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Original Article

Lobaplatin arrests cell cycle progression, induces apoptosis and alters the proteome in human cervical cancer cell Line CaSki

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Abbreviations:
MTT, Methylthiazolyldiphenyl-tetrazolium bromide
TEMED, Tetramethyl ethylene diamine
SDS, Sodium dodecyl sulfate
Tris, Tris(hydroxymethyl)aminomethane
IPG, Immobilized PH gradient
PI, Propidium iodide
IEF, Isoelectric focusing
DTT, Dithiothreotol
PAGE, Polyacrylamide gel electrophoresis
CBB, Coomassie brilliant blue
2-DE, Two-dimensional gel electrophoresis
PMF, Peptide mass fingerprint
LC-MS/MS, Liquid chromatography mass spectrometry/mass spectrometry
Mw, Molecular weight
pI, Isoelectric point

ABSTRACT

Cervical cancer is one of the most common gynecologic tumors. There is an upward trend in the incidence. The objective of this research was to explore the effect of lobaplatin on cervical cancer CaSki cells proliferation, cell cycle and apoptosis and analysis of the differential expressed proteins of CaSki cells after exposed to lobaplatin. Our findings have shown that lobaplatin inhibits cell proliferations in human cervical cancer CaSki cells in dose- and time-dependent manner. Flow cytometry assay confirmed that lobaplatin affected cervical cancer cell survival by blocking cell cycle progression in S phase and G0/G1 phase and inducing apoptosis in dose- and time-dependent manner. Lobaplatin treatment reduced polypyrimidine tract-binding protein 2, ribose-phosphate pyrophosphokinase, hypothetical protein, terminal uridylyltransferase 7, ubiquitin specific protease 16 and heterogeneous nuclear ribonucleoprotein A2/B1 expression and increase zinc finger protein 91, zinc finger protein, C-X-C motif chemokine 10 precursor, stromal cell protein and laminin subunit alpha-4 expression. Some of the differentially expressed proteins may be associated with antitumor effect of lobaplatin. Lobaplatin showed a good antitumour activity in in vitro models of human cervical cancer cells. These results indicate that lobaplatin could be an effective chemotherapeutic agent in human cervical cancer treatment by inducing apoptosis, cell cycle arrest and changing many kinds of protein molecule expression level.

1. Introduction

Cervical cancer is one of the most common gynecologic tumors. Drugs in the comprehensive treatment of cervical cancer occupy more and more important position. National Comprehensive Cancer Network (NCCN) guidelines recommend platinum as the basic drugs for chemotherapy [1].

Because of cisplatin, severe renal toxicity and gastrointestinal side effects limits its application in clinic. But platinum, as the third generation of platinum antineoplastic agent, with its lighter side effects, incomplete cross resistance to cisplatin and stronger antitumor activity, has preliminary shown its important role in tumor treatment.

Some clinical trials indicated that lobaplatin had antitumor effects on human oesophageal cancer, ovarian cancer, breast cancer and small cell lung cancer, colorectal carcinoma, hepatocellular carcinoma, and cholangiocarcinoma, etc [2–7].

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But there was still no research to study lobaplatin effects on cervical carcinoma. In order to provide evidence for the clinical application of lobaplatin in cervical carcinoma treatment, the effect of lobaplatin was assessed in human cervical carcinoma cell line CaSki and the underlying molecular mechanisms were explored in the present study.

2. Materials and methods

2.1. Materials

All the chemicals used were of analytic grade. Lobaplatin was obtained from Hainan Chang'an International Pharmaceutical Co., Ltd (China). MTT, acrylamide, SDS, TEMED and Tris were obtained from Sigma. Bio-Lyte and IPG strip was obtained from BIO-RAD (USA).

2.2. Cell culture

CaSki cells (human cervical carcinoma cell line) were obtained from the cell bank of the Type Culture Collection of Chinese Academy of Sciences (Shanghai, China), and were cultured in RPMI-1640 medium (Hyclone, Logan, UT, USA) with 10% fetal bovine Serum (Hyclone), 100 U/mL penicillin, 100 μg/mL streptomycin, and 2 mM L-glutamine and kept at 37 °C in a humidified atmosphere (MCO-175, SANYO, Japan) with 5% CO_2.

2.3. Proliferation assay

Cytotoxicity of lobaplatin to CaSki cell was examined using cell proliferation assay. Cells were seeded in a 96-well microtiter plate at 5×10^4 cells/well, and cultured for 24 h prior to exposure to lobaplatin (0, 2, 4, 6, 8, 12, 16, 24, 32 μg/mL) for 24, 48 and 72 h, followed by incubation with 20 mL (5 mg/mL) MTT for 4 h. Then, the supernatant was removed following centrifugation, and 100 μL DMSO was added. Absorbance at 490 nm was measured with a microplate reader (Model 550, Bio-Rad, Hercules, CA, USA). The inhibitory rate of cell proliferation was calculated by the following formula: inhibition rate (IR) = [1 – (OD treated/OD control) × 100%]. The 50% inhibitory concentration (IC_{50}) was calculated by non-linear regression fit of the mean values of the data obtained in triplicate independent experiments. Six wells were used for concentrations of 0,2,6 and 12 μg/mL. And cell morphologic changes were assessed using a phase-contrast microscope (TE2000-U, Nikon. Japan) after 24,48 and 72 h treatment with lobaplatin.

2.4. Cell cycle distribution analysis

The effect of lobaplatin on CaSki cell cycle distribution was determined by fluorescence activated cell sorting (FACS) analysis. Cells were exposed to different concentrations (0, 2, 6 and 12 μg/mL) of lobaplatin respectively for 24, 48 and 72 h. After that, the cells were collected, washed twice with PBS and fixed with 70% ethanol solution at 4 °C overnight. After centrifugation, the cell pellets were stained with 5 mg/mL PI and 1 mg/mL RNaseA at 4 °C for 30 min. The samples were analyzed by FACS Can flow cytometer (Becton Dickinson, Franklin Lakes, NJ, USA) with Cell Quest software. The distributions of the cell cycle were analyzed using ModFit LT software. All the experiments were repeated and yielded similar results.

2.5. Cell apoptosis analysis

Cell apoptosis analysis was performed using Annexin V-FITC Apoptosis Detection Kit (KeyGEN Biotech, China). CaSki cells were exposed to different concentrations of lobaplatin (0, 2, 6 and 12 μg/mL) for 24, 48 and 72 h. After that, 5×10^5 cells were collected by centrifugation and resuspended in 500 μL of 1 × binding buffer. Then, 5 μL of Annexin V-FITC and 5 μL of PI were added to the cell suspension. After incubation at room temperature for 5 min in the dark, the cells were then analyzed by flow cytometry (Becton Dickinson, Franklin Lakes, NJ, USA). All the experiments were repeated and yielded similar results.

2.6. Protein extraction

Proteins were extracted from CaSki cells of the control and lobaplatin (6 μg/mL) treated groups for 48 h. Briefly, 1.5×10^6 cells were dissolved in 100 μL of sample lysis buffer containing 7 M urea, 2 M thiourea, 4% [w/v] CHAPS, 65 mM DTT, 2% [v/v] Bio-Lyte pH [3–10], 2% protease inhibitor cocktail, mixed by vortexing and kept in an ice bath for 30 min. After freezing and thawing in liquid nitrogen for 3 times, the samples were centrifuged at 15,000 rpm and 4 °C for 1 h, and the supernatant was collected. 2-D Clean-Up Kit (GE Healthcare Life Sciences) had been used to purify protein according to the manufacturer's instructions and Bradford method had been used to determine the concentration of protein. Then, the protein stored at −80 °C until use for 2-DE.

2.7. 2-DE and image analysis

About 700 μg protein dissolved in 350 μL rehydration buffer was applied to IPG strips (17 cm, pH 3–10, Bio-Rad), which was allowed to hydrate for 13 h (20 °C). Subsequently, IEF was performed at using a Protean IEF Cell (Bio-Rad) under the following conditions: 250 V for 1 h with a slow increase in voltage, 500 V for 1 h with a slow increase in voltage, 1000 V for 1 h with a slow increase in voltage, 10,000 V for 5 h with a linear increase in voltage, and maintained at 10,000 V until 60,000 Volt–hours was reached. After IEF, the strips were equilibrated for 15 min in equilibration buffer I (0.375 M Tris–HCl pH 8.8, 6 M urea, 2% SDS, 20% glycerol, 1% DTT), then equilibrated in buffer II containing 2.5% iodoacetamide instead of DTT for 15 min. The strips were transferred onto 12% polyacrylamide gels for SDS–PAGE. Electrophoresis was performed using the PROTEAN II xi Cell system (Bio-Rad) at 10 mA per gel for 30 min, followed by 30 mA until the bromophenol blue marker reached the end of the gel. Gels were run in triplicate for each sample. The gels were stained with modified colloidal CBB G-250 [8] and were scanned using UMAX 2100XL scanner. Image and statistical analysis was performed with PDQuest 8.0.1 (Bio-Rad) as previously reported [9]. In the quantitative analysis, 2.0 and 0.5 were chosen as the upper and lower limits, respectively. Student's *t*-test and a significance level of 95% were used for the statistical analysis of the gels. Each sample was performed in triplicate gels.

2.8. LC-MS/MS and database retrieval

Protein spots were manually excised from the gel, and digested as previously reported [9]. The following steps performed by the Institute of Zoology of Chinese Academy of Sciences. Proteins were identified by PMF based on LC-MS/MS (LCQ Deca Xp plus, USA) and database searching from National Centre for Biotechnology Information (NCBI).

2.9. Statistical analysis

Statistical calculations were carried out with the SPSS 17 for Windows software package. The results are expressed as the mean ± SD. One-way analysis was used for statistical analyses and $P < 0.05$ were considered to be significant.