HSA templated self-generation of gold nanoparticles for tumor vaccines delivery and combinational therapy

Dan Zhang [a], Pengran Liu [b], Xianya Qin [c], Cheng Lu [a], Fuqian Wang [a], Xin Xiong [a], Chuanqi Huang [a], and Zhiping Zhang [c]*

[a]Department of Pharmacy, Wuhan No. 1 Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, China

[b]Department of Orthopedics, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, 430022, China.

[c]Tongji School of Pharmacy, Huazhong University of Science and Technology, Wuhan 430030, China

E-mail: zhipingzhang@mail.hust.edu.cn

Materials and methods

1.1 Materials

Auric chloride trihydrate ($HAuCl_4 \cdot 3H_2O$, 99.9%) and human serum albumin (HSA) were purchased from Sigma Aldrich (St. Louis, MO, USA). $Hgp100_{25\text{-}33}$ (KVPRNQDWL) and cysteine-terminated hgp $100_{25\text{-}33}$ (KVPRNQDWLC, hgp100-SH) were synthesized by Bioyears gene biotechnology Co., Ltd (Wuhan, China). 6-Diamidino-2-phenylindole (DAPI) was obtained from Nanjing KeyGen Biotech. Inc., China. Prodium Iodide (PI) was purchased from Invitrogen Corporation, USA.

1.2 Cell line and cell culture

B16F10 cells (purchased from cell bank, shanghai) were cultured in Dulbecco's Modified Eagle Medium containing 10% fetal bovine serum, 100 $IU \cdot mL^{-1}$ of penicillin and 100 $\mu g \cdot mL^{-1}$ of streptomycin at a humidified atmosphere incubator with 5% CO_2 at 37 °C.

1.3 Animals

Six to eight weeks of C57BL/6 mice were purchased from the Experimental Animal Center of Hubei provincial academy of preventive medicine, China. All mice were raised under specific pathogen-free (SPF) condition in the Animal Center of

Huazhong University of Science and Technology, China. All animal procedures were conducted in compliance with the regulations of Chinese law and the local Ethical Committee Quantita. All the animal studies were approved by the Institutional Animal Care and Use Committee at Tongji Medical College, HUST, China.

1.4 Preparation of HSA-H NP, g-HSA-H NP and HSA-H@AuNP

The preparation of HSA-H NP was conducted according to the reported method with minor modification [1-4]. Typically, 20 mg HSA was dissolved in 2 mL deionized water with pH adjusted to 8.3. 2 mg hgp100 was then suspended in the HSA solution and stirred at room temperature for 30 min. Then ethanol was added into the suspension dropwise at the speed of 0.05 $mL \cdot min^{-1}$. The supernatant was continually stirring overnight to volatilize the ethanol. The acquired solution was firstly centrifuged at 2000 g for 10 min and then the supernatant was centrifuged at 13000 g for 20 min to pellet the HSA-H NP. The acquired nanoparticles were washed twice with PBS.

As for the preparation of g-HSA-H NP, 8 μL 0.25% glutaraldehyde was added to the HSA-H NP and stirred at 4 ^{o}C overnight. To purify the g-HSA-H NP, centrifugation was repeated for twice at 13000 g for 20 min.

As for the preparation of HSA-H@AuNP, 50 mM $HAuCl_4$ was added to the HSA-H NP after addition of 2.5 mM ascorbic acid and incubated at 37 ^{o}C overnight. To purify the HSA-H@AuNP, centrifugation was repeated for twice at 13000 g for 10 min.

1.5 Characterization of HSA-H@AuNP

The morphology of the nanoparticles was observed by TEM (Tecnai G2 20 TWIN, USA) at an accelerating voltage of 100 keV. The hydrodynamic diameter and zeta potential of nanoparticles suspended in 1×PBS were measured by dynamic light scattering (DLS) (Zeta Plus, Brookhaven Instruments, USA).

1.6 Photothermal profiles of HSA-H@AuNP

A series of 1 mL of HSA-H@AuNP solution were placed in 1.5 mL tubes, irradiated with NIR (808 nm, 2.0 $W \cdot cm^{-2}$) at different time point of 0, 1, 2, 3, 4 and 5 min, and photographed with an IR thermal camera (FLIR System E40, Boston, MA, USA).

1.7 Drug loading and cumulative release of hgp100 in HSA-H@AuNP

To detect the drug loading of hgp100 in HSA-H@AuNP, the nanoparticles were disintegrated with acetonitrile. Hgp100-SH was measured by high performance liquid chromatography (HPLC, Hitachi HPLC system, JAPAN). A C18 column (4.6×250 mm, 5 μm, Kromasil) was used for sample separation. The measurement was performed at a constant flow rate of 1 mL/min at room temperature and the absorption of hgp-SH was record at 220 nm. The cumulative release of hgp100 from HSA-H@AuNP was conducted in pH 7.4 PBS exploiting an orbital shaker (37 °C, 100 rpm). The HSA-H@AuNP + NIR group was irradiated by an 808 nm laser illumination with power of 2 $W \cdot cm^{-2}$ at 0 min. The released drug was collected by centrifugation at different time points, and fresh medium was supplemented for continuous release. The hgp100 concentration was then determined using HPLC.

1.8 Cellular uptake of HSA-H@AuNP

The internalization of nanoparticles was evaluated using confocal laser scanning microscope (710META, Zeiss, Germany) and flow cytometer (Accuri C6, BD, USA). 3×10^5 B16F10 cells were seeded in 6-well plates and cultured to 80% confluency. The culture medium was then replaced by fluorescence labeled free hgp100, HSA-H NP and HSA-H@AuNP suspended in fresh medium and continuously cultured in conditioner. The cells of different time points were washed with PBS twice and fixed with 4% paraformaldehyde for 15 min followed by staining with DAPI for 5 min, and then observed by confocal microscope.

1.9 Intracellular retention of HSA-H@AuNP

The internalization of nanoparticles was evaluated using confocal laser scanning microscope (710META, Zeiss, Germany). $1x10^5$ DCs were seeded in 24-well plates and cultured to 80%. The culture medium was then replaced by FITC labeled free hgp100, HSA-H NP and HSA-H@AuNP suspended in fresh medium and continuously cultured in conditioner for 6 h. Then the culture medium was discarded and rinsed by PBS twice. 20 μM DiI was utilized to label the cell membrane. The cells were continuously cultured with complete medium for 72 h and the retention of nanoparticles of different time points were observed by confocal microscope.

1.10 Therapeutic effect on tumor

5×10^4 B16F10 cells were subcutaneously injected in the left flank of female C57BL/6 mice. When tumors grow up to 4-6 mm, tumor-bearing mice were divided into six groups randomly. PBS, free hgp100, HSA@AuNP and HSA-H@AuNP with or without NIR were administered at day 0 and day 7. Then the tumor was exposed to an 808 nm NIR laser at 2.0 $W\cdot cm^{-2}$ for 1 min at 12 h post each injection. The tumor size was measured every other day. Mice were sacrificed on day 12 and tumors were investigated through hematoxylin and eosin (H&E) staining and TUNEL assay. The tumor volume was calculated according to the formula, $width^2\times length\times0.5$.

1.11 Immunization assay

C57BL/6 mice were administered with various formulations three times as described above. At 48 h after the last administration, the lymph nodes, spleen and tumors were collected and analyzed with a flow cytometer (LSR II, BD, USA). The acquired lymphocytes were stained with anti-CD11c-PE and anti-CD86-FITC for DC maturation and anti-CD3e-Cy5.5, anti-CD8a-FITC and anti-CD4-PE antibodies for T cell analysis.

1.12 Statistical analysis.

Data were represented as mean ± standard deviation (SD). Comparisons among groups were performed using one-way or two-way ANOVA analysis. The difference was considered statistically significant when $p < 0.05$.

Supporting information

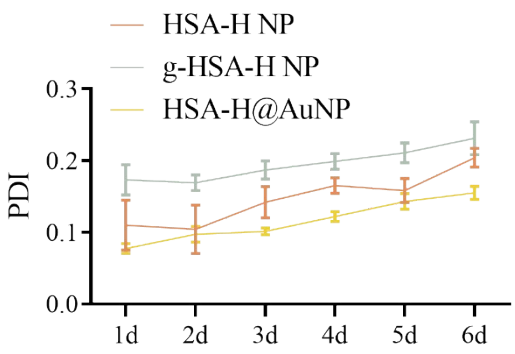

Figure S1. Polydispersity index (PDI) of nanoparticles.

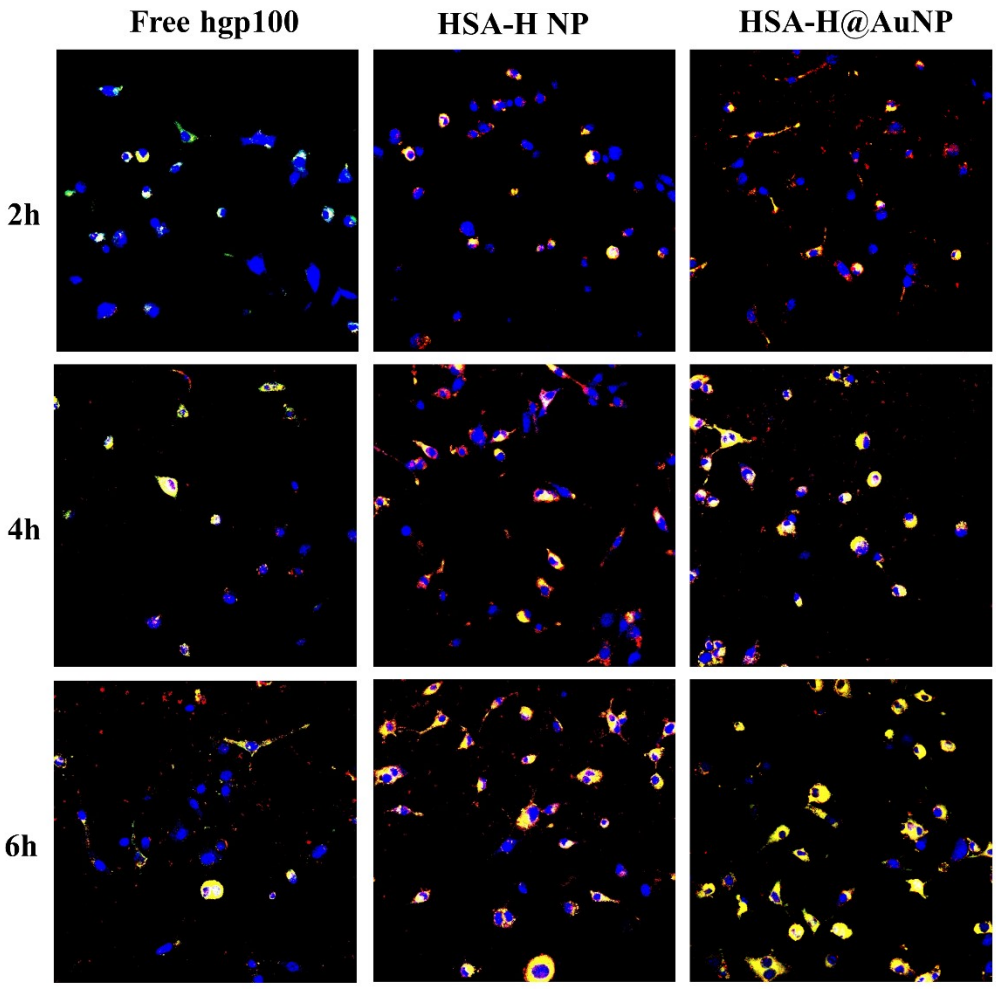

Figure S2. Merged images of internalization of the nanoparticles in B16F10 cells. Blue, DAPI labeled cell nucleus, Red, DiI labeled cell membrane, Green, FITC labeled peptides.

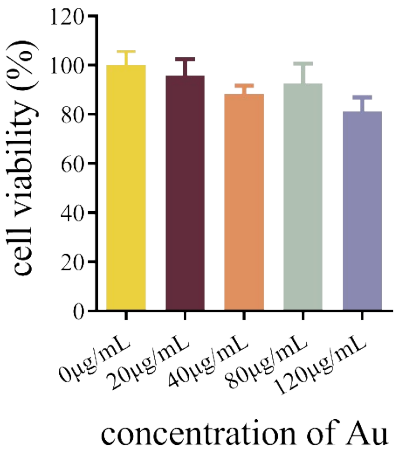

Figure S3. Cell viability of DC2.4 cells treated with HSA-H@AuNP with different

concentration of Au.

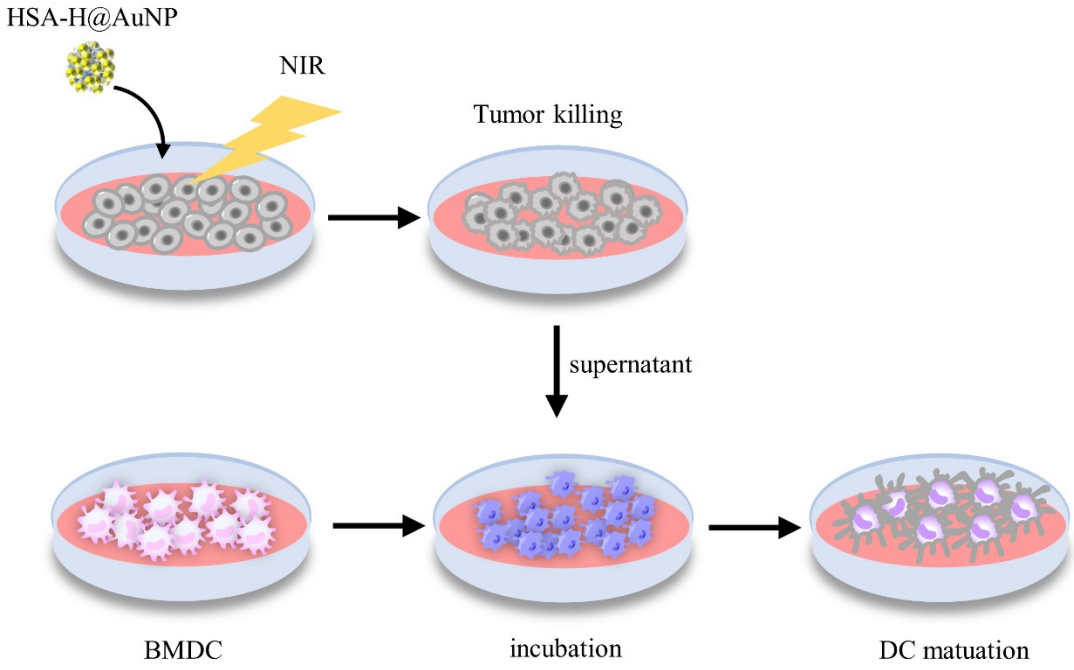

Figure S4. Scheme of pulsing DCs *in vitro* with different formulations treated tumor cells.

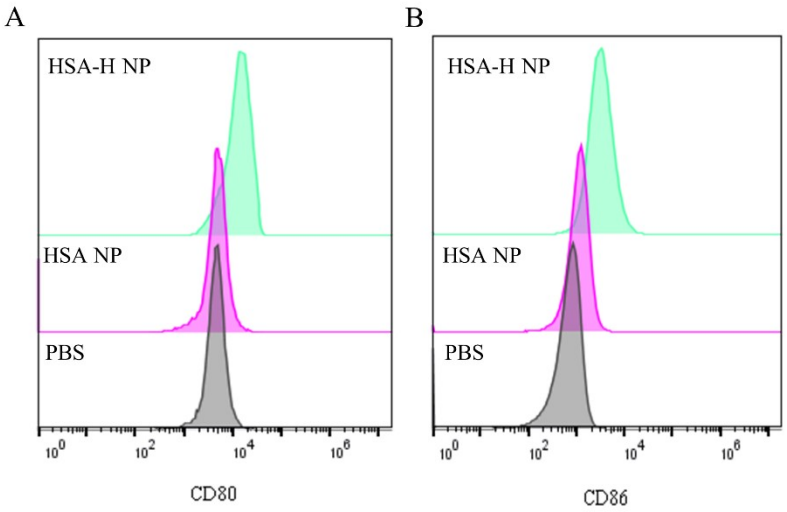

Figure S5. Expression of CD80 and CD86 on DCs treated with HSA NP and HSA-H NP.

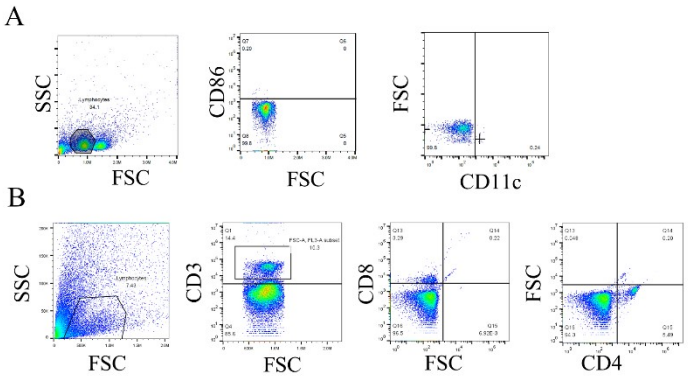

Figure S6. Gating strategy. Gating method for flow cytometry analysis of A) APC-CD11c and FITC-CD86, B) PE-Cy5.5-CD3, PE-CD4 and FITC-CD8. Data were analyzed by Flowjo Version10.7.2.

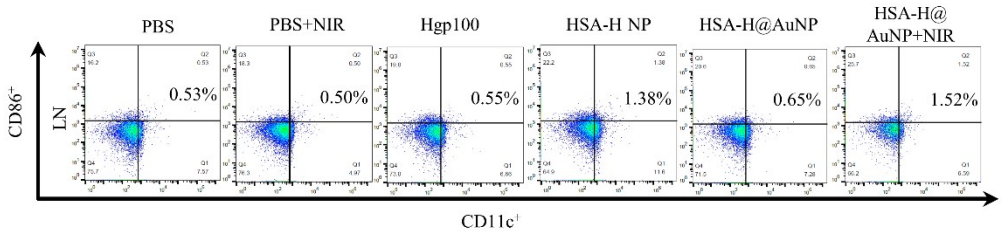

Figure S7. The maturation ($CD86^+CD11c^+$) of DCs in LNs.

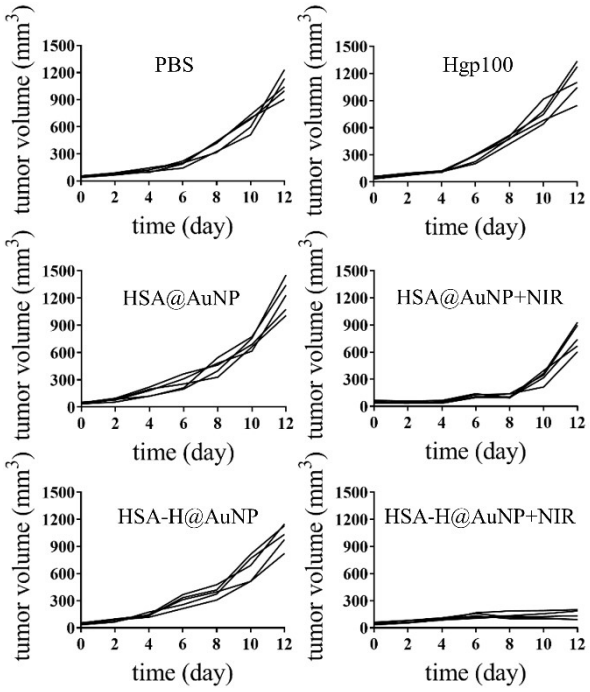

Figure S8. Body weight of mice with different treatments (n=5).

References

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2. N. Qu, Y. Sun, Y. Li, F. Hao, P. Qiu, L. Teng, J. Xie and Y. Gao, *Biomed Eng*, 2019, **18**, 11.
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