Supplementary information

for

Nucleus-targeted DNA tetrahedron as nanocarrier of metal complex for enhanced glioma treatment

Yiqiao Tian[#], Yanyu Huang[#], Pan Gao, Tianfeng Chen*

Department of Chemistry, Jinan University, Guangzhou 510632, China.

*E-mail: tchentf@jnu.edu.cn.

These authors contributed equally to the work.

1. Materials and methods

1.1 Materials and cell lines

[Ir(ppy)₂phen]⁺PF₆ (IrPP) was synthesized according to our previous study¹. Dulbecco's modified Eagle's medium (DMEM) medium and fetal bovine serum (FBS) were bought from Gibco® Thermo Fisher Scientific Inc.

U251 and U87 human glioma cells, HBM human brain microvascular endothelial cells and CHEM-5 human glial cells were purchased from American Type Culture Collection (ATCC, Manassas, Virginia).

1.2 The assembly of DNA nanocarriers

The DNA tetrahedron was synthesized according to the methods of Andrew J. Turberfield *et al.*² with aptamer modification. The nucleotides of aptamers were preliminary linked to the 5' terminal of DNA tetrahedron strands by Sangon Biotech (Shanghai) Co., Ltd. The DNA sequences were listed as follows: S1(5')GGCTATAGCACATGGGTAAAAGACAGGCAGTTGAGACGAACATTCCT AAGTCTGAAATTTATCACCCGCCATAGTAGACGTATCACC (3') S2(5')GGTGGTGGTGGTTGTGGTGGTGGTGGTGGCTTGCTACACGATTCAGACTT AGGAATGTTCGACATGCGAGGGTCCAATACCGACGATTACAG (3') S3(5')GGTGGTGGTGGTGGTGGTGGTGGTGGTGGGGGTGATAAAACGTGTAGCAA GCTGTAATCGACGGGAAGAGCATGCCCATCCACTACTATGGCG (3') S4(5')GGCTATAGCACATGGGTAAAACGACCCTCGCATGACTCAACTGCCTG GTGATACGAGGATGGGCATGCTCTTCCCGACGGTATTGGAC (3')

The assembly of DNA nanocarriers was performed as we previously described³.

1.3 Loading of IrPP on Apts-DNA

For IrPP loading, 1 mM IrPP and Apts-DNA were mixed for 24 h. Afterwards, the mixed solution was centrifuged for 2 times at 8000 rpm to remove the unloaded IrPP and DNA tetrahedron. Finally, the obtained Apts-DNA@Ir was used for chemical characterization and biological determination.

1.4 Characterization of Apts-DNA@Ir

The zeta potential of IrPP, Apts-DNA and Apts-DNA@Ir was analyzed by dynamic light scattering (Malvern Instruments Limited). Atomic force microscopy (AFM) was used to characterize the morphology of Apts-DNA and Apts-DNA@Ir. The images were analyzed by AFM Nanoscope Software.

1.5 Stability evaluation of Apts-DNA@Ir

Apts-DNA@Ir was incubated in DMEM (containing 10% FBS) or human plasma for 7 days. The hydrodynamic diameter of Apts-DNA@Ir was measured by dynamic light scattering (Malvern Instruments Limited).

1.6 Binding assay of IrPP to Apts-DNA

The interaction mode between IrPP and Apts-DNA was analyzed by measuring the UV-vis and fluorescence spectra of IrPP (25 μ M) with continuous titration of 0.25 μ M Apts-DNA as we previously described³.

1.7 MTT assay

The cell viability of U251, U87, HBM and CHEM-5 cells after treatment with different concentrations of IrPP, AS-DNA@Ir, MUC-DNA@Ir and Apts-DNA@Ir for 72 h was measured by MTT assay⁴.

1.8 Cellular uptake efficacy of Apts-DNA@Ir

The cellular uptake efficacy of Apts-DNA@Ir (4 μ M) in U251 and CHEM-5 cells was quantified by the fluorescence of IrPP with the excitation and emission wavelength at 416 nm and 580 nm respectively.

1.9 Flow cytometric analysis

Flow cytometric analysis was performed to analyze the distribution of cell cycle in U251 and CHEM-5 cells after treatment with different concentrations of IrPP or Apts-DNA@Ir for 48 h⁵.

1.10 Mitotracker & Hoechst staining

Mitotracker & Hoechst staining assay was performed to visualize the DNA fragmentation caused by IrPP and Apts-DNA@Ir at an equivalent concentration of 4 μ M as we previously described³.

1.11 Intracellular trafficking of Apts-DNA@Ir

The localization of Apts-DNA@Ir in U251 cells was measured by cytoskeletal staining. Briefly, U251 cells were seeded at a density of 5×10^4 cells/mL for 24 h. After attachment, 8 µM of IrPP or Apts-DNA@Ir was incubated with U251cells for 12 h. the cytoskeleton was stained with Alexa Fluor 555 (red) and Hoechst 33342 (blue) respectively. At different time points, the green fluorescence of IrPP from Apts-DNA@Ir was observed under a fluorescence microscope (EVOS® FL Auto Imaging System, AMAFD1000).

1.12 Determination of intracellular ROS overproduction

The generation of ROS species including hydroxyl and peroxyl was measured by a fluorogenic dye 2'-7'dichlorofluorescin diacetate (DCFH-DA). The generation of hydroxyl and peroxyl was quantified by the 2'-7'dichlorofluorescein (DCF) with the excitation and emission wavelength at 485 nm and 525 nm respectively. The relative DCF generation was calculated by the following equation.

Relative DCF generation (%) = $\frac{F_{\text{Sample}}}{F_{\text{Control}}} \times 100\%$

F_{Control} is the fluorescence intensity of DCF in control group.

F_{Sample} is the fluorescence intensity of DCF in drug treatment group.

The ${}^{1}O_{2}$ generation of Apts-DNA@Ir was quantified by the probe of 1,3-diphenylisobenzofuran (DPBF), which can react with ${}^{1}O_{2}$ and cause a decrease in the fluorescence intensity of DPBF at the excitation and emission wavelength at 410 nm and 484 nm respectively⁶. The generation of ${}^{1}O_{2}$ was calculated by the following equation.

Relative ¹O₂ generation (%) =
$$\frac{F_{Control} - F_{Sample}}{F_{Control}} \times 100\%$$

F_{Control} is the fluorescence intensity of DPBF in control group.

 $F_{\text{Sample}} \, \text{is the fluorescence intensity of DPBF}$ in drug treatment group.

1.13 Western blot analysis

The expression of MUC-1, nucleolin and VM-associated signals in different cells was detected by Western blot analysis as we previously described⁷.

1.14 In Vitro Cell Migration Assay

Wounding healing assay was conducted to evaluate the anti-metastasis ability of Apts-DNA@Ir as we previously described⁸.

1.15 Statistical analysis

All the experiments were carried out at least for 3 times. All data were conducted at least in triplicate and were expressed as mean \pm SD. The difference between two groups was analyzed by two-tailed Student's t test. Difference with P < 0.05 (*) or P < 0.01(**) was considered statistically significant. Different letters represented significantly different means in three or more groups (P < 0.05, Tukey's test, one-way ANOVA).

References:

- T. F. Chen, Y. A. Liu, W. J. Zheng, J. Liu and Y. S. Wong, *Inorg. Chem.*, 2010, 49, 6366-6368.
- A. S. Walsh, H. Yin, C. M. Erben, M. J. Wood and A. J. Turberfield, ACS Nano, 2011, 5, 5427-5432.
- Y. Y. Huang, W. Huang, L. Chan, B. W. Zhou and T. F. Chen, *Biomaterials*, 2016, 103, 183-196.
- Y. Y. Huang, L. Z. He, W. Liu, C. D. Fan, W. J. Zheng, Y. S. Wong and T. F. Chen, *Biomaterials*, 2013, 34, 7106-7116.
- 5. Y. Huang, L. He, W. Liu, C. Fan, W. Zheng, Y. S. Wong and T. Chen,

Biomaterials, 2013, 34, 7106-7116.

- 6. Z. H. Song, Y. Z. Chang, H. H. Xie, X. F. Yu, P. K. Chu and T. F. Chen, *NPG Asia Mater.*, 2017, **9**, 1-16.
- B. W. Zhou, Y. Y. Huang, F. Yang, W. J. Zheng and T. F. Chen, *Chem. Asian J.*, 2016, **11**, 1008-1019.
- 8. Y. Y. You, H. Hu, L. Z. He and T. F. Chen, *Chem. Asian J.*, 2015, **10**, 2743-2753.

2. Figures



Figure S1. Zeta potential of IrPP, Apts-DNA and Apts-DNA@Ir. Values were expressed as means \pm SD of triplicate.



Figure S2. Hydrodynamic diameter of Apts-DNA@Ir in DMEM (containing 10% FBS) or in human plasma. Values were expressed as means \pm SD of triplicate.



Figure S3. FT-IR spectra of Apts-DNA, IrPP and Apts-DNA@Ir.



Figure S4. Representative images of mitochondrial fragmentation in U251 cells after treatment with Apts-DNA@Ir, as measured by Mitotracker & Hoechst-staining assay. The white arrows indicated the fragmented sites of mitochondria in cells, scale bar = $10 \mu m$.



Figure S5. Intracellular trafficking of IrPP in U251 cells for 12 h. U251 cells were stained with Alexa Fluor 555 (cytoskeleton) and Hoechst 33342 (nucleus) before the treatment of 8 μ M IrPP for 12 h, scale bar = 20 μ m.



Figure S6. Overproduction of ROS in U251 cells caused by Apts-DNA@Ir. (a) ROS overproduction in U251 cells after treatment of IrPP, DNA@Ir, MUC-DNA@Ir, AS-DNA@Ir and Apts-DNA@Ir at 5 μ M. (b) ROS overproduction in U251 cells treated by Apts-DNA@Ir at 2.5, 5.0 or 7.5 μ M. Values were expressed as means \pm SD of triplicate.

Complex	IC ₅₀ (mM)			
	U251	U87	CHEM-5	Safety index ^a
IrPP	3.35	18.9	2.33	0.69
AS-DNA@Ir	3.05	-	4.47	1.47
MUC-DNA@Ir	4.81	-	7.27	1.51
Apts-DNA@Ir	1.68	9.5	7.74	4.61

 Table S1. Safety indicies of IrPP and Apts-DNA@Ir towards different cells.

a: Safety index = $IC_{50}(CHEM-5)/IC_{50}(U251)$