Electronic Supplementary Information (ESI)

Gold(III) porphyrin complex as an anti-cancer candidate to inhibit growth of cancer-stem cells

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1. General experimental procedure

A. Materials. All chemicals were purchased form Sigma-Aldrich, unless otherwise noted. The solvents used for synthesis were of analytical or HPLC grade. The gold(III) porphyrin complexes gold-**1a** [*Chem. Commun.*, **2003**, 1718], a hydroxyl-substituted gold-**1a** [*Cancer Res.*, **2010**, *70*, 329], and a glycosylated gold(III) porphyrin [*Chem. Eur. J.*, **2010**, *16*, 3097] were prepared according to the reported methods.

B. Instrumentation. ^{1}H-NMR spectra were recorded on a Bruker AVANCE 400 Fourier-Transform NMR spectrometer (chemical shift in ppm). Positive-ion ESI (electrospray ionization) mass spectra were recorded on a Finnigan LCQ mass spectrometer. Elemental analyses were conducted in the Institute of Chemistry, The Chinese Academy of Sciences, Beijing. Gold-**1a** and TMZ were reconstituted in dimethyl sulfoxide (DMSO) for *in vitro* studies and in PET solution (PET = 6% polyethylene glycol 400; 3% ethanol; 1% Tween 80; 90% phosphate-buffered saline, pH 7.0) for *in vivo* studies.

C. Cell line and cell culture. U-87 MG human glioblastoma cell line and SKOV-3 cell line were purchased from the American Type Culture Collection (Manassas, VA). Minimal Essential Medium (MEM), Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (DMEM/F12), fetal bovine serum (FBS), trypsin-EDTA, human epidermal growth factor (EGF), human basic fibroblast growth factor (bFGF) and B-27 Supplement were products of Invitrogen (Carlsbad, CA). The U-87 MG cells were maintained as monolayer culture in MEM supplemented with 10% FBS (complete medium) at 37°C, 5% CO_2.

D. Sphere formation assay. U-87 MG/ SKOV-3 cells were seeded at 3×10^3 cells per well in 0.5 ml DMEM/F12 supplemented with 20 ng/ml EGF, 20 ng/ml bFGF and 1× B-27 Supplement with or without gold-**1a** (0.05, 0.1 and 0.5 μM) or TMZ (50 and 500 μM) in a 24-well Ultra Low Cluster Plate (Costar). The concentration of DMSO was identical in all wells, and was 0.5%. The cells were incubated at 37°C for 5 days. The spheres were photographed under an inverted microscope at 40× magnification. Four fields were randomly chosen and the relative areas occupied by the spheres were measured using ImageJ (National Institutes of Health, Bethesda, MD).

E. Cell viability assay. SKOV-3 cells were seeded at a density of 1×10^4 cells per well in 100 μl of complete medium in a 96-well plate 24 hours before treatment. The cells were treated with different concentrations of the gold complexes for 48 hours. The concentration of DMSO was identical in all wells and its final concentration was 0.01%. 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 hours. Two hundred microliters of acidic isopropanol (0.04 M hydrochloric acid in isopropanol) was then added to each well to dissolve the formazan complexes. Finally, the absorbance of the converted dye was measured at a wavelength of 580 nm. Each condition was done in triplicate and the data were shown as mean ± SEM from two independent experiments.

F. microRNA expression profiling. U-87 MG cells were seeded at 1×10^6 cells per dish in 10 ml complete medium in a 10-cm dish 24 hours before treatment. Cells were untreated or treated with gold-**1a** at 0.1 μM or 0.2 μM in 20 ml medium for 5 days. The concentration of DMSO was identical in all dishes (0.5%). Total RNA, including small RNAs, was then extracted using miRNeasy Mini Kit (Qiagen, Valencia, CA). miRNA expression profiling was performed using Stem Cell MicroRNA qPCR Array with QuantiMir™ (System Biosciences, Mountain View, CA). Expression levels of a total of 95 miRNAs with published implications in cancer, cell development and apoptosis were measured by the array, using U6 transcript as control.

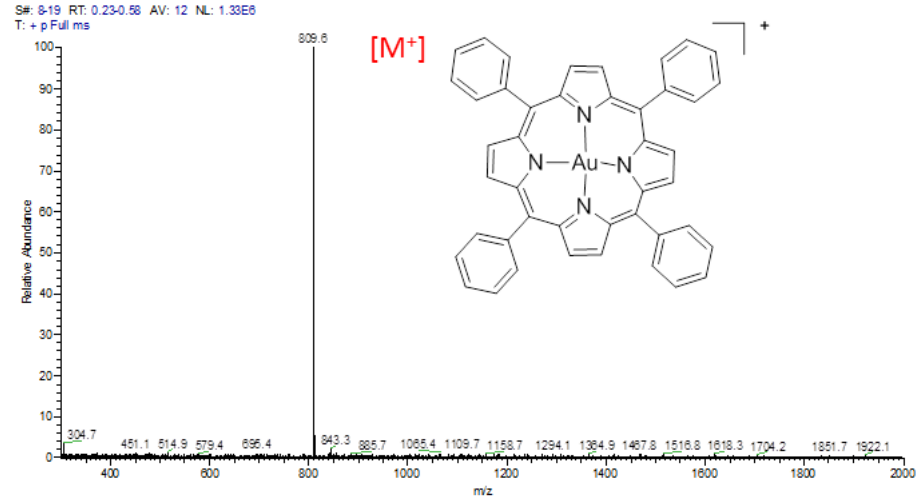
G. Quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR). U-87 MG cells were seeded at 3×10^5 cells per well in 2 ml complete medium in a 6-well plate 24 hours before gold-**1a** treatment. Cells were untreated or treated with 0.5 μM gold-1a for 24 or 48 hours. The concentration of DMSO was identical in all wells (0.005%). Total RNA was then extracted using miRNeasy Mini Kit (Qiagen). 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. Forward and reverse primers for human GAPDH were 5' TGGCGCTGAGTACGTCGTGG 3' and 5' TGGGGGCATCAGCAGAGGGG 3' respectively. Forward and reverse primers for human NANOG were 5' ACCTTGGCTGCCGTCTCTGGC 3' and 5' GCAAAGCCTCCCAATCCCAAACAAT 3' respectively. Forward and reverse primers for human MYLIP were 5' AGCGGGCTCTACCGAGCGAT 3' and 5' GTGGCCCTTCAAGTCACGGCT 3' respectively. Forward and reverse primers for human

CDKN1A were 5' CGGCTGGGGATGTCCGTCAGA 3' and 5' TCGCGTCTCAGCTGCTCGCT 3' respectively. Three hundred nanograms of each total RNA sample was reverse transcribed to cDNA using NCode™ miRNA First-Strand cDNA Synthesis Kit (Invitrogen). The resulting first strand cDNA was diluted 10-fold and 1 μl of the diluted cDNA was then subjected to quantitative polymerase chain reaction (qPCR), using FastStart Universal SYBR Green Master (Roche, Indianapolis, IN). The data were shown as mean ± SEM from three independent experiments.

H. Statistical analysis. Relative areas occupied by the spheres, cell viabilities and relative expression levels were compared by two-tailed Student's t test (MS Excel). $P < 0.05$ was considered statistically significant.

2. Figures and Table

(a) **Gold-1a**



(b) **Gold-1a + GSH**

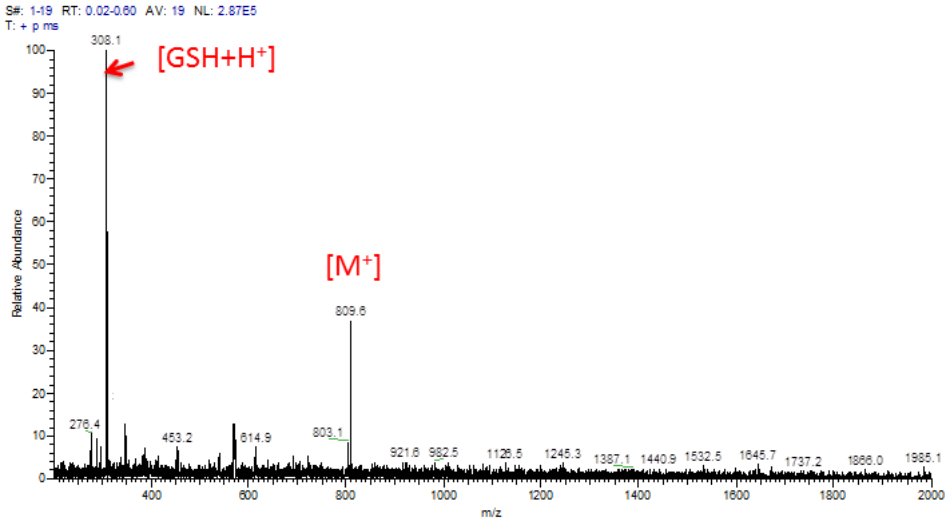


Fig. S1 ESI-MS (positive mode) of (a) gold-**1a** and (b) gold-**1a** incubated with glutathione (GSH, 2 mM) for 24 h in phosphate-buffered saline (pH 7.0)

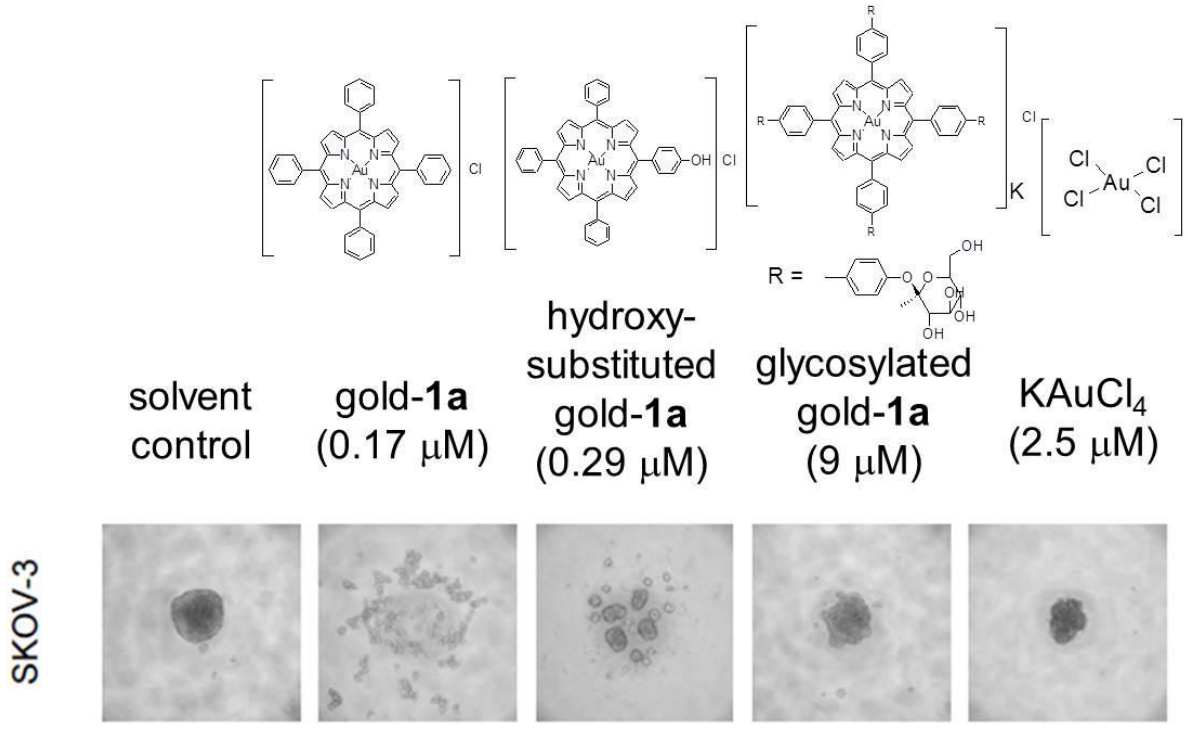


Fig. S2 The inhibitory activities of gold-**1a**, hydroxyl-substituted gold-**1a**, glycosylated gold-**1a** and $KAuCl_4$ at 1/10 of their corresponding cytotoxic IC_{50} values in inhibiting sphere formation by SKOV-3 ovarian cancer cells. Representative pictures from three independent experiments. Data are shown as mean ± SEM from three independent experiments. *, $p < 0.05$, compared to solvent control.

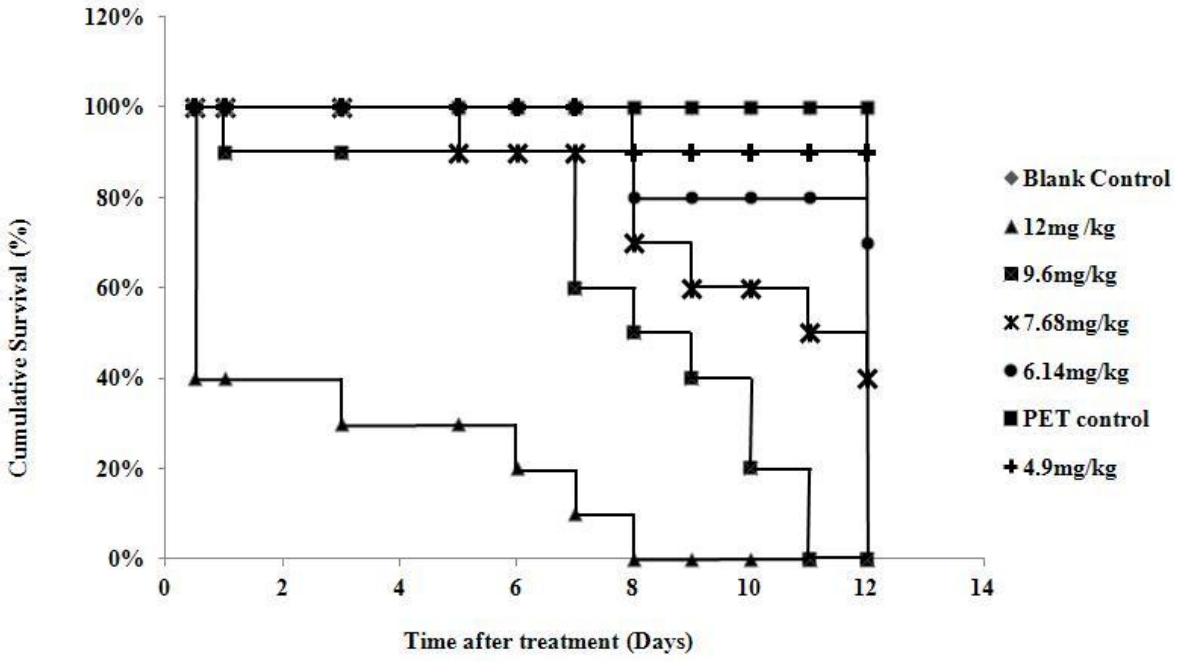


Fig. S3 Survival curves of mice injected intravenously with different dosages of gold-**1a** for 12 days.

Table S1 Polychromatic erythrocytes (PCE) of blood cells harvested from bone marrow of each mice treated with PET solvent (for 24 h), gold-**1a** (for 24 or 48 h) or cyclophosphamide (for 24 h) were examined to determine the frequency of micronucleated PCE (expressed % micronucleus rate).

Agent	Dose(mg/kg)	Mice (n)	PCE (n)	Micronucleus rate (%)
PET control	0	10	2000	0.75±0.486
Gold-**1a** (24h)	0. 85	10	2000	0.65±0.784
Gold-**1a** (24h)	1.7	10	2000	0.50±0.471
Gold-**1a** (24h)	3.4	10	2000	0.60±0.516
Gold-**1a** (48h)	3.4	10	2000	0.75±0.791
cyclophosphamide (24h)	80	10	2000	46.85±9.815**

*P<0.05, **P<0.01

Full reference of reference #13

R. Stupp, W. P. Mason, M. J. van den Bent, M. Weller, B. Fisher, M. J. Taphoorn, K. Belanger, A. A. Brandes, C. Marosi, U. Bogdahn, J. Curschmann, R. C. Janzer, S. K. Ludwin, T. Gorlia, A. Allgeier, D. Lacombe, J. G. Cairncross, E. Eisenhauer, R. O. Mirimanoff; European Organisation for Research and Treatment of Cancer Brain Tumor and Radiotherapy Groups; National Cancer Institute of Canada Clinical Trials Group. *N. Engl. J. Med.*, 2005, **352**, 987.