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Radiosensitization in Cancer Treatment with Gold nanoparticles through Synergistic Sonoporation

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***Abstract*—We demonstrated synergistic radiosensitization in human liver cancer using gold nanoparticle-encapsulated microbubbles (AuMB) and ultrasound. Microbubbles-mediated sonoporation enhanced cellular uptake of gold nanoparticles (AuNP). Higher cavitation signals were associated with more DNA damages and inferior cancer cell survivals after mega-voltage (MV) X-ray irradiation.**

Keywords—radiosensitization; gold nanoparticle; sonoporation; cavitation.

I. Introduction

Radiotherapy (RT) as one of the powerful anti-cancer modalities, is utilized to manage 40% patient who is cured [1]. However, ionizing irradiation itself not a selective anti-tumor treatment; the main challenge is to increase its therapeutic efficacy without increasing injuries dealt with the surrounding healthy tissues. Numerous research programs have focused on combining gold nanoparticle (AuNP) with RT. Through its unique physiochemical properties, AuNP has widely used as a radiosensitizer in the research field, and promising results with *in vitro* and *in vivo* application have been reported [2,3]. The main mechanism supports radiosensitization is the enhanced photoelectrical and Compton effects, which leads to subsequent emissions of secondary electrons deposit dose in local regions and generate more damages on DNA double strands. However, the dose enhancement of AuNP is most prominent with kilovoltage (kV) radiation therapy, which is inherent with shallow penetration. Megavoltage (MV) X-ray, in contrast to kV X-ray, is essential to provide skin-sparing and adequate dose deposition to central tumors in clinical situations. There are some appealing demonstrations of AuNP-mediated radiosensitization with clinically utilized MV energies. Nevertheless, those promising results rely on the high systemic concentration of AuNP [4], or further modification of AuNP with specific targeting moieties such as Trastuzumab [5], glucose [6] or goserelin [7] for a specific tumor type. Investigation on AuNP-mediated radiosensitization with MV X-ray under more generalized condition is warranted. In the present study, human hepatocellular carcinoma, to which radiotherapeutic effects are typically unsatisfactory [8], is selected to assess the radiosensitization of gold nanoparticle with MV X-ray.

Sonoporation is the use of sound, typically ultrasonic frequencies, to modify the permeability of the cell plasma membrane and has been exploited to enhance intercellular delivery of therapeutic materials or genes [9]. The principle of successful sonoporation is cavitation. Cavitation is the process of microbubble formation, resonance, and destruction, during which the destruction of microbubble under acoustic filed would disrupt both the endothelial wall and tumor cell membrane. In our previous works, it has been successfully demonstrated that delivery of or gene therapy [9] and AuNP [10,11] could be greatly enhanced by combining microbubbles and sonoporation, which employs acoustic cavitation on MB. We hypothesize that sonoporation with AuMB) will augment the delivery of AuNP and potentiate radiosensitization in MV X-ray.

II. Experimental Setup

Production and characterization of AuNP and AuMB

The AuNR was synthesized by the electrochemical conversion of an anodic gold material into particles within an electrolytic cosurfactant system. The synthesized AuNR was with an average aspect ratio of four— 10 nm in diameter and a length of 40 nm. The peak of optical absorption was 814 nm.

AuMB was produced through the emulsion method as followed. A digital sonicator is utilized to sonicate the pre-mixed solution—which contained 4% (w/v) HSA human serum albumin (HSA; Octapharma, Vienna, Austria), 3.6 nM AuNPs, phosphate-buffered saline (PBS) and C_3F_8 gas—for 3 minutes. The concentration and size distribution of produced AuMB was measured by cell counters/analyzer (Coulter MultiSizer III, Beckman Coulter, USA). AuMB with a median size ranging from 1-3 μm was selected by varying the setup of centrifugation.

System setup

The experiment was performed with Huh7 human hepatocellular carcinoma obtained from JCRB cell bank (Okayama, Japan), which is inherently radioresistant. The measurement setup is shown in Figure 1. A cylindrical cavity 5

mm in diameter and 2 cm deep was made in an agarose phantom (2% concentration) as a container for the AuMB solution. A 1-MHz cylindrical focused transducer (V303, Panametrics-NDT) was used to induce cavitation, and an unfocused 10-MHz transducer (V312, Panametrics-NDT) was used to receive signals from the AuMB during ultrasound stimulation. The two transducers were positioned perpendicular to each other, and both are focused at the cavity. The –6-dB focal zone of the 1-MHz transducer covers the entire cavity. In this study, the total sonoporation time was 5 minutes, with a pulse repetition frequency of 100 Hz. The pulse duration was 30 μs in the high-intensity arm (H_US) or 10 μs for low-intensity arm (L_US).

Cavitation measurements

The inertial cavitation was measured as inertial cavitation dose (ICD). The broadband signals received by the 10-MHz transducer were quantified for the root-mean-square (RMS) value of the spectrum between 9.5MHz and 10.5MHz. A plot of the RMS amplitude as a function of time represents the ICD. Finally, the background (the measured RMS amplitudes from PBS alone) was subtracted, and the resulting amplitude was referred to as the differential ICD (dICD). In this study, the dICD was calculated from an average of 2-second stimulation (200 pulses).

Irradiation of cells

Two hours after sonoporation, Huh7 cells in culture flasks were irradiated with different doses of radiation (0 — 10 Gy), using a Synergy Elekta 6-MV photon linear accelerator (Elekta AB, Inc, Stockholm, Sweden). The irradiated cells were surrounded with water equivalent bolus that could bring scattered photons of lower energy for full AuNR-induced effect.

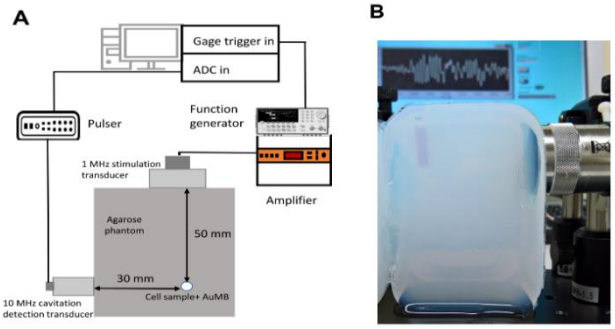


Fig.1 System setup of ultrasound treatment and cavitation measurement (A) Illustration of in vitro treatment and ICD measurement system. (B) water equivalent agarose phantom with pre-specified cell well.

Colony formation assay

Cell suspension treated with AuMB-sonoporation were diluted and seeded in six-well plates with an appropriate number of cells. After an interval of 120 minutes, the cells were irradiated with different doses of radiation (0 Gy–10 Gy).

γ-H2AX immunofluorescence microscopy

After irradiation of 4 Gy, cells were incubated for one hours, washed and fixed, permeabilized, incubated with the antibody (fluorescein isothiocyanate [FITC] conjugated anti-phospho-histone γ-H2AX [Ser139; 1:1500; Millipore, Billerica, MA, USA]) and mounted. In each sample, γ-H2AX foci were counted per nucleus using a Zeiss Axio Imager A1 fluorescence microscope at high magnification; an average number of γ-H2AX foci for at least 50 nuclei was calculated. The average number of γ-H2AX foci per nucleus was the total number of double-strand breaks per nucleus.

III. Results

The median size of AuMB was 1.61 μm with a concentration of 3.0×10^8 /ml. The dCID measured was measured and calculated every 30 seconds for the 5-minute ultrasound stimulation. The dICD was apparently higher with high-intensity ultrasound compared to that with low-intensity ultrasound through the whole course (Fig. 2).

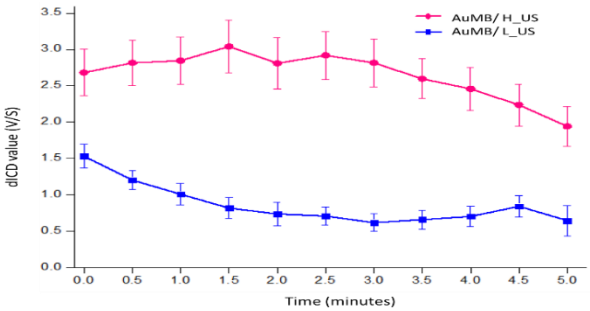
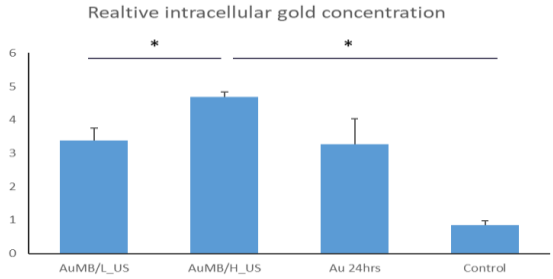


Fig. 1 dICD values measured during 5-minutes sonoporation with high-intensity (pulse duration: 30 μ s) vs. low intensity (10 μ s) acoustic stimulation

To assess the efficiency of sonoporation, we additionally



incubated Huh7 cells with 3.6 nM AuNP in regular medium for 24 hours as a comparative arm. The intracellular uptake of AuNP was assessed via inductively coupled mass spectrometry (ICP-MS). Huh7 cell solution after sonoporation was dissolved with aqua regia for 4 hours and then quantified by ICP-MS. Sonoporation with longer pulse-duration (H US) was associated with significantly higher cellular gold uptake compared to short-pulse acoustic stimulation (Fig. 3). Notably, the intracellular concentration of gold via 5-minute sonoporation was comparable, if not higher than, with 24-hours incubation of AuNP

Fig.3 Quantitative analysis of intracellular uptake of gold nanoparticles by inductively coupled plasma mass-spectroscopy. High-intensity acoustic sonoporation (pulse duration: 30 μ s) was associated with significantly higher intracellular compared to low intensity (10 μ s) acoustic stimulation and negative control. Each column represents the mean ± SD from three independent experiments. * $P < 0.05$.

After ultrasound stimulation with AuMB, cells were plated and irradiated. After 10 days of incubation, the clonogenic assay was performed. Fig. 4 showed Huh7 cell survival decreased in a dose-dependent manner with irradiation (2–10 Gy) Five-minute

AuMB sonoporation significantly enhanced the radiation-induced reduction in cell survival at a high dose of irradiation (>6 Gy)

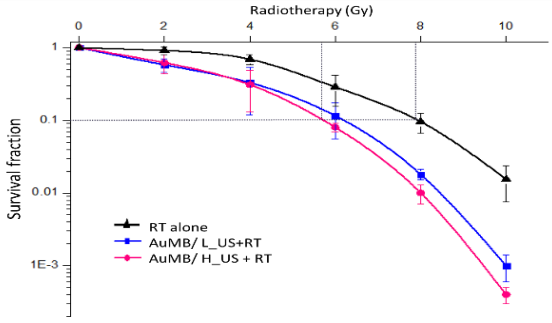


Fig.4 The number of colonies in each well containing more than50 cells was counted for the quantitative results of the clonogenic assays. Points: mean (n = 3); Bars: standard deviation

We then investigated whether the increased uptake of AuNP via sonoporation modulated radiosensitization through increased DNA damages. The assessment of double-stranded DNA breaks was quantified via calculating the number of γ-phosphorylated histone H2AX (γ-H2AX), 1 hour after irradiation of 4 Gy. Radiation of 8 Gy was served as the positive control. Fig. 5 demonstrates that pre-irradiation treatment by AuMB sonoporation produced significantly more immunofluorescence foci compared to RT of 4 Gy alone at the time point of 1 hour after radiation. The number of foci in high-intensity ultrasound arm was comparable with 8 Gy irradiation.

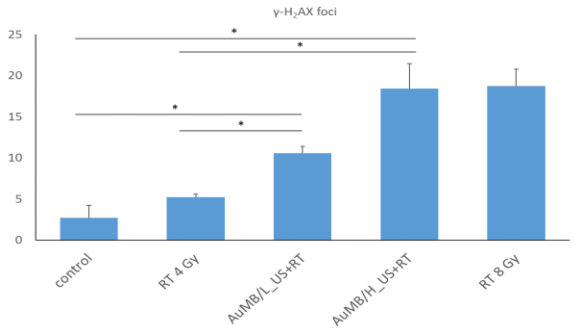


Fig. 5 The number of γ -H2AX foci counted in 50 cells 1 hour and 16 hours after the treatment. Data presented are the mean number of foci per cell in each group. Points: mean (n = 3); Bars: standard deviation

IV. Discussion

This study demonstrated that sonoporation with AuNP-encapsulated microbubble achieved megavoltage radiosensitization through increasing intracellular concentration of AuNP. The accumulation of AuNP in Huh7 cells increases the yield of short-range secondary electrons generated after irradiation and enhances the DNA double-strand breaks damages. We derived the dose modifying ratio (DMR) as following for quantification of radiosensitizing power of AuNR.

$$DMR_{x\%} = \frac{D_{without\ AuNP}}{D_{AuNP}} \quad \text{(for x\% survival)}$$

In our colony formation assay, we obtained a DMR of 1.38., which is comparable with literature [2,7,12,13]. Notably, previous researches immerse and incubate the cells with AuNP, whether with tumor-targeted modifications or not, for 24 hours before MV irradiation. By contrast, we achieved similar results with 5-minutes of sonoporation before irradiation. This strategy could advance GNP-enhanced radiation therapy from their current pre-clinical setting to clinical trials and eventual routine usage.

The concentration of intracellular cellular gold and γ -H2AX foci after irradiation was significantly increased with sonoporation by higher intensity ultrasound (longer pulse period). The clonogenic data also confirmed the worse survival by combining AuMB/high-intensity ultrasound and irradiation. Therefore, the dICD obtained during sonoporation could serve as a treatment predictor. Further *in vivo* experiment is warranted.

V. Conclusion

Sonoporation with AuNP-encapsulated microbubbles enhances gold internalization within cancer cells and promotes effective radiosensitization in human liver cancer cells with megavoltage radiation.

VI. References

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