Functional Blockage of Cancer-associated Fibroblast with Ultrafine Gold Nanomaterials Brought Unprecedented Bystander Anti-tumoral Effect

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1. Materials and methods

Synthesis of 3-nm GNPs

GNPs (3-nm) were synthesized by the DMSA reduction of $HAuCl_4$. Briefly, a 500-mL beaker was cleaned in aqua regia and rinsed with DDI water. Next, 72 mg of DMSA was fully dissolved in 180 mL of DDI water, and then 20 mL of 0.01 M $HAuCl_4$ solution (0.08 g in $HAuCl_4 \cdot 3H_2O$ in 20 mL of DDI water) was added and stirred for approximately 10 minutes. Finally, the solution was placed in a dialysis bag overnight and freeze-dried.

Synthesis of 15-nm, 30-nm, 50-nm and 80-nm GNPs

GNPs (15-nm, 30-nm, 50-nm and 80-nm) were synthesized by the citrate reduction of $HAuCl_4$. The amount of $HAuCl_4$ solution and citrate solution determines the size of the nanoparticles (Table S1). $HAuCl_4$ solution was heated to boiling while stirring in a three-necked flask. Next, a small amount of trisodium citrate solution was quickly added to the auric solution. After the color changed from yellow to black, heating was continued heating for 10 minutes. Finally, heating was stopped and the solution was cooled under ambient conditions.

Table.S1 Approximate amount of $HAuCl_4$ and citrate and the corresponding sizes of nanoparticles

GNP size	$HAuCl_4$ amount	Citrate amount
15 nm	**1 mM, 100 mL**	**38.8 mM, 10 mL**

30 nm	**0.25 mM, 50 mL**	**0.01 g/mL, 350 μL**
50 nm	**0.25 mM, 50 mL**	**0.01 g/mL, 260 μL**
80 nm	**0.25 mM, 50 mL**	**0.01 g/mL, 210 μL**

Real-time PCR (qPCR)

Total RNA was isolated from untreated CAFs/GNPs-treated CAFs and NFs using the RNeasy Plus Mini Kit (QIAGEN) according to manufacturer's protocol. Isolated RNA was then reverse transcribed using the RevertAid First Strand cDNA Synthesis Kit (Thermo), followed by real-time PCR (qPCR) using FastStart Universal SYBR Green Master (Rox) (Servicebio) and gene-specific primers. The details of the primers used are provided in Supplementary Table S2.

Table.S2 List of qPCR primers used in this study

Gene name	Primer sequence
GAPDH-FW	GGAGCGAGATCCCTCCAAAAT
GAPDH-RV	GGCTGTTGTCATACTTCTCATGG
Vimentin-FW	GACGCCATCAACACCGAGTT
Vimentin-RV	CTTTGTCGTTGGTTAGCTGGT
N-cadherin-FW	TCAGGCGTCTGTAGAGGCTT
N-cadherin-RV	ATGCACATCCTTCGATAAGACTG
FSP-1-FW	GATGAGCAACTTGGACAGCAA
FSP-1-RV	CTGGGCTGCTTATCTGGGAAG
α-SMA-FW	AAAAGACAGCTACGTGGGTGA

α-SMA-RV	GCCATGTTCTATCGGGTACTTC

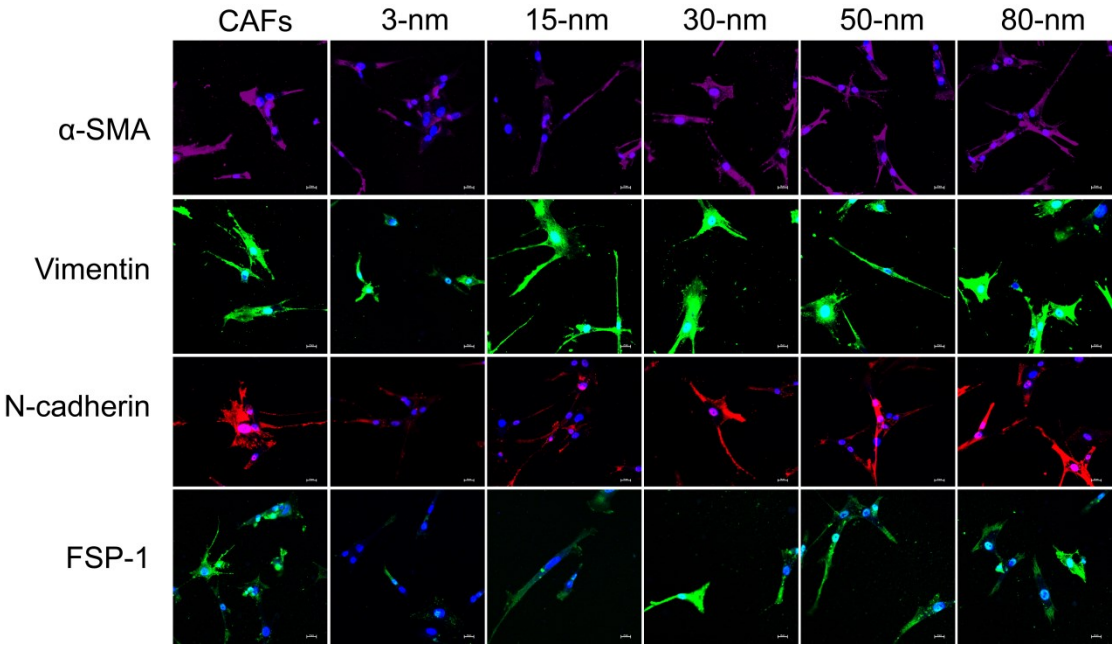

Figure.S1 Protein expression level of N-cadherin, Vimentin, α-SMA, and FSP-1 in CAFs treated with 15 μg/mL of GNPs as detected by immunofluorescence.

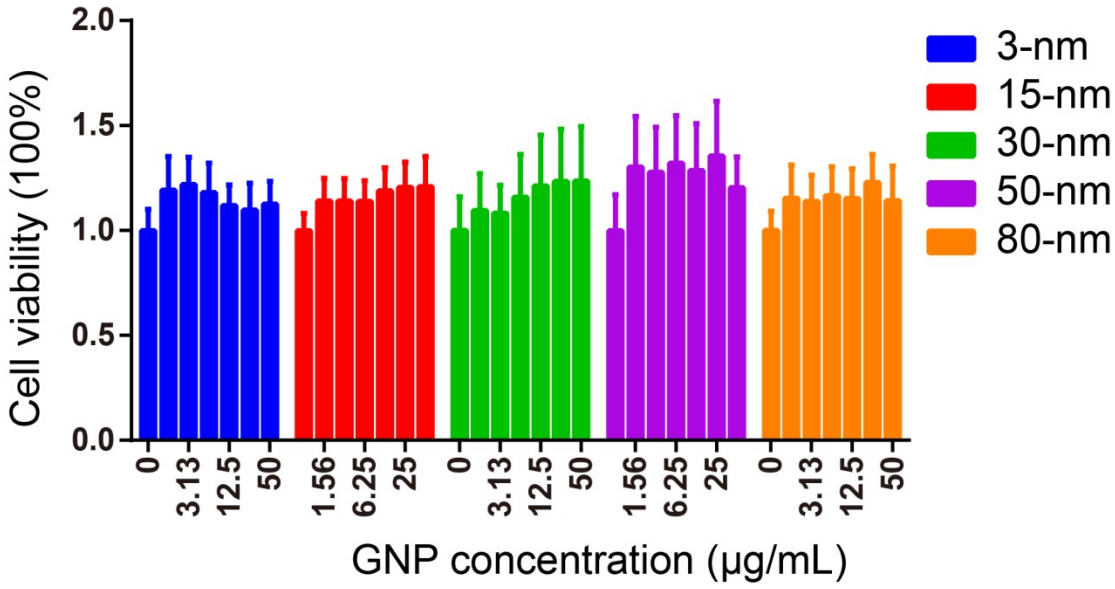

Fig.S2 The CCK-8 results showed no significantly effect of the GNP concentration from 1.56 μg/mL to 50 μg/mL on the proliferation of OSCC tumor cells.

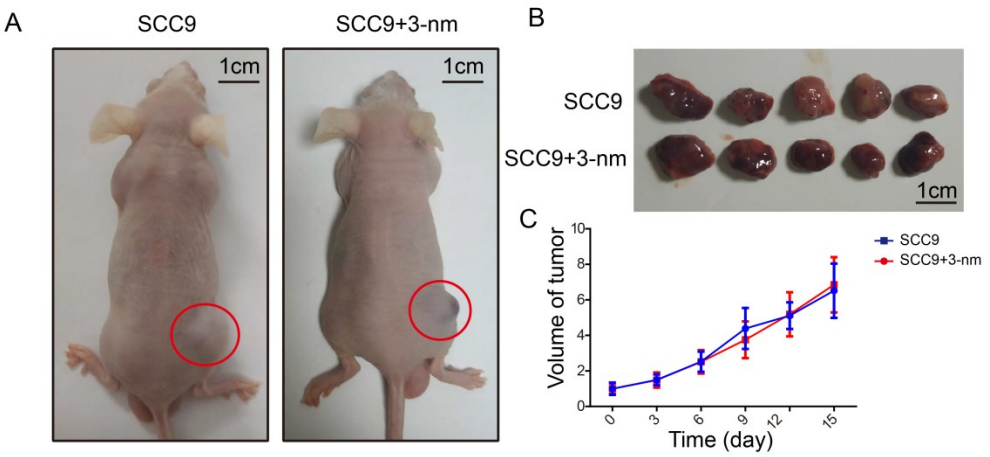

Fig.S3 GNPs at 3-nm did not inhibit the proliferation of OSCC tumor cells *in vivo*.