tumor invasion and metastasis, can alleviate the toxic effects of chemoradiotherapy, and also can sensitize tumors to anti-tumor drugs. However, the ginsenoside Rg3 monomer has low solubility in the human body, thus resulting in low effectiveness. Several studies indicated that the nanometer-sized particle carrier can improve the solubility of the drug, therefore increasing the body’s absorption and improving the bioavailability of the drug.

MicroRNAs (miRNAs) are a class of noncoding single-stranded RNA molecules that affect various biological behaviors. The mechanism of action is mainly through the interaction with 3′-UTR to affect the mRNA expression of target genes. Many studies have shown that [2] the change of miRNA expression will lead to the upregulation or down-regulation of its target gene mRNA expression and then affect cell proliferation and apoptosis. In addition, the abnormal expression of miRNAs can also induce a series of
human diseases, including cancer. Previous studies have shown that miR-192 expression is significantly increased in non-small-cell lung cancer tissues. It is thus speculated that miR-192 is related to the occurrence and development of non-small-cell lung cancer. The tumor suppressor gene phosphatase and tensin homolog (PTEN) is a dual-specific protein and lipid phosphatase, which can block the PI3K signaling pathway by activating AKT to inhibit cell proliferation and survival. Therefore, PTEN has a certain inhibitory effect on cancer [3]. Through prediction, it was found that PTEN may be a downstream target gene of miR-192. It is, therefore, speculated that miR-192 might affect the occurrence and development of non-small-cell lung cancer by regulating the expression of PTEN. Therefore, in this study, the nanoparticle carriers covered with ginsenoside Rg3 monomer were prepared to investigate its effect on the malignant behavior of human non-small-cell lung cancer cell line H125 and its underlying mechanism.

2. Material and Methods

2.1. Cell Lines and Reagents. In this study, human normal lung epithelial cells BEAS-2B were purchased from the Chinese Academy of Sciences Cell Bank, and human non-small-cell cancer cells H125 were purchased from FuHeng Cell Center, Shanghai, China. Ginsenoside Rg3 (Shenyi Capsule) was purchased from Jilin Yatai Pharmaceutical Co., Ltd.; fetal bovine serum and DMEM medium were purchased from Suzhou Laisa Biotechnology Co., Ltd.; the MTT kit was purchased from Beijing Kanglong Kangtai Biotechnology Co., Ltd.; the Transwell chamber was purchased from American BD Company; the Tunel kit and RIPA cell lysis were purchased from Shanghai Biyuntian Biotechnology Co., Ltd.; the Lipofectamine 2000 kit and reverse transcription kit were purchased from Shanghai Kemin Biotechnology Co., Ltd.; the ECL development kit was purchased from China Solarbio, and the dual-luciferase reporter gene detection kit was purchased from Beijing YuanPingHao Biotechnology Co., Ltd.

2.2. Preparation of Nanoparticles. Gelatin and hyaluronic acid nanoparticles were prepared in an electrostatic field preparation system. In addition, 4 μL of the prepared ginsenoside Rg3 solution (25 mg/mL) was separately added to 996 μL of prepared gelatin and hyaluronic acid nanoparticles (final concentration of ginsenoside Rg3 was 100 μg/mL) for their coating in the electrostatic field preparation system. The coverage of ginsenoside Rg3 was then determined by HPLC.

2.3. Cell Culture and Transfection. H125 cells were seeded in the DMEM medium (containing 10% fetal bovine serum, 100 U/L penicillin, and 100 mg/L streptomycin) and cultured in a constant temperature incubator at 37°C with 5% CO₂. miR-192 inhibitor, miR-NC, pc-PTEN, pc-NC, and sh-PTEN were provided by Shanghai Jima. The cells in the logarithmic growth phase were transfected with miRNA mimics and siRNAs according to the Lipofectamine 2000 kit. After 48 h of transfection, the cells were harvested for subsequent experiments. The primer sequences used in this study are shown in Table 1.

2.4. MTT Experiment. The single-cell suspension was prepared by trypsinization of H125 cells in the logarithmic growth phase. The cell concentration was adjusted to 5 × 10^3 cell/well and inoculated into a 96-well plate. The cells were then cultured in an incubator at 37°C with 5% CO₂. After 24 h, the cells were treated according to the experimental design and then cultured at 37°C with 5% CO₂ for 24 h. The cells were washed twice with PBS buffer solution and then cultured in a DMEM medium containing 10% fetal calf serum for 48 h. 20 μL (5 mg/L) MTT solution was added to each well, and the proliferation activity of H125 cells was measured in strict accordance with the instructions of the MTT kit.

2.5. Transwell Experiment. The upper chamber of the Transwell chamber was coated with Matrigel, and the H125 cells in the logarithmic growth phase were taken and seeded at a concentration of 1 × 10^5 cell/well in the upper chamber of the Transwell chamber containing a serum-free medium. The DMEM medium containing 10% fetal bovine serum was added to the lower chamber. The cells were removed 24 h after inoculation, fixed with formaldehyde, stained with crystal violet for 5 min, washed 3 times with PBS buffer, photographed, and counted.

2.6. Cell Scratch Test. 5 × 10^5 cells were added to each well of a 6-well plate. When the cell fusion rate reached 95%, the cell membrane was streaked and washed slowly with PBS buffer 3 times, the delineated cells were removed, and serum-free medium was added. The plate was incubated in a 37°C, 5% CO₂ incubator for 24 h and photographed after 24 h, and the scratch distance was determined using ImageJ.

2.7. Western Blotting. H125 cells were transferred to EP tubes, lysed on ice for 30 min using the RIPA cell lysis, and then centrifuged at 14,000 rpm for 5 min at 4°C. The supernatant was extracted and a loading buffer was added to the supernatant. The sample was then denatured at 100°C for 10 min and then stored at −20°C. 25 μg of protein sample was then loaded and electrophoresed at 40 A for 100 min. After that, it was then transferred to the membrane at 100 V for 90 min. After adding the primary antibody, the membrane was incubated at 4°C overnight and then incubated at room temperature for 2 h after the addition of the secondary antibody. Image development was performed strictly based on the ECL kit instructions and the grayscale analysis of protein bands was performed using ImageJ software.

2.8. RT-qPCR. H125 cellular RNA was extracted and 2 μL of the extracted RNA was added to 98 μL of ddH₂O to measure the RNA content and purity based on the absorbance value obtained through an ultraviolet spectrophotometer. The
cDNA was then synthesized according to the instructions of the reverse transcription kit. The PCR reaction was carried out according to Table 2; specifically, the reaction conditions were as follows: 95°C, 10 min; 95°C, 20 s; 60°C, 1 min, for a total of 40 cycles.

2.9. Detection of Apoptosis by TUNEL Assay. After the treated sample was rinsed with PBS buffer, the liquid surrounding the sample was removed. 100 μL of the TdT enzyme reaction solution was added to each sample, the reaction was carried out in the dark at 37°C for 60 min, and then the diluted SSC solution was added to terminate the reaction. After rinsing with PBS buffer, the samples were blocked in 0.3% hydrogen peroxide/PBS and incubated for 4 min at room temperature. 100 μL of the streptavidin-HRP working solution was then added, and the reaction was carried out at 37°C for 60 min. The BAD chromogenic solution was added, followed by rinsing the cells with PBS buffer 3 times, and then sealed with a neutral resin and observed under a microscope.

2.10. Dual-Luciferase Reporter Gene Assay. After the 3′-UTR fragment of PTEN gene was amplified and inserted into the double luciferase reporter gene plasmid, the positive clone was screened, followed by sequencing, amplification, and purification of the plasmid. The miR-192 plasmid and the empty vector were cotransfected with well-grown H125 cells, and the cells were selected for subsequent experiments.

2.11. Statistical Analysis. SPSS (version 22.0) statistical software was used for the data analysis, the data obtained in this study were expressed as x ± s, the t-test was used for comparison between groups, and the comparison among multiple groups was analyzed by one-way ANOVA. The difference was statistically significant when P < 0.05 or P < 0.01.

3. Results

3.1. Nanoparticle Detection and Vector Screening. The prepared gelatin nanoparticle carriers and hyaluronic acid nanoparticle carriers were found uniformly distributed, uniform in size and spherical in shape under the electron transmission microscopy, and the particle sizes of the nanoparticle carriers were 4.5 ± 0.75 nm and 16.4 ± 4.2 nm, respectively. As shown in Figures 1(a) and 1(b), the results of MTT experiments showed that cell viability of H125 cells was significantly reduced when cocultured with 20 μL gelatin nanoparticle carrier suspension (P < 0.05), while similar significantly reduced cell viability of H125 cells was observed, and when cocultured with 40 μL hyaluronic acid nanoparticle carrier suspension (P < 0.05). After coculture of H125 cells with gelatin polymer or HA polymer, the activity of H125 cells is similar to that of gelatin or HA nanoparticles; see Figure 1(c). After coating ginsenoside Rg3, Gelatin-Rg3 nanoparticles and hyaluronic acid HA-Rg3 nanoparticles showed a small amount of particle agglomeration, and the particle sizes of the nanoparticles were significantly different from that before coating (P < 0.01); the particle sizes were 40.3 ± 5.8 nm and 31.2 ± 8.4 nm, respectively. The coverage of ginsenoside Rg3 on gelatin and hyaluronic acid nanoparticles measured by HPLC was 13.715 ± 1.985% and 36.410 ± 6.472%, respectively. Therefore, to reduce the impact of nanoparticle carriers on H125 cells, HA-Rg3 nanoparticles were selected for subsequent experiments.

3.2. Effect of HA-Rg3 Bio-Polymerized Nanoparticles on the Expression Level of miR-192. As shown in Figure 2(a), the expression level of miR-192 in H125 cells was significantly higher than that of BEAS-2B cells. However, the expression levels of miR-192 in H125 cells were significantly downregulated after cocultured with ginsenoside Rg3 and HA-Rg3 bio-polymerized nanoparticles (P < 0.05), and the expression levels of miR-192 achieved lowest when H125 cells were cocultured with HA-Rg3 (P < 0.01) as shown in Figure 2(b).

3.3. Effect of miR-192 on Malignant Behavior of H125 Cells. The results of RT-qPCR showed that the expression level of miR-192 in H125 cells was significantly downregulated compared with the control group after the transfection of miR-192 inhibitor (P < 0.01), as shown in Figure 3. And the knockdown of miR-192 significantly inhibited the activity of H125 cells (P < 0.01) as shown in MTT (Figure 4(a)), significantly inhibited the invasion of H125 cells (P < 0.05) as shown in Transwell results (Figure 4(b)), and also significantly inhibited the migration ability of H125 cells (P < 0.05) as shown in the results of cell scratch assay (Figure 4(c)). Furthermore, knockdown of miR-192 also significantly promoted the apoptosis of H125 cells (P < 0.01) as shown in Figure 4(d).

3.4. PTEN Is the Target Gene of miR-192. By using the ENCORI database, we predicted that the 3′-UTR end of

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### Table 1: Primer sequences.

| Gene name | Primer sequence
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>miR-192</td>
<td>5′-GGGCTGACCTATGAAATG-3′</td>
</tr>
<tr>
<td></td>
<td>5′-CAGTGGTGTCGTGGAGT-3′</td>
</tr>
<tr>
<td>U6</td>
<td>5′-ACGATGCACCTGTCGATCA-3′</td>
</tr>
<tr>
<td></td>
<td>5′-TCTTTCAACACGGAGGACAG-3′</td>
</tr>
</tbody>
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### Table 2: RT-qPCR reaction system (20μL).

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Volume (μL)</th>
</tr>
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<tbody>
<tr>
<td>SYBR premix Ex Taq (2x)</td>
<td>10</td>
</tr>
<tr>
<td>Forward primer (5 pmol/L)</td>
<td>1</td>
</tr>
<tr>
<td>Reverse primer (5 pmol/L)</td>
<td>1</td>
</tr>
<tr>
<td>cDNA</td>
<td>0.5</td>
</tr>
<tr>
<td>RNase-FREE WATER</td>
<td>7.5</td>
</tr>
</tbody>
</table>
PTEN mRNA has a partial base sequence binding to the 5′-UTR end of miR-192 as shown in Figure 5(a). Western blotting results (Figure 5(b)) showed that the expression level of PTEN was significantly downregulated in H125 cells (P < 0.05), whereas the knockdown of miR-192 significantly enhanced the expression of PTEN in H125 cells (P < 0.05). Moreover, as shown in Figure 5(c), the results of the dual-luciferase assay verified that PTEN is the target gene of miR-192.

3.5. Mechanism of Action of HA-Rg3 Bio-Polymerized Nanoparticles on Non-Small-Cell Lung Cancer. The MTT assay showed that the proliferation activity of H125 cells in group I and group II was significantly lower than that in the NC group (P < 0.01), and the proliferation activity of H125 cells in group III and IV was significantly inhibited (P < 0.001), whereas the proliferation activity of H125 cells in the V group was not significantly different from that in the NC group. Moreover, the activity of H125 cells in group II was significantly inhibited when compared with group I (P < 0.05) as shown in Figure 4(a). Transwell results (Figure 4(b)) showed that the invasiveness of H125 cells in group I, II, III, and IV was inhibited compared with the NC group, and the difference was statistically significant (P < 0.01). The scratch test results (Figure 4(c)) showed that the invasiveness of H125 cells in group I, II, III, and IV was inhibited compared with the NC group, and the migration ability of H125 cells in group I was significantly lower than that of
group I ($P < 0.05$). The apoptosis results of Tunel assay (Figure 4(d)) showed that the number of apoptosis of H125 cells in group I, group II, group III, and group IV increased in different degrees compared with the NC group, and the difference was statistically significant ($P < 0.05$). There was no significant difference in the number of apoptosis of H125 cells in the V group compared with the NC group, and again the number of apoptosis of H125 cells in group II was significantly lower than that of group I ($P < 0.05$).

4. Discussion

Lung cancer is primary bronchial carcinoma, of which non-small-cell lung cancer is the most common type of cancer,
accounting for 75% to 80% of the total number of lung cancer patients [4]. Due to the lack of early specific symptoms, lung cancer often develops into the advanced stage at the clinical diagnosis. Although surgery can be used to treat early-stage lung cancer, 70% of patients cannot be cured [4]. Recently, radiotherapy and chemotherapy are becoming mainstream methods for the treatment of lung cancer. However, with that comes the toxic side effects and higher recurrence rate, and it is also demanding in terms of the patient’s physical quality. In recent years, the advantages of traditional Chinese medicine in cancer treatment have gradually gained attention, which can reduce the side effects of radiotherapy and chemotherapy on patients, prolong the quality lifespan of patients, and alleviate the drug resistance of radiotherapy and chemotherapy on patients, prolong the...

Ginsenoside Rg3 is the active ingredient extracted from ginseng. The main component of which is tetracyclic tri-terpenoid saponin, which can inhibit the formation of tumor blood vessels, block the invasion and metastasis of tumors, and improve the immunity and quality of life for the cancer patients. Wu et al. [8] reported that ginsenoside Rg3 can inhibit the proliferation of thyroid cancer in vitro and in vivo and significantly inhibit the metastasis of thyroid cancer. Dai et al. [5] showed that ginsenoside Rg3 monomer and HA-Rg3 nanoparticles may inhibit non-small-cell lung cancer by downregulating the...
expression level of miR-192 in H125 cells and thus promoting the expression of PTEN in H125 cells.

**Data Availability**

The data used to support the findings of this study are included within the article.

**Conflicts of Interest**

The authors declare that they have no conflicts of interest.

**Authors’ Contributions**

Weizheng Zhou and Chengliang Cai contributed equally to this study.

**Acknowledgments**

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**References**


