One stop radiotherapeutic targeting of primary and distant osteosarcoma to inhibit cancer progression and metastasis using 2DG-grafted graphene quantum dots

Fu-I Tung1,2, Lu-Jun Zheng3,*, Kai-Ting Hou4,*, Chih-Sheng Chiang4, Ming-Hong Chen5,6,*, Tse-Ying Liu4,*

1. Dr. Fu-I Tung
Department of Orthopaedic Surgery, Taipei City Hospital, Yangming branch
Taipei City 112, Taiwan
E-mail: fui.tung@gmail.com

2. Dr. Fu-I Tung
Department of Health and Welfare, College of City Management, University of Taipei,
Taipei City 112, Taiwan
E-mail: fui.tung@gmail.com

3. MS Lu-Jun Zheng
Department of Medical Imaging, National Taiwan University Hospital,
Taipei, Taipei City 112, Taiwan
E-mail: k12388877@gmail.com

4. Prof. Tse-Ying Liu
Department of Biomedical Engineering, National Yang Ming University,
Taipei City 112, Taiwan
E-mail: tyliu5@ym.edu.tw

5. Ming-Hong Chen
Department of Neurosurgery, Taipei Municipal Wanfang Hospital,
Taipei, Taiwan, ROC.
Email: chen.minghong@gmail.com

6. Ming-Hong Chen
School of Medicine, College of Medicine,
Taipei Medical University, Taipei, Taiwan, ROC.
Email: chen.minghong@gmail.com

7. Ming-Hong Chen
Graduate Institute of Nanomedicine and Medical Engineering, Taipei Medical University, Taipei, Taiwan, ROC
Email: chen.minghong@gmail.com

* the authors contributed equally to this work.

Fig. S1 The optical properties of the GQDs in aqueous solution. (A) UV-vis absorption spectra. (B) Fluorescent spectra under 360 nm laser excitation.

Fig S2. Quantitative analysis of 2DG on the GQD by fluorescamine assay. The calibration curve of 2DG using fluorescamine assay at an emission of 460 nm shows a linear relationship between the fluorescence intensity and the concentration of 2DG.
**Fig. S3** The specific targeting ability of 2DG-g-GQDs was examined by glucose uptake assay. 143B and hFOB 1.19 cells were incubated with 2DG for 24 h before incubation with 2DG-g-GQDs. The slight uptake of 2DG-g-GQDs (green) indicated that introduced of 2DG prior to 2DG-g-GQDs inhibited the cell association. Hoechst (blue), actin (red), 2DG-g-GQD (green). The scale bar is 20 μm.
Fig. S4 Micro PET images of (A) sagittal, (B) coronal, (C) transverse. As shown, in situ osteosarcoma was successfully implanted in (nu/nu) mice.

Fig. S5 In vivo biodistribution of GQDs and 2DG-g-GQDs was semi-quantified in various organs of tumour-bearing nude mice.
Fig. S6 (a) Immunofluorescence staining of γ-H2AX for monitoring DNA double strand break (DSB) in 143B cells. Cells were treated with-without X-ray radiation (6 Gy) in the presence or absence of 2DG-g-GQDs (1 mg mL$^{-1}$). DAPI (blue), γ-H2AX and 2DG-g-GQDs (green). Scale bar = 20 μm. (b) The fluorescence intensity in each nucleus was measured by ImageJ and further analyzed by two-way ANOVA multiple comparison test. In short, the area of nuclei was selected through the color threshold (color space: RGB). The intensity of green channel in the selected area were measured after splitting channels.