Supporting Information

Materials and Methods

2.15. Immunofluorescence analysis

Ex vivo cultured cells were treated with 3-MA (20 μM), and carboplatin (25 μM), for 24h. Next, cells were fixed in 4% formaldehyde for 15 min at room temperature prior to cell permeabilization with 0.1% Triton X-100 (4°C, 10 min). Cells were saturated with PBS containing 2% BSA for 1 h at room temperature and processed for immunofluorescence with PD-L1 antibody, respectively, at 4 ^{o}C overnight. Then, they were incubated with Alexa Fluor 488-conjugated anti-rabbit IgG antibody (1:100). Between all incubation steps, cells were washed three times for three minutes with PBS containing 0.2% BSA. Fluorescence signals were analyzed using a Carl Zeiss fluorescent microscope at a 100× magnification, with excitation and emission wavelengths of 488 nm and 520 nm, respectively, using image analysis software.

2.16. TILs isolation

Peripheral blood mononuclear cells (PBMC) from lung cancer patients were isolated by Ficoll density gradient centrifugation. Fresh tumor tissues were used for the isolation of TIL and non-tumor-infiltrating lymphocytes (NIL). In brief, fresh tumor tissues were washed three times in RPMI 1640 before being cut into small pieces. The specimen were then collected in RPMI 1640 containing 1 mg/ml collagenase IV (Sigma-Aldrich, St. Louis, MO, USA) and 10 mg/ml DNase I (Roche, Basel, Switzerland) and mechanically dissociated by using the gentle MACS Dissociator (Miltenyi Biotec, Auburn, CA, USA). Dissociated cell suspensions were further incubated 1 h at 37C under continuous rotation and filtered through 70 μm cell strainers to obtain cell suspensions. Cells were gently minced and passed through 70-μm cell strainers to obtain cell suspensions. The cell suspensions were then used for FACS analysis.

2.17. Transwell Migration assay

For the transwell migration assay, LC/LC-DR treated cells were placed in 0.2 ml RPMI 1640 without fetal bovine serum (FBS) on the top chamber of each insert (BD

Biosciences, NJ) with or without 40 μl of 1 mg/ml Matrigel. The lower chamber was filled with 600 μl of RPMI 1640 medium with 10% FBS to act as the nutritional attractant. Twenty-eight hours later, the migrant cells that had attached to the lower surface were fixed with 20% methanol and stained for 20 min with crystal violet. The membranes were then carved and embedded under cover slips with the cells on the top. Cells in three different fields of view at 100× magnification were counted and expressed as the average number of cells per field of view. All assays were performed in triplicates.

2.18. Cell cycle analysis

Flow cytometric analysis was performed to define the cell cycle distribution for chloroquine treated (20 μM), carboplatin treated (25 μM) and control drug resistant LC-DR cells for 24 h. In brief, cells grown in 100 mm culture dishes were harvested, resuspended in cold PBS, and fixed with 9 volumes of 70% ethanol. Cells were then stained for total DNA content with a solution containing 50 μg/mL propidium iodide, 50 μg/mL RNaseA, 0.1% Triton X-100 and 0.1 mmol/L EDTA in PBS for 30 min at 37 °C. Cell cycle distribution was analyzed with a FACScan flow cytometer (BD Biosciences, San Jose, CA).

2.19. Th1 Cytokine detection

A multiplex bead-based cytokine assay (Biorad, Hercules, CA) method was used for the detection of Th1 (IL-2, TNF- β, IFN-γ) cytokines from the clinical samples of chemoresistant or chemosensitive lung cancer patients (n=45 for each group). A two-way anova multiple comparisons test was implemented for statistical analysis using Graph pad 6.0 software.