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Inhibition effects of gold nanoparticles on proliferation and migration in hepatic carcinoma-conditioned HUVECs

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ABSTRACT

Tumor angiogenesis is a complicated process based upon a sequence of interactions between tumor and vessel endothelial cells. Tumor conditioned medium has been widely used to stimulate endothelial cells in vitro angiogenesis. This work was aimed to investigate the effects of gold nanoparticles (GNPs) on angiogenesis in hepatic carcinoma-conditioned endothelial cells. Human umbilical vein endothelial cells (HUVECs) were cultured with conditioned medium (CM) from the human hepatocarcinoma cell line HepG2 (HepG2-CM), and then treated with different concentrations of GNPs. The effects of GNPs on the viability, migration and active VEGF level of HUVECs were investigated by MTT assay, wound healing assay and transwell chamber assay, and ELISA assay, respectively. The data showed that GNPs significantly inhibited HUVECs proliferation and migration induced by HepG2-CM, and also reduced the levels of active VEGF in the co-culture system. Then, the alterations in morphology and ultrastructure of HUVECs detected by atomic force microscopy (AFM) showed that there appeared obvious pseudopodia, larger membrane particle sizes and much rougher surface in HUVECs after HepG2-CM treatment, which were all reversed after GNPs treatment. Changes in cytoskeleton of HUVECs determined by immunocytochemistry demonstrated that GNPs treatment remarkably inhibited the activation effect of HepG2-CM on HUVECs, which was associated with the disruption of actin filaments induced by GNPs. This study indicates that GNPs can significantly inhibit HepG2-CM activated endothelial cell proliferation and migration through down-regulation of VEGF activity and disruption of cell morphology, revealing the potential applications of GNPs as antiangiogenic agent for the treatment of hepatic carcinoma.

Tumor angiogenesis plays vital roles in tumor progression and cancer metastatic process, which is a complex process based upon a sequence of interactions between tumor cells and endothelial cells (ECs).[1] Previous studies have showed that tumor conditioned medium (CM) played an important role in tumor angiogenesis, which can not only promote the mitogenic and anti-apoptotic activity of ECs,[2] but also induce angiogenesis of ECs, such as their migration and tube formation.[3,4] CM is rich in varieties of pro-angiogenic factors, such as vascular endothelial growth factor (VEGF), basic fibroblast growth factors (bFGF), platelet derived growth factor (PDGF), epidermal growth factor (EGF), and so on.[5] Among all these pro-angiogenic factors, VEGF is considered to play the most critical role in tumor angiogenesis.[6]

Currently, the research on nanomaterials in biomedicine is rapidly emerged and widely developed, especially the applications of gold nanoparticles (GNPs), which have been shown to be particularly attractive.[7] With small particle size, good biocompatibility and low cytotoxicity, GNPs have exhibited good prospects for further biological applications.[8] Most importantly, GNPs have been reported to possess the potential potency of antiangiogenic by interacting with the heparin-binding domain of VEGF and bFGF.[9] And, in their work, they verified gold nanoparticles inhibited anti-angiogenic effects induced by VEGF,[9] while, we proved that gold nanoparticles inhibited antiangiogenic effects induced by tumor-conditioned medium (HepG2-CM). Our previous studies have demonstrated that GNPs can inhibit angiogenesis of hepatocellular carcinoma xenografts in nude mice,[10] but the effect of GNPs on hepatic carcinoma conditioned endothelial cells in vitro angiogenesis is still unknown. The current study was designed to investigate the effect of GNPs on angiogenesis of human umbilical vein endothelial cells (HUVECs) induced by hepatic carcinoma-conditioned (HepG2-CM). Atomic force microscopy (AFM) has been proved to be a powerful, quantitative tool for nanoscale imaging of cell surfaces. As a novel nanotechnology tool, AFM can provide the surface topography of cell at the nanometric scale, which allows us to better understand the biophysical functions of the cells in their physiological environment.[11,12]

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In this work, HUVECs were co-cultured with HepG2-CM to imitate the microenvironment of ECs in tumor angiogenesis process, and intervened by different concentrations of GNPs. We detected the abilities of cell proliferation and migration by MTT, wound healing assay and transwell chamber assay, as well as the levels of active VEGF in co-culture system was detected by ELISA assay. In addition, we also imaged the ultratructure of HUVECs surface and the actin filaments in cytoskeleton using AFM and LSCM, aiming at further exploring the probable mechanism of GNPs in tumor angiogenesis.

The GNPs were prepared and characterized as follows:[13] in a typical experiment, 50 ml of aqueous solution contain 0.01 mol/l chloroauric acid 5 ml, 1% sodium citrate 10 ml was added to the system after heating and stirring to boiling, then kept heating and vigorous stirring until the solution reached a wine red color. GNPs thus formed were filtered through a 0.22 μm filter and used for experiments. Finally, they were characterized by UV–vis Absorption Spectroscopy (Perkin–Elmer, USA) and Transmission Electron Microscopy (TEM, Philips, Holland). The prepared GNPs with wine red color showed a peak in the region of 521 nm in the UV–visible spectrum (Fig. 1A). TEM images showed the GNPs particles were spherical and monodisperse, the mean diameter was about 15 nm (Fig. 1B).

To examine the optimal concentration of HepG2-CM to stimulate HUVECs, the viability of cells was measured by MTT assay. As shown in Figure 2A, the proliferation rates of HUVECs were 8.63 ± 0.88%, 15.83 ± 1.43%, 24.82 ± 2.19%, 35.53 ± 0.78%, 36.74 ± 1.22% for 6.25%, 12.5%, 25%, 50% and 100% HepG2-CM treatment, respectively, implying that HepG2-CM treatment could significantly promote the proliferation of HUVECs. We found that when HepG2-CM concentration reached up to 50% (V/V), the promotion effect tended to be stabilized, so we selected 50% (V/V) of HepG2-CM for stimulating HUVECs in the following experiments, and detected the inhibition effect of GNPs to HepG2-conditioned HUVECs viability. Figure 2B showed the inhibition effect of GNPs on the proliferation of HUVECs induced by HepG2-CM. After treated with different concentration of GNPs (1, 2 and 4 nmol/L), the inhibition rates were increased to 9.29 ± 0.52%, 16.50 ± 2.07% and 22.71 ± 1.38% when co-culture with HepG2-CM, while no difference in control group without HepG2-CM. It showed that GNPs could inhibit HUVECs proliferation induced by HepG2-CM significantly, but could not inhitbit alone. In other word, GNPs in our experiment were non-cytotoxic to HUVECs, which were proved in our previous work.[14] As a result, the proliferation of HUVECs could be promoted by HepG2-CM, which was consistent with others work,[15] but GNPs could suppress this tendency significantly.

The effect of GNPs on the cell cycle of HUVECs induced by HepG2-CM was determined by flow cytometry (BD Inc, USA) through PI staining, and the results were presented in Figure 3. The percentage of HUVECs in S phase was 20.6% for control group, which increased to 35.5% after the exposure of HepG2-CM. But the percentage of HUVECs in S phase turned to be 20.6% and 18.2%

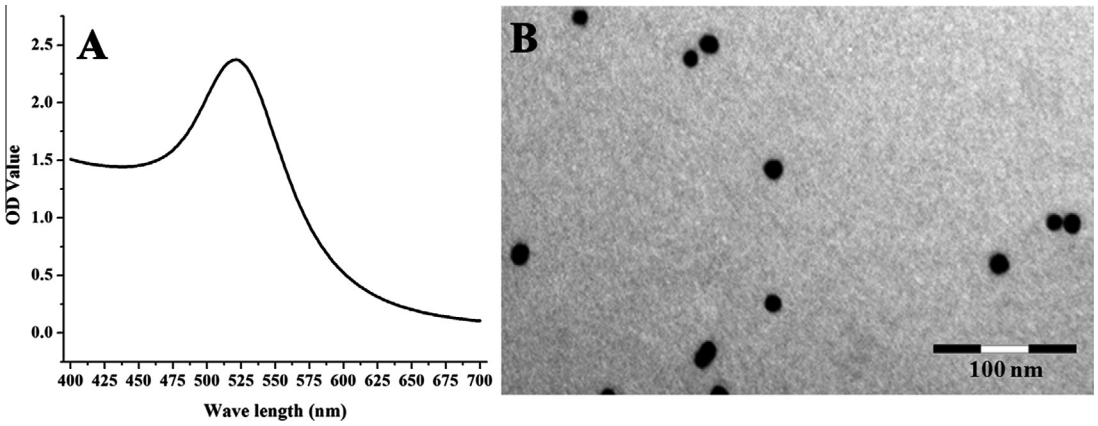

Figure 1. Characterization of GNPs we prepared. (A) UV–visible spectrum and (B) TEM image of GNPs.

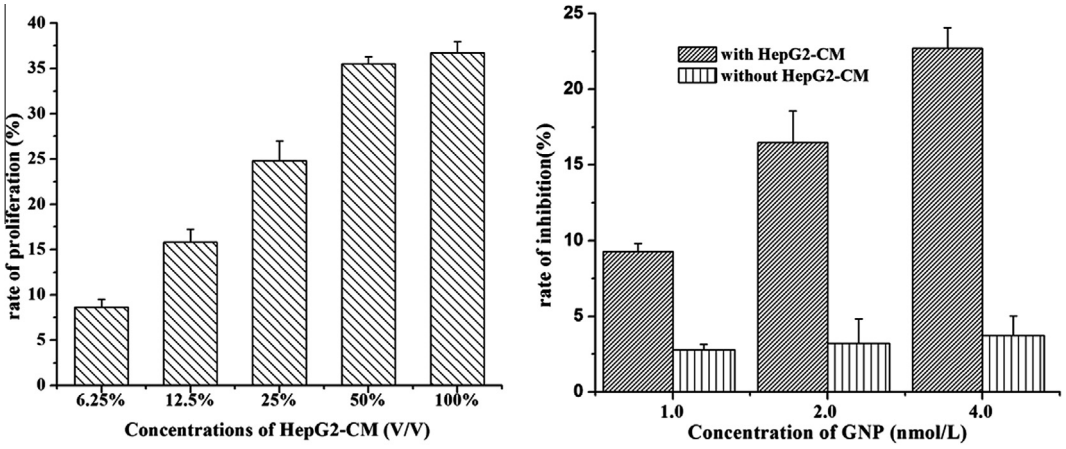

Figure 2. Viability of HUVECs determined by MTT assay. (A) Promotion effect of 6.25%, 12.5%, 25%, 50% and 100% HepG2-CM (V/V) treatment for 48 h on the proliferation of HUVECs. (B) Inhibition effect of 1, 2 and 4 nmol/L GNPs exposure for 48 h on the proliferation of HUVECs induced by HepG2-CM (50%).