Electronic supplementary information:

Aptamer-functionalized graphene oxide for high efficient loading and cancer cell-specific delivery of antitumor drug

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1. Cell culture

A549 (human adenocarcinoma cell, NSCLC), NCI-H157 (squamous carcinoma cell, NSCLC), NCI-H520 (squamous carcinoma cell, NSCLC), NCI-H1299 (large cell carcinoma cell, NSCLC), NCI-H446 (SCLC), MCF-7 (human breast carcinoma cell), and HeLa (human cervical carcinoma cell) cell lines were obtained from the cell bank of type culture collection of the Chinese Academy of Sciences (Shanghai, China) and cultured at 37 °C in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin in a 5% CO_2 environment. After growing to 90% confluence, the cells were washed with PBS (0.145 M NaCl, 1.9 mM NaH_2PO_4, 8.1 mM K_2HPO_4, pH 7.4), the culture medium replaced the culture medium by 1 mL PBS, and the cell number was estimated by a hemocytometer.

2. MTT assay

MTT assays were used to evaluate cellular viability. For this purpose, A549 cells were seeded in 96-well plates with a density of 1×10^4 cells/well. After 24 h incubation, the medium was replaced with fresh medium containing 20 μg/mL A1–GO/ADC complexes and incubated at 37 °C for 24 h. Then, 10 μL of MTT solution (5 mg/mL in PBS, pH 7.4) was added into each well and incubated for 4 h at 37 °C. Afterward, 100 μL of dimethylsulfoxide (DMSO) was added into each well. Absorbance was recorded at 550 nm on a Synergy 2 microplate reader (Biotek, USA). The viabilities of the cells incubated with the complexes were obtained by comparing with those of the cells not being incubated with the complexes (their viability was taken as 100% as a control).

3. LDH assay

To assess the cell membrane integrity, lactase dehydrogenase (LDH) release assay was performed. Cancer cells were placed in 96-well plates (~1×10^4 cells/well) and incubated for 24 h. The A1–GO/DAC complexes (at 20 μg/mL) were introduced to each well and incubated for 4 h at 37 °C, the plates were equilibrated at room temperature for 30 min, 100 μL of CytoTox-ONE reagent (Sigma) was added to each well and the plates were incubated for 10 min at room temperature. The fluorescence

signal at 590 nm was recorded with an excitation source of 560 nm on a Synergy 2 microplate reader (Biotek, USA). LDH release was expressed relative to the basal LDH release from untreated cells.

4. The hydrogen bonds formed between GO and DAC at different pH

Under neutral condition, four kinds of hydrogen bonding can be formed between –COOH of GO and –OH of DAC, –COOH of GO and $–NH_2$ of DAC, –OH of GO and –OH of DAC, and –OH of GO and $–NH_2$ of DAC. Under acidic conditions, $–NH_2$ of DAC forms $–NH_3^+$ (pK_a of $–NH_2$ in DAC molecule is ~7.6[1]) and therefore cannot participate in hydrogen bonding. In this case, two kinds of hydrogen bonding can occur between –COOH of GO and –OH of DAC, and –OH of GO and –OH of DAC. Furthermore, H^+ in solution competes with the hydrogen-bond-forming groups and then weakens the above hydrogen-bonding interaction. Under basic conditions, –COOH of GO exists as $–COO^–$ (pK_a of –COOH in GO is ~6.6[2]) and cannot form a hydrogen bond with –OH or $–NH_2$ groups of DAC. Therefore, two kinds of hydrogen bonding interaction can occur between –OH of GO and –OH of DAC, and –OH of GO and $–NH_2$ of DAC. Thus, the strongest hydrogen-bonding interaction between GO and DAC is expected under neutral conditions, and the highest loading of DAC on GO is therefore obtained.

References

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2 B. Konkena and S. Vasudevan, *J. Phys. Chem. Lett.*, 2012, **3**, 867-872.

5. Figures

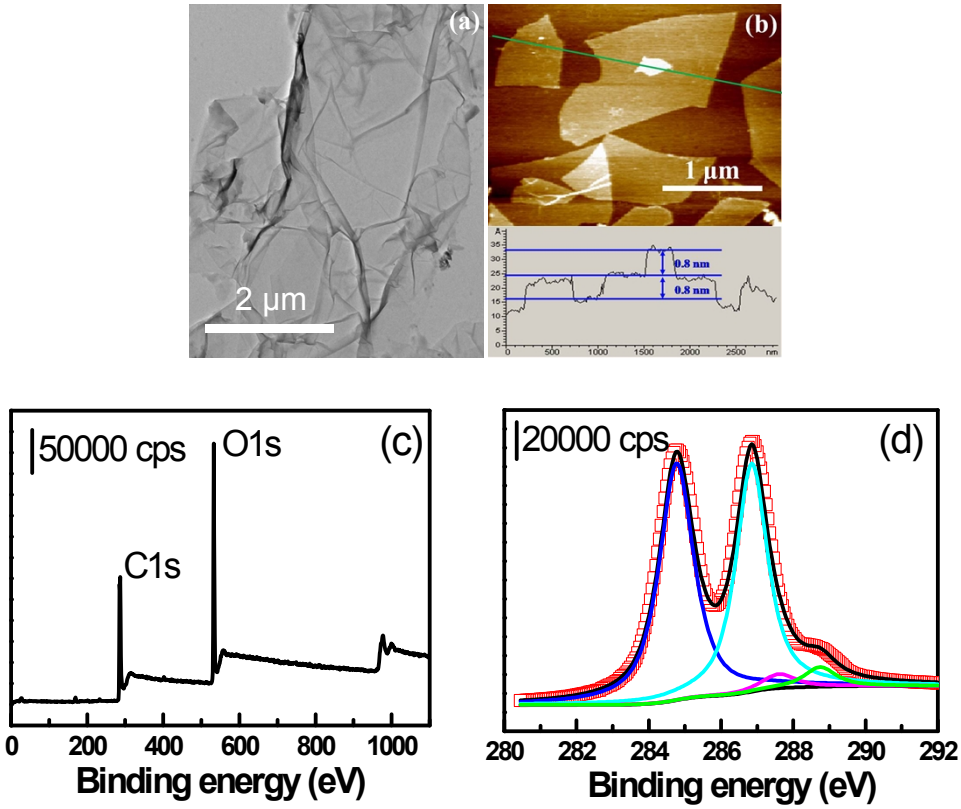

Fig. S1 Typical TEM (a), AFM (b) images, and XPS (c) spectra of the prepared GO sheets. (d) is XPS spectrum of C1s in GO and their related curve-fitted components. The TEM image was recorded on a JEOL–2010 microscope with an accelerating voltage of 80 kV. The AFM image was recorded with a Nanoscope IIIa scanning probe microscope (Digital Instruments) using a tapping mode at ambient temperature (23 ± 2 °C) with a humidity of 30%. XPS was measured with an ESCALAB 250 XPS spectrometer (VG Scientifics) using the monochromatic Al Kα line at 1486.6 eV. The binding energies were calibrated with respect to C1s peak of 284.6 eV. The deconvolution analysis was performed using the XPS PEAK program (version 4.0).

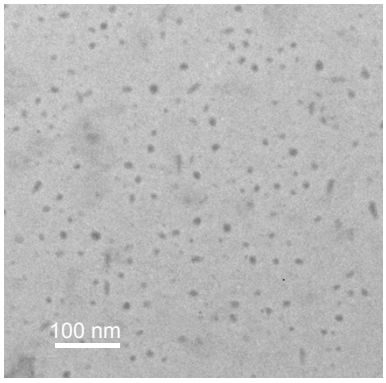

Fig. S2 TEM image of the small pieces of GO, which were obtained by cutting the synthesized GO with sonicating and hydrothermally treating.

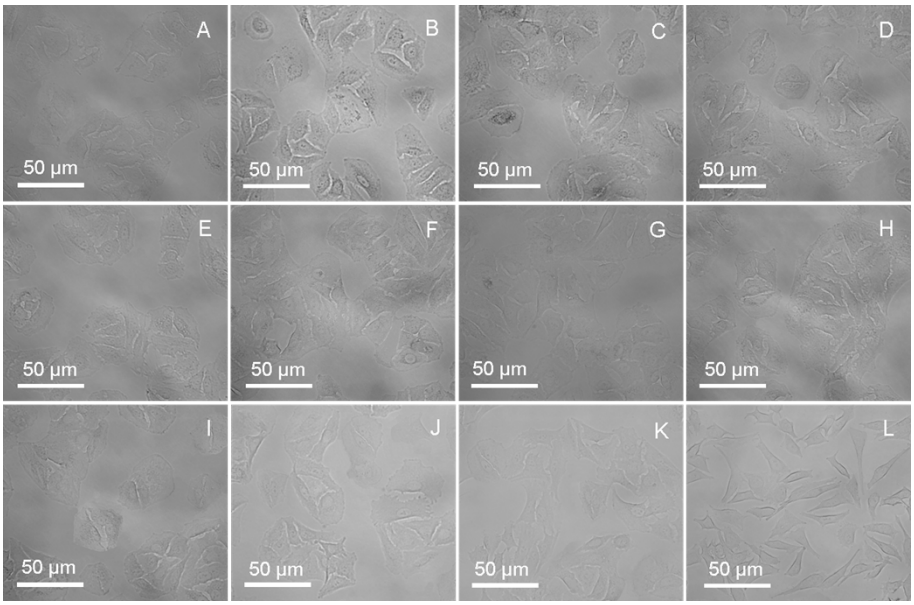

Fig. S3 BF images A549 cells before (A) and after incubation with 20 μg/mL A1–GO/DAC complexes (B) for 2 h. (C)–(F) show the bright field images of A549 cells after 2 h incubation with 20 μg/mL A1–GO (C), GO (D), GO/DAC (E), and free DAC (F), respectively. (G)–(L) show the bright field images of NCI-H157 (G), NCI-H520 (H), NCI-H1299 (I), NCI-H446 (J), MCF-7 (K), and HeLa cells (L), respectively, after 2 h incubation with A1–GO/DAC complexes under a dose of 20 μg/mL.

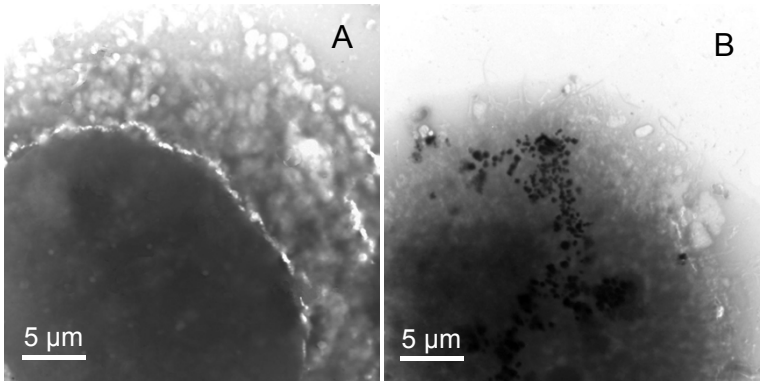

Fig. S4 TEM images of the part area of A549 cells before (A) and after (B) incubated with 20 μg/mL A1–GO/DAC for 2 h.

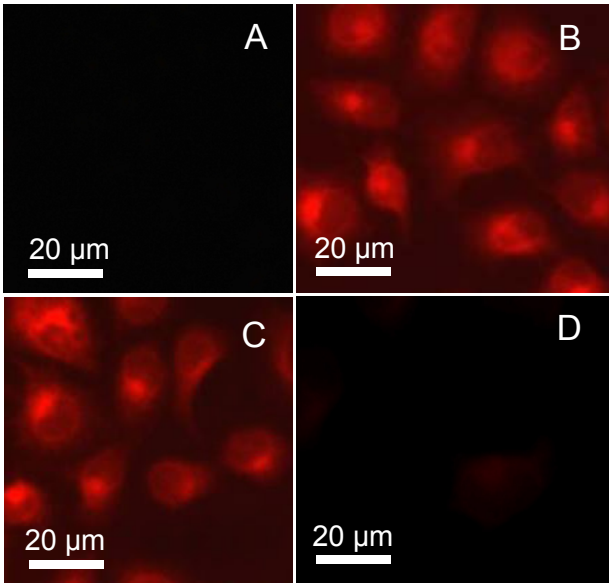

Fig. S5 Fluorescence image of A549 cells (A). (B)–(D) show the fluorescence images of the cells after incubated with Rh6G-labeled A1–GO/DAC (B), A1–GO (C), and GO (D) respectively. Before recording the images, the cells were washed thrice with PBS. The images were captured under an AXIO microscope (AxioObserver A1, Carl Zeiss) with an excitation at 543 nm.

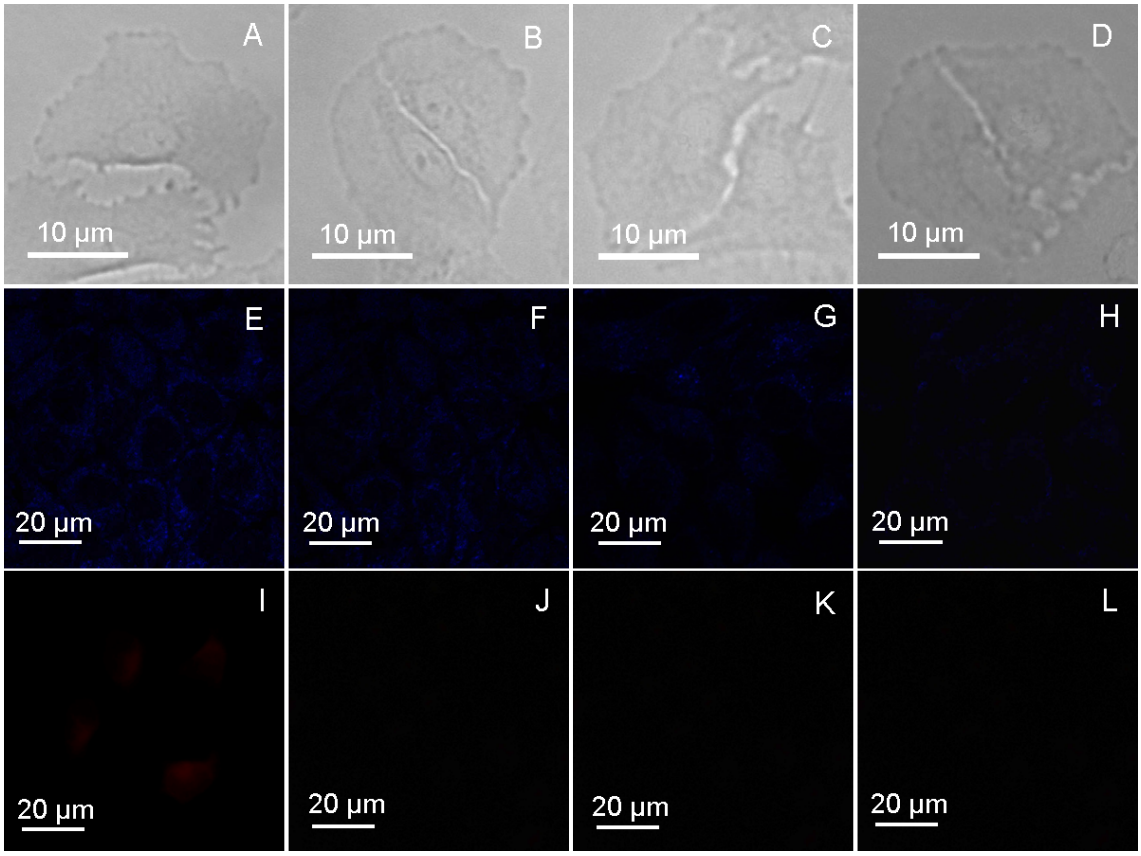

Fig. S6 (A)–(D) BF images of A549 cells after incubated with 20 μg/mL A2–GO/DAC (A), A3–GO/DAC (B), sgc8–GO/DAC (C), and tdo5–GO/DAC (D) for 2 h. (E)–(H) CLSM images A549 cells after incubated with 20 μg/mL A2–GO/DAC (E), A3–GO/DAC (F), sgc8–GO/DAC (G), and tdo5–GO/DAC (H) for 2 h. The images were captured under an excitation at 405 nm. (I)–(L) Fluorescence image of A549 cells after incubated with 20 μg/mL Rh6G-labeled A2–GO/DAC (I), A3–GO/DAC (J), sgc8–GO/DAC (K), and tdo5–GO/DAC (L) for 2 h. The images were captured under an excitation at 543 nm. Before recording these images, the cells were washed thrice with PBS.

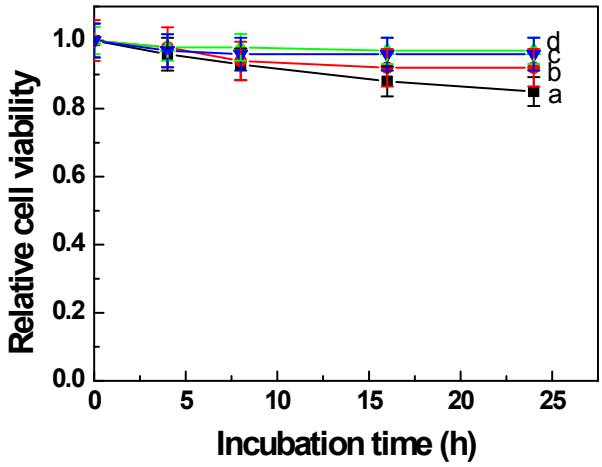

Fig. S7 Incubation time-dependent cell viability of A549 cells incubated with 20 μg/mL A2–GO/DAC (a), A3–GO/DAC (b), sgc8–GO/DAC (c), and tdo5–GO/DAC (d). Error bars were based on three measurements.

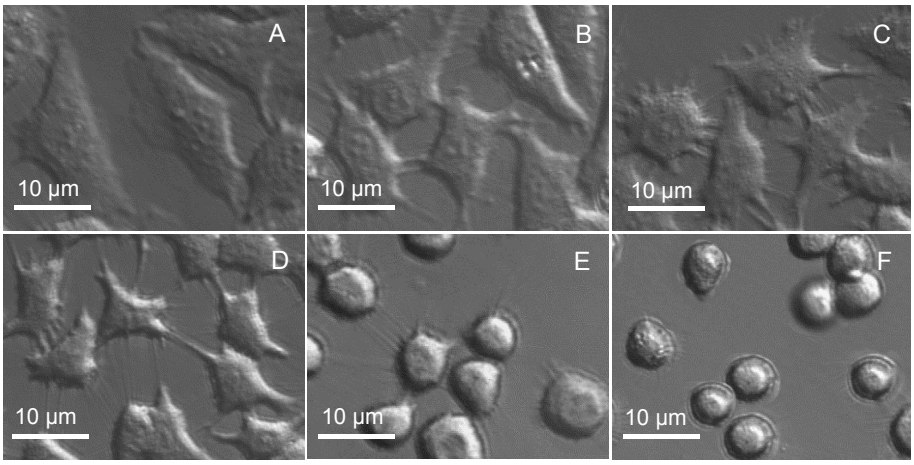

Fig. S8 DIC images showing the A549 cell natural death process. The images were recorded after the cells stayed at ambient temperature for 0 (A), 4 (B), 8 (C), 16 (D), 20 (E), and 24 h (F).