Electronic Supplementary Information (ESI)

# Gold(III) complexes inhibit growth of cisplatin-resistant ovarian cancer in association with upregulation of proapoptotic *PMS2* gene

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## A. Materials and Method.

### Materials

Analytical grade organic solvents were used in all experiments unless otherwise stated. Gold(III) porphyrin complexes $[Au^{III}(TPP)]Cl$ (gold-**1a**, wherein $[TPP]^{2-}$ = *meso*-tetraphenylporphyrinato ligand) and $[Au^{III}(OEP)]Cl$, **2**, wherein $H_2OEP$ = octaethylporphyrin) [*Chem. Eur. J.*, 2010, **16**, 3097]; a gold(III)-NHC complex ($[Au^{III}(CNC)(IMe)]CF_3SO_3$ (**3**, wherein HCNCH= 2,6-diphenylpyridine, IMe = 1,3-dimethylimidazol-2-ylidene) [*Chem. Commun.*, 2010, **46**, 3893]; a gold(I)-phosphine complex ($[(CNC)Au^{I}_3(\mu\text{-dppm})_2]Cl$, **4**, wherein dppm = 1,1-bis(diphenylphosphino)methane) [*Chem. Commun.*, 2011, **47**, 9318]; and a gold(I)-thiourea complex ($[Au^{I}(TU)_2]Cl$, **5**, wherein TU = 1,3-bis(4-methoxyphenyl)imidazolidine-2-thione) [*Chem. Commun.*, 2010, **46**, 7691] have been prepared and characterized as reported previously.

Cisplatin was purchased from Sigma-Aldrich (St. Louis, MO). All gold complexes and cisplatin were reconstituted either in PET diluent (60% polyethylene glycol 400, 30% ethanol, 10% Tween 80) and then diluted with phosphate-buffered saline (PBS) for *in vivo* studies or reconstituted in dimethyl sulfoxide (DMSO) and then diluted with cell culture medium for *in vitro* studies. Cisplatin was freshly prepared for each experiment and was immediately diluted in PBS (for *in vivo* experiments) or in culture medium (for *in vitro* experiments) upon reconstitution.

### Cell lines, growth media and growth conditions

RPMI1640 medium, fetal bovine serum (FBS) and trypsin-EDTA were purchased from Invitrogen (Carlsbad, CA). Human ovarian cancer cell line A2780 and the cisplatin-resistant derivative A2780cis were obtained from Sigma-Aldrich and were maintained as monolayer cultures in RPMI1640 medium supplemented with 10% FBS (complete medium) and complete medium with cisplatin (1 μM), respectively, at 37°C with 5% $CO_2$.

### Ovarian cancer xenograft model

Female BALB/cAnN-nu (nude) mice, 4–7 week old, were purchased from Charles River Laboratories (Wilmington, MA). Mice were maintained under specific pathogen-free conditions. All animal experiments were conducted under the guidelines approved by the Committee on the

Use of Live Animals in Teaching and Research of the University of Hong Kong. To establish tumors, $5 \times 10^6$ A2780 cells and $2.5 \times 10^6$ A2780cis cells suspended in PBS (100 μl) were injected into the right and left back flanks of each mouse respectively by subcutaneous injection. When the tumor volumes reached about 50 $mm^3$ (2–3 days after tumor inoculation), the mice were divided into the following four groups with at least three mice per group: (A) solvent control; (B) cisplatin (2 mg/kg); (C) gold-**1a** (0.75 mg/kg) and (D) gold-**1a** (1.5 mg/kg). Mice were treated with cisplatin, gold-**1a** or solvent control according to the body weight once every 2–3 days by intraperitoneal injection until the mice were sacrificed. Tumor sizes were measured once every 2–3 days using a digital caliper and tumor volumes were calculated by the formula $V = ab^2 \times 0.52$, where a and b were the longest and the shortest diameters of the tumor. The volume of PET diluent injected was kept ≤3 μl per injection in all mice, and mice in all groups received the same amount of PET diluent.

**Histological analysis**

All mice were sacrificed 15 days after treatment before the tumor volumes reached 1000 $mm^3$. Tumors were excised, fixed in 4% paraformaldehyde for 16 h, and then embedded in paraffin for histological studies. Paraffin-embedded tissues were sectioned into 5 μm slices. For the detection of apoptotic cells, *in situ* Cell Death Detection Kit (Roche, Penzberg, Bavaria, Germany) was used and the experiments were conducted according to the instruction of the manufacturer. The tissue sections were monitored under an inverted microscope at 200× magnification. At least five microscopic fields were randomly chosen for each section and the number of apoptotic cells was counted.

**Cell viability assay**

For the cell viability assay, cells were seeded at a density of $3 \times 10^4$ cells per well in 100 μl of complete medium in a 96-well plate 24 h before treatment. The cells were treated with different concentrations of different gold complexes or cisplatin for 48 h. The concentration of DMSO was identical in all wells and its final concentration was ≤ 1%. At the end of the incubation period, 10 μl of 5 mg/ml MTT solution was added to each well, and the cells were incubated at 37°C for 3 h. Two hundred microliters of acidic isopropanol (0.04 M hydrochloric acid in isopropanol) was added to each well to dissolve the formazan complexes. The absorbance of the

converted dye was measured at a wavelength of 580 nm. The number of viable cells at the time of assay was read from standard curves. Each condition was done in triplicate and the data were shown as mean ± standard error of the mean (SEM) from at least three independent experiments.

**Soft agar colony formation assay**

Ten thousand A2780 or A2780cis cells were mixed with solvent control, or with gold-**1a** or cisplatin at various concentrations in complete medium (2 ml) with soft agar (0.35%) per well in a 6-well plate coated with RPMI1640 medium with 0.7% agar. The final concentration of DMSO was identical in all wells (0.04%). After the soft agar with cells was solidified, complete medium (1 ml) supplemented with solvent control, or with gold-**1a** (0.05–2 μM) or cisplatin (2–25 μM) was added onto the soft agar. The cells were incubated under the conditions of 37°C, 5% $CO_2$ for 14 days. Colonies were observed under an inverted microscope at 50× magnification. The relative area occupied by these colonies was estimated using Photoshop CS4 as a pixel value. Four microscopic fields were randomly chosen for each well and the data were shown as mean ± SEM from three independent experiments.

**Cell migration assay**

To conduct cell migration assay, cells were seeded at $6 \times 10^5$ per well in a 6-well plate in 2 ml complete medium 24 h before wound induction. The cells were treated with solvent control or with gold-**1a** or cisplatin at various concentrations for 28 h after wound induction. The final concentration of DMSO was identical in all wells (0.1%). At 0 and 28 h after wound induction, the cells were observed under an inverted microscope at 50× magnification. The percentage of cell migration was calculated by the formula $(x-y)/x \times 100\%$, where x and y were the relative widths of the wounds at 0 and 28 h respectively. The data were shown as mean ± SEM from three independent experiments.

**Quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR)**

Six hundred thousand of A2780 or A2780cis cells per well were seeded onto 6-well plates 24 h before treatment. The cells were treated with solvent control or approximately double the $IC_{50}$ values of different gold complexes and cisplatin at 48 h. The exact concentrations applied were: gold-**1a** (**1**): 0.5 μM; **2**: 2 μM; **3**: 1.8 μM; **4**: 30 μM (the complex precipitated in culture medium

at this concentration); **5**: 14 μM for A2780; 28 μM for A2780cis and cisplatin: 25 μM for A2780; 100 μM for A2780cis for 24 h. The concentration of DMSO was 0.1% for wells treated with gold-**1a**, **2**, **3** or cisplatin, and was 0.56% for wells treated with **4** or **5**. Total RNA was extracted using RNeasy Mini Kit (Qiagen, Valencia, CA). Oligos were purchased from Integrated DNA Technologies (Coralville, IA). The expression profiles of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were used to normalize those of the target genes. Primer sequences for human GAPDH were 5' TGGCGCTGAGTACGTCGTGG 3' and 5' TGGGGGCATCAGCAGAGGGG 3'. Primer sequences for human *PMS2* were 5' GCCACGCATCGGCGAAGGTT 3' and 5' GGGCGGGGGTAGGGGGTTTT 3'. One microgram of each total RNA sample was reverse transcribed to cDNA using ThermoScript™ RT-PCR System (Invitrogen). The resulting first strand cDNA was diluted 10-fold with water, and one microlitre of the diluted cDNA was subjected to quantitative polymerase chain reaction (qPCR), using FastStart Universal SYBR Green Master (Roche, Indianapolis, IN). The data were shown as mean ± SEM from at least three independent experiments.

**Suppression of *PMS2* by small hairpin RNA**

To suppress *PMS2* by small hairpin RNA, plasmids expressing small hairpin RNAs (shRNAs) targeting the coding region of human *PMS2* were purchased from Sigma-Aldrich. Three different plasmids were transfected into A2780cis cells, including the empty vector and two plasmids targeting different coding regions of *PMS2*. Stable cells were selected in complete medium supplemented with 6 μg/ml puromycin and stable clones were pooled. The resulting stable cell lines were designated as A2780cis [shcontrol], A2780cis [shPMS2-1686] and A2780cis [shPMS2-2136].

**Statistical analysis**

Tumor volumes, number of apoptotic cells, cell viabilities, relative areas occupied by colonies, percentage of cell migration and relative expression levels were compared by two-tailed Student's t test (MS Excel). $P < 0.05$ was considered statistically significant.

**B. Table and Figures.**

**Table S1**. Cytotoxic $IC_{50}$ values (48 h) of gold complexes and cisplatin in A2780 and A2780cis.

| **Complexes** | **$IC_{50}$ (μM)** | |
|---|---|---|
| | **A2780** | **A2780cis** |
| gold-**1a** | 0.340 ± 0.012 | 0.303 ± 0.003 |
| **2** | 0.893 ± 0.070 | 1.23 ± 0.14 |
| **3** | 0.863 ± 0.205 | 0.740 ± 0.032 |
| **4** | 13.5 ± 2.5 | 22.2 ± 4.5 |
| **5** | 6.44 ± 0.11 | 16.3 ± 1.3 |
| $KAu^{III}Cl_4$ | 67.2 ± 5.8 | > 100 |
| cisplatin | 7.93 ± 0.47 | 34.9 ± 5.4 |

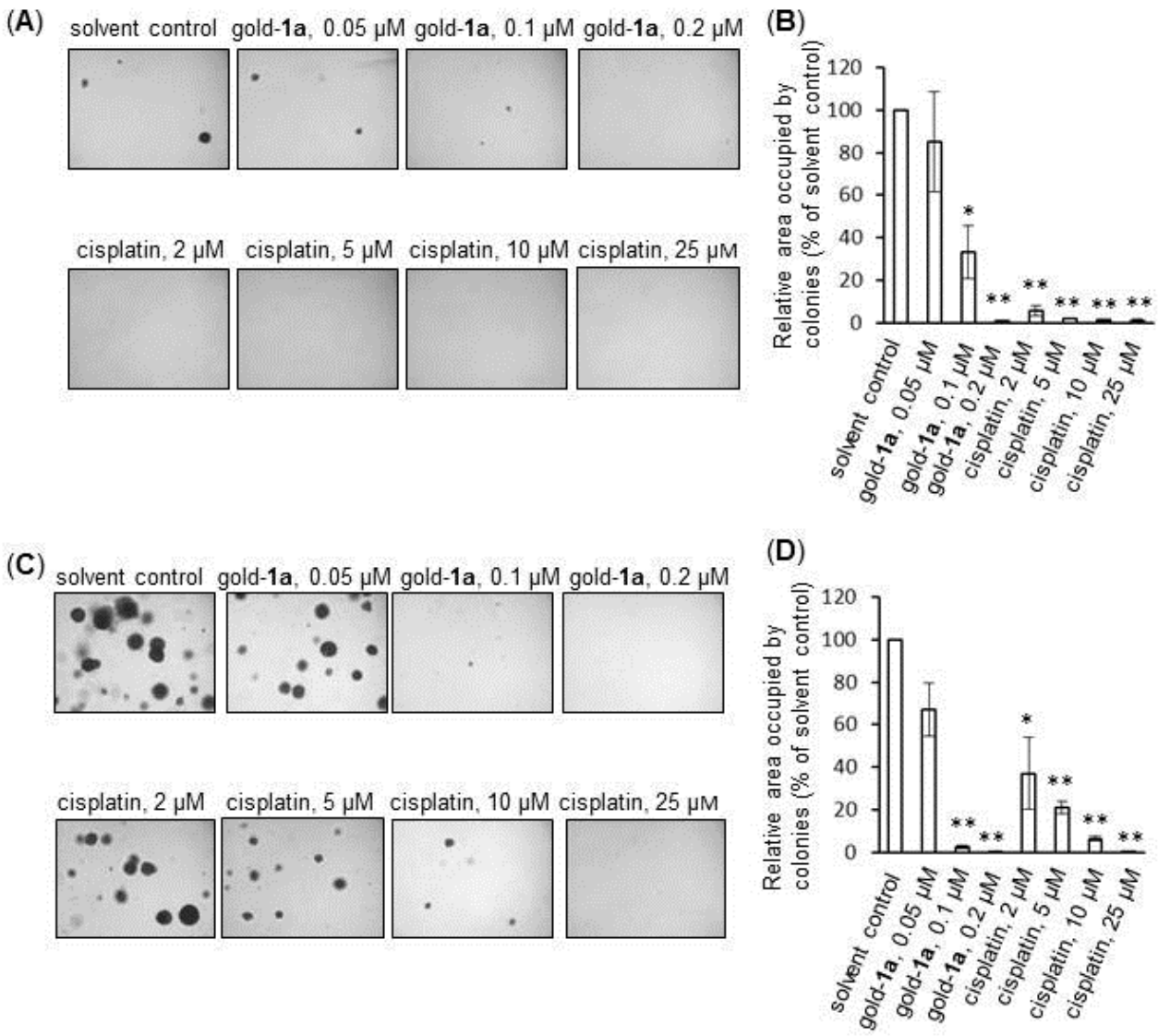


**Fig. S1.** (**A**, **C**) Soft agar colony formation assay of A2780 (**A**) and A2780cis (**C**) cells in response to different concentrations of gold-**1a** or cisplatin. Pictures shown were taken at 50× magnification. Representative pictures from three independent experiments. (**B**, **D**) Graphical representation of relative area occupied by colonies in response to gold-**1a** or cisplatin of A2780 (**B**) and A2780cis (**D**), compared to solvent control. Data are shown as mean ± SEM from three independent experiments. *, $p < 0.05$, compared to solvent control; **, $p < 0.001$, compared to solvent control.

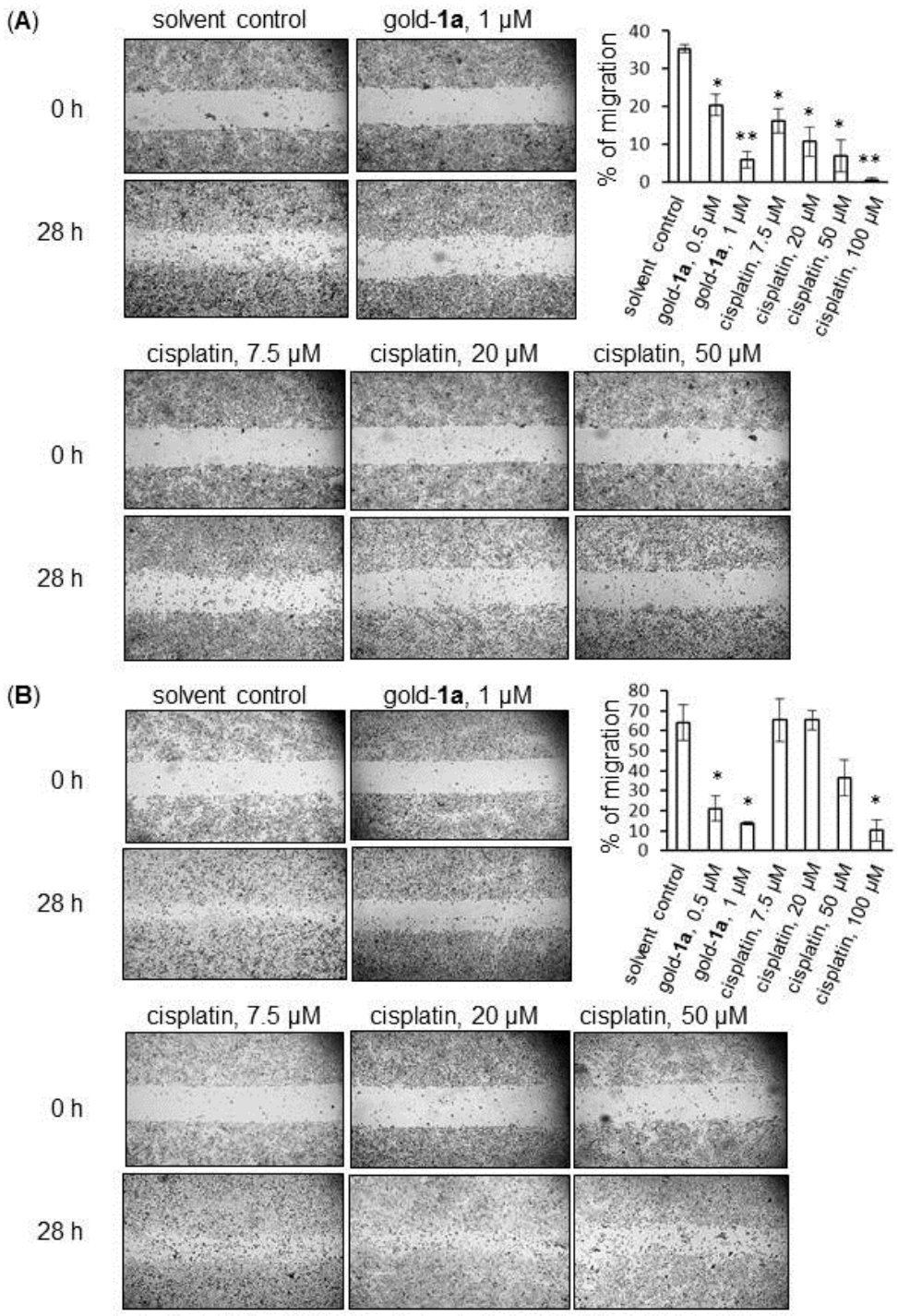


**Fig. S2.** Cell migration assay of A2780 (**A**) and A2780cis (**B**) cells in response to gold-**1a** or cisplatin treatment. Pictures shown were taken at 50× magnification. Data are shown as mean ± SEM from three independent experiments. *, $p < 0.05$, compared to solvent control; **, $p < 0.001$, compared to solvent control.