Supplementary information

Chemerin promotes proliferation and migration of ovarian cancer cells by upregulating expression of PD-L1

Chenxi GAO[1], Jinming SHI[1], Jingxin ZHANG[2], Yin LI[2]✉, Yi ZHANG[1]✉

[1]*Department of Gynecology, the First Affiliated Hospital of China Medical University, Shenyang 110001, China*
[2]*Department of Integration of Chinese and Western Medicine, School of Basic Medical Sciences, Peking University, Beijing 100191, China*

✉Yi ZHANG, syzi@163.com; Yin LI, yinli@bjmu.edu.cn

Materials and methods

Clinical specimens

All clinical specimens were collected from the Department of Gynecology of the First Affiliated Hospital of China Medical University. The use of human specimens was in accordance with the ethical standards of the ethics committee of China Medical University and with the Helsinki Declaration, and was approved by the ethics committee of China Medical University (AF-SOP-07-1.1-01).

Cell culture and transfection

HO-8910 and HO-8910PM ovarian cancer cells were purchased from the Cell Bank of Shanghai Institute of Cell Biology, Chinese Academy of Sciences, and cultured in 10% fetal bovine serum and Dulbecco's modified Eagle's medium in a cell incubator at 37 °C with 5% CO_2. CD274 siRNA (5'-CCACACACUGUAGUAGUAGATT-3') and control siRNA (5'-UUGUAGUAGUAGUAGGTT-3') were purchased from Gemma Gene (Shanghai, China). siRNA was transfected into HO8910 and HO8910PM cells by Lipofectamine 2000 (Thermo Fisher Scientific, Waltham, MA, USA). The transfection effect was detected by western blotting.

ELISA

Following the instructions of the manufacturer, levels of chemerin in serum and ascites were detected by enzyme linked immunosorbent assay using the Human Chemerin ELISA Kit (Abcam, Cambridge, UK, cat no. SEA945Hu, sensitivity ≤0.059 ng/mL).

Immunohistochemistry

Slides were individually incubated with PD-L1 (CST, Beverly, MA, cat no.10084-MM36-P) or chemerin (Abcam, Cambridge, UK, cat no. bs-10410R) antibody (1:100) in a humid chamber at 4 °C overnight. Secondary antibody staining was performed with a biotin-labeled horse anti-rabbit antibody (1: 200) for 1 h at room temperature, followed by incubation with a streptavidin-biotin horseradish peroxidase complex (Vector Laboratories, Burlingame, CA, USA). Immunoreactivity was detected using diaminobenzidine (DAB) substrate (peroxide substrate kit, SK-4100; Vector) for 2–5 min. Slides were then counterstained with Mayer haematoxylin before dehydration and mounting.

Western blotting

The cells were dissolved in RIPA buffer (50 mm Tris-HCl, 150 mmol/L NaCl, 1% NP-40, 0.5% deoxycholate, 0.1% SDS). A BCA Protein Analysis Kit (PIERCE, Rockford, IL) was used to determine protein concentration. The same amount of protein was separated by SDS-PAGE and transferred to a PVDF membrane by immunoblotting. The primary antibody was added and cells were incubated overnight at 4 °C in a chromatography freezer. On the second day, the secondary antibody was added and cells were incubated at room temperature for 2 h. Then, the chemiluminescent developer was dripped onto the cells, which were exposed in the darkroom for development.

Flow cytometry

Based on the antibody specification, the expression of PD-L1 in ovarian cancer cells stimulated by chemerin was detected by flow cytometry. Cells were collected and suspended in fixation/Permeabilization solution (BD Cytofix/Cytoperm kit-BD pharmingen), and PD-L1 staining with Alexa Fluor 647-tagged anti-PD-L1 antibody was performed according to the manufacturer's instructions.

Proliferation experiment

A Water Soluble Tetrazolium (WST)-1 assay was used to determine the effect of chemerin on HO-8910 cell proliferation. Briefly, 1×10^3 cells/well were incubated in 96-well plates overnight, starved in serum-free medium for 24 h, and treated with indicated reagents. Cells were incubated with 10 μL of WST-1 reagent (BioVision Research Products, Milpitas, CA, USA) for 2 h and absorbance was measured at 450 nm using the Bio-Rad iMark Microplate Absorbance Reader (Bio-Rad, USA).

Scratch-wound assay

For the scratch-wound assay, cells were cultured until they reached confluence. An area of cells was removed with use of a sterile pipette tip and then incubated at 37 °C for the indicated times. The furthest distance that cells migrated from the wound edge was measured and relative

wound closure was calculated.

Cytoskeleton staining

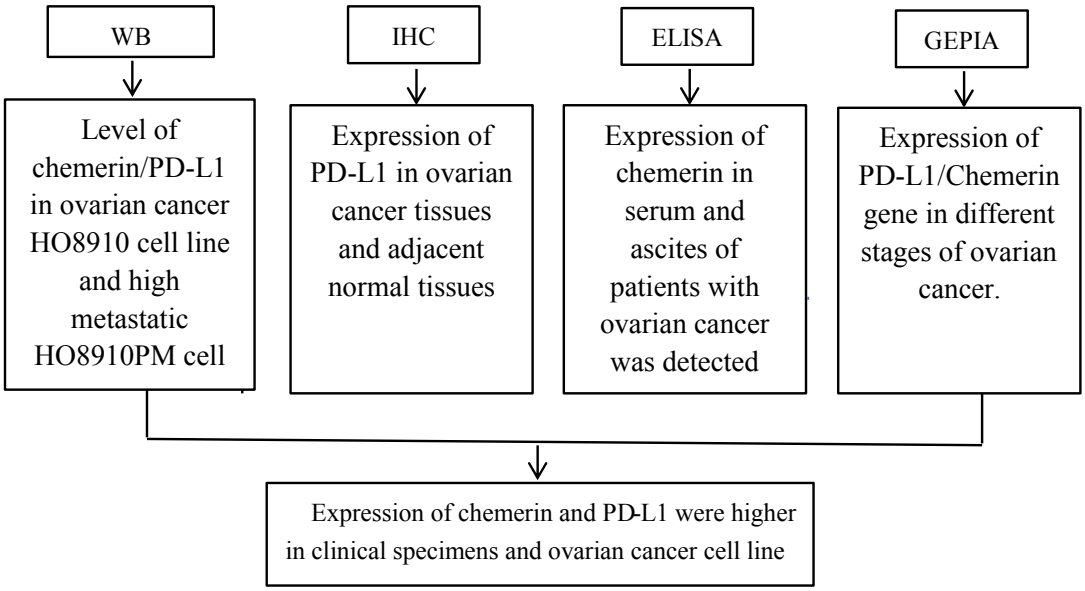
Normal and PD-L1 knockdown cells were washed with PBS (pH 7.4), fixed with 3.7% paraformaldehyde for 10 min, washed with PBS, incubated with 0.1% Triton X-100 for 5 min, washed with PBS, stained with DAPI 1:1000 for 5 min, and incubated with ghost pen cyclopeptide 1:1000 for 30 min. Images were recorded by confocal laser microscopy at 1000· magnification.

Statistical analysis

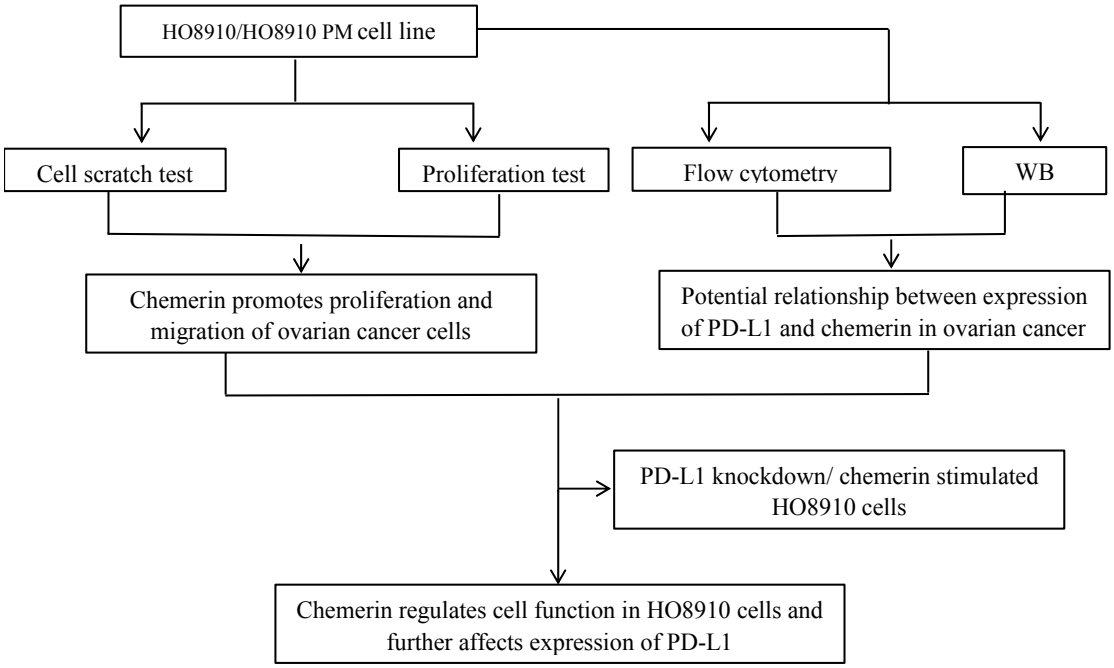
All data are expressed as means±SEM or are original data representing one of at least three independent experiments. One-way ANOVA followed by the Newman-Keuls post hoc test were used to compare multiple groups. The unpaired Student t-test was performed to compare two groups. $P<0.05$ was considered statistically significant.

Experiment procedure

Clinical



In vitro


HO8910/HO8910 PM cell line
Cell scratch test
Proliferation test
Flow cytometry
WB
Chemerin promotes proliferation and migration of ovarian cancer cells
Potential relationship between expression of PD-L1 and chemerin in ovarian cancer
PD-L1 knockdown/ chemerin stimulated HO8910 cells
Chemerin regulates cell function in HO8910 cells and further affects expression of PD-L1