Combinational photochemotherapy of organoplatinum(II) metallacage based nanoparticles on liver cancer stem cells

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Supplementary Figure 1. The average hydrophilic diameters of MNPs in PBS and FBS after different time periods of incubation.
Supplementary Figure 2. (A) The ratio of CD133+ subset and CD44+ subset of CD133- cells and CD133+ Huh7 CSCs after MACS sorting. (B) The image of Huh7 CSCs after MACS sorting and followed 3D non-adherent culture with a magnification of 100 times. (C) Fluorescence imaging of Huh7 cells and Huh7 CSCs stained with Nanog, Sox2, Oct4 (green), respectively, to show the expression of stem cell markers.
The cell nuclei were stained by DAPI (blue). The images were magnified 400 times. (D) Comparison of spheroid formation and (E) clone formation between Huh7 cells and Huh7 CSCs. * p<0.05. The image of tumor sphere was magnified 100 times. (F) The apoptosis, migration, living status, ROS production and mitochondria injury of CCLP-1 CSCs received only light irradiation (0.5 W/cm², 1 min). (G) Fluorescence images of CCLP-1 CSCs received different treatment. Dead cells were stained with red color (PI) while alive cells were stained with green color (fluorescein diacetate, FDA).
Supplementary Figure 3. The cell uptake investigation of MNPs in CCLP and CCLP CSCs. Representative photograph of positive cells in CCLP CSCs and CCLP NC groups after incubation with MNPs for 12 and 36 hours. (BF = bright field, the images and Fluorescence images were magnified 100 times).
Supplementary Figure 4. (A) Cytotoxicity comparison of Huh7 CSCs incubated with PNP, cPt or MNP with or without irradiation of 638 nm light (0.5 W/cm², 1 min), respectively. (B) The migration and (C) apoptosis of Huh7 CSCs treated with cPt, PNP, PNP + cPt, MNP with or without irradiation of 638 nm light (0.5 W/cm², 1 min), respectively. The images of migration were magnified 100 times. * p<0.05, ** p<0.01, *** p<0.001. (D) Fluorescence image of Huh7 CSCs treated with cPt, light only or MNP with or without irradiation of 638 nm light (0.5 W/cm², for 10 s, 60 s and 120 s, respectively). Dead cells and alive cells were stained in red and green, respectively.
Supplementary Figure 5. (A) ROS production caused by MNP in Huh7 CSCs. Fluorescence images (Green: DCF fluorescence; blue: DAPI fluorescence) and (B) quantitative determination via FACS in the Huh7 CSCs received different treatment. The fluorescence images were magnified 200 times. * p<0.05, ** p<0.01. (C) The survival rate of Huh7 CSCs received different treatment. (D) Detection of mitochondria
injury caused by MNP in Huh7 CSCs. Fluorescence images reflected compact of mitochondria membrane of the Huh7 CSCs received different treatment. The images were magnified 200 times. (E) The images and (F) Fluorescence images of Huh7 CSCs spheroids treated with MNP only, cPt, light only, and MNP with irradiation of 638 nm light (0.5 W/cm², 1 min). The white arrows denote the dead cancer cells in tumor sphenoid. The images above and Fluorescence images were magnified 200 times while images below were magnified 400 times.
Supplementary Figure 6. Representative photograph of mice bearing the subcutaneous tumors formed by CCLP-1 CSCs received various therapeutic treatment. Scale bar is 2 cm.