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*Research Article*

# Astragaloside IV Suppresses the Effects of Hepatocellular Carcinoma Cells on Proliferation, Angiogenesis, and Invasion in Human Umbilical Vein Endothelial Cells by Controlling Exosomes by Inhibiting Rab27a

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Hepatocellular carcinoma (HCC) is the third most common cancer worldwide, and invasion and metastasis are the leading causes of death. Astragaloside IV (AS-IV), a kind of traditional Chinese medicine, has been widely used in cancer therapy. Cell Counting Kit-8, tube formation, and Transwell analyses were performed to determine cell proliferation, invasion, and angiogenesis of human umbilical vein endothelial cells (HUVECs) under the treatment of Hep3B or SK-Hep1 cells-derived exosomes or Hep3B or SK-Hep1 cells with or without AS-IV treatment. Then, Rab27a overexpression in Hep3B cells was selected to determine the function of AS-IV on the regulation of cell proliferation, invasion, and angiogenesis of HUVECs. Ultimately, the injection of Hep3B cell-derived exosomes or treatment with AS-IV on nude mice was used to comprehensively investigate AS-IV functions in vivo. Hep3B or SK-Hep1 cells promoted proliferation, invasion, and angiogenesis of HUVECs, which were inhibited by AS-IV therapy. The concentrations of Hep3B and SK-Hep1 cells-derived exosomes were markedly reduced after AS-IV therapy. Meanwhile, Rab27a expression was significantly decreased in SK-Hep1 or Hep3B cells after AS-IV therapy, and it inhibited the effects of AS-IV on Hep3B-induced proliferation, invasion, and angiogenesis of HUVECs. Tumor growth was also suppressed by AS-IV, which was accompanied by the decreased expression levels of Ki67 and CD34. Overall, this study demonstrated that AS-IV inhibits the effects of HCC cell-derived exosomes on proliferation, invasion, and angiogenesis of HUVECs via inhibiting Rab27a expression. *Practical Applications*. To our knowledge, this is the first study to explore the efficacy of AS-IV against HCC cell-induced proliferation, invasion, and angiogenesis of HUVECs to reveal the underlying mechanism. In this study, CCK-8, Transwell, and tube formation assay detection show that AS-IV has an inhibitory effect on HCC cell-induced proliferation, invasion, and angiogenesis of HUVECs by inhibiting Rab27a, which helps in the development of novel nutraceutical/functional food against HCC progression and thus could improve the quality of life in patients with HCC.

## 1. Introduction

Hepatocellular carcinoma (HCC) is a public health problem, which approximately 700 000 patients were newly diagnosed and suffered from this disease [1]. Overall, >50% of HCC cases at 5 years caused liver tumors, which is the third cancer-related death among malignant cancers [2, 3]. Given its high recurrence and metastasis rate, conventional chemotherapy and radiation therapy are limited for treating HCC [4, 5]. In the last decade, studies have reported molecular investigation about HCC therapy, which identified some aberrant signaling pathways and key molecular targets for the treatment of HCC. For example, Kaempferol has displayed anticancer activity by suppression of cancer cell proliferation and invasion [6]. In addition, clinical experiments show that sorafenib is efficacious for patients with HCC by promoting the apoptosis of HCC cells [7]. Recently, the loss of cell cycle control is a characteristic feature of HCC [8].

An exosome is a vesicle structure found in various cells, which contain RNA, dsDNA, and other small proteins [9]. The tumor cell-derived exosomes can be taken up by neighboring tissues and cells, which causes cell fate alteration, tumor invasion, and immunity response [10, 11]. Recent study has reported that exosomes extracted from gemcitabine-treated pancreatic cancer cells enhance cancer progression [12]. Meanwhile, Hsp20 overexpression promotes exosome secretion and presents autocrine- and paracrine-like protective effects on diabetic hearts [13]. Moreover, the distinct contents of exosomes from various cell types function as key mediators on regulating molecular processes [14, 15]. Exosomal miR-1247-3p derived from tumor cells mediates inflammatory response by activating the NF-$\kappa$B signaling pathway [16]. Thus, the exosome technology may provide an effective therapy for controlling tumor progression.

Generation and release of exosomes involves several Rab GTPase proteins, such as Rab11, Rab27, and Rab35 [17]. Rab27a controls exosome secretion in several types of cancer, increases proliferation and invasion, decreases apoptosis, and results in resistance to the cisplatin therapy [18–20]. Previous studies have reported that Rab27a promoted cell proliferative, migratory, invasive, and epithelial-mesenchymal transition phenotypes in HCC [21, 22].

Astragaloside IV (AS-IV) is the main pharmacological component of Astragalus, an herb used in traditional Chinese medicine that exhibits cardiovascular and cerebrovascular effects [23, 24]. Previous studies have shown that AS-IV has a strong antioxidant ability by removing free radicals or decreasing lipid peroxidation [25]. In addition, AS-IV exerts anti-inflammation and antidiabetes effects [26]. In these experiments, AS-IV prevented cancer cell proliferation and migration of MG-63 and U-20S cells. AS-IV also significantly inhibited the production of some inflammatory cytokines, such as tumor necrosis factor-$\alpha$, interleukin-4, and interferon [27, 28].

## 2. Materials and Methods

*2.1. Cell Culture.* Hep3B and SK-Hep1 cells were obtained from American Type Culture Collection. The cells were cultured in Dulbecco's modified eagle medium containing 10% fetal bovine serum and incubated at 37°C under 5% $CO_2$. The cocultures of human umbilical vein endothelial cells (HUVECs) with hepatocellular carcinoma (HCC) cells were made in Transwell chamber systems. HUVECs were inoculated into the lower chamber, whereas HCC cells pretreated with or without an exosome secretion inhibitor GW4869 (20 $\mu$m) or AS-IV (20 or 50 $\mu$m; Yuanye Biomart, Shanghai, China) were added to the upper chamber. Following the coculture for 10 days, the functions of HUVECs were analyzed.

*2.2. Exosome Experiment.* Exosomes were extracted from the HCC cell culture medium. Then, the supernatant was passed through a filter, followed by ultracentrifugation. The purity of exosomes was examined by transmission electron microscopy and Western blot analysis. The antibodies used were CD63 (Bioss, bsm-52384R), TSG101 (Abcam, ab125011), and Alix (Bioss, bs-6767R). The size and number of exosome particles were examined by nanoparticle tracking analysis (NTA) using ZetaView 8.04.02 software.

Interaction between HUVECs and exosomes was analyzed using a PKH-67 green fluorescent labeling kit (Sigma-Aldrich, St. Louis, MO, USA) according to the manufacturer's protocol, and HUVECs were treated with 50 or 100 $\mu$g/mL HCC cell-derived exosomes for 48 h.

*2.3. Cell Transfection.* Rab27a CDs were cloned into the pLVX-Puro plasmid (Clontech Laboratories, Inc., Mountain View, CA, USA). 293 T cells were transfected with three vectors, namely, pLVX-Puro-Rab27a, psPAX2, and pMD2G, using Lipofectamine 2000 (Invitrogen). Then, the recombinant lentivirals were transduced into HCC cells. pLVX-Puro transduction was used as a negative control.

*2.4. Cell Proliferation.* Cell Counting Kit-8 (CCK-8; Dojindo, Japan) was used to detect cell proliferation. In brief, HUVECs were planted into 96-well culture plates with a primary density of $3 \times 10^3$ cells per well. At the specified time points, CCK-8 solution was added to the cells at 37°C for 2 h incubation, and the absorbance was scanned by using a microplate reader.

*2.5. Transwell Assay.* Matrigel-coated Transwell chambers were used to assess HUVEC invasion. The HUVECs resuspended in a cell culture medium without fetal bovine serum were inoculated into the upper chamber. Dulbecco's modified eagle medium containing 10% fetal bovine serum was added to the lower chamber. After 24 hours of incubation, uninvaded cells were removed and the remaining cells were fixed with 4% paraformaldehyde and stained with 0.1% crystal violet.

*2.6. Tube Formation Assay.* HUVECs were placed on 96-well plates coated with Matrigel® Growth Factor Reduced Basement Membrane Matrix (Corning, Corning, NY, USA). After 6 hours of incubation, image of the tubule branches was taken under a microscope and calculation was performed using Image J.

*2.7. Quantitative Reverse-Transcription Polymerase Chain Reaction (qRT-PCR).* The total RNA was extracted from Hep3B and SK-Hep1 cells using the TRIzol reagent (Invitrogen). Then, 1 $\mu$g of the total RNA was reverse-transcribed to synthesize cDNA utilizing the PrimeScript RT Reagent Kit (Takara). qRT-PCR was performed using Maxima SYBR Green/ROX qPCR (Thermo Fisher Scientific). Sequences of primers are as follows: Rab11-F, AGCGATGGCTGAAAG AAC; Rab11-R, GTTAAAATTGTCTGAAAAGCAG; Rab27a-F, AGTGAAAGAGGAGGAAGCC; Rab27a-R, TCATTATCAGGTCCAGAAGC; Rab27-F, TTGGCAACA AGGCAGACC; Rab27-R, TTCCACCATTGACAGTAT

CAGG;Rab35-F, CTCATCATCGGCGACAGC; Rab35-R, AGGTGATGGTGCGGAAGC; GAPDH-F, AACGGATTT GGTCGTATTG; and GAPDH-R, CTGGAAGATGGTGAT GGG. mRNA expression levels were quantified with GAPDH as the reference gene.

*2.8. Western Blot.* Cells were collected from Hep3B cells, lysed on ice with lysis buffer, and quantified using a Bicinchoninic Acid Protein Assay Kit (Beyotime Biotechnology). Sodium dodecyl sulfate-polyacrylamide gel was used to separate protein from homogenate which are then transferred electrophoretically to polyvinylidene fluoride membranes (Millipore). Once the transfer of the proteins was completed, the membranes were blocked with 5% skimmed-milk solution and incubated overnight at 4°C with the primary antibody, anti-Rab27a (bsm-51334M, Bioss), anti-E-cadherin (ab231303, Abcam), anti-N-cadherin (ab76011, Abcam), and anti-GAPDH (#5174, CST). Secondary antibodies were labeled with horseradish peroxidase. Visualization was detected using an ECL detection system (Bio-Rad, CA, USA).

*2.9. In Vivo Tumor Formation.* A total of $5 \times 10^6$ Hep3B cells were subcutaneously injected into the right flank of 4-week-old male BALB/c nude mice. Hep3B-derived exosomes (10 $\mu$g/mouse every 3 days) were intravenously injected after 7 days of cell injection. After 12 days of cell injection, AS-IV (50 mg/kg, every day) was intraperitoneally injected. Tumor growth was monitored for 33 days, and the tumor volume was detected every 3 days. On day 33, the tumors were surgically removed, weighed, and processed ($n = 5$ per group). Mice were subjected to euthanasia if they had breathing difficulty, weight loss >10% of the body weight, or display pain or distress. The hospital's Animal Care and Use Committee approved the animal research.

*2.10. Immunofluorescence.* Tumor tissues were fixed in 10% buffered formalin and embedded in paraffin, and 5 $\mu$m sections were subjected to antigen repair by citrate buffer (pH 6.0) at 95°C for 15 min. The sections were incubated with primary antibodies targeting Ki67 (ab243878 Abcam) and then incubated with Alexa Fluor 488-labeled Goat Anti-Rabbit IgG ($H + L$) (A0423, Beyotime Biotechnology) for 1 h at room temperature. DAPI was used to visualize cell nuclei. Observation was performed using the Olympus multifunction microscope (Tokyo, Japan).

*2.11. Microvessel Density Evaluation.* The expression of CD34 in xenograft tumors was evaluated by EnVision immunohistochemistry. The regions with the highest angiogenesis were identified, and microvessels were stained in three random views of the "hot spot." As a measure of microvessel density, the mean value was recorded for each case.

*2.12. Statistical Analysis.* The analysis results are shown as the mean ± standard deviation (SD) for each experimental group ($n = 3$ or 5). For comparisons between experimental groups, the one-way analysis of variance was performed. $P < 0.05$ was considered statistically significant.

## 3. Results

*3.1. Hep3B and SK-Hep1 Cells Heighten Cell Proliferation, Invasion, and Angiogenesis of HUVECs.* To examine the effect of HCC cells on HUVECs, the HUVECs were cocultured with Hep3B and SK-Hep1 cells using a Transwell coculture system. First, the proliferation of HUVECs was analyzed using the CCK-8 assay. As shown in Figure 1(a), HUVEC proliferation in Hep3B and SK-Hep1-cocultured groups was significantly increased and this trend was reversed by the exosome inhibitor GW4869. In addition, the Transwell and tube formation assays showed that HUVECs cultured with Hep3B or SK-Hep1 cells exhibited higher invasion and angiogenesis rates than that cultured alone, and this effect was inhibited by GW4869 (Figures 1(b)–1(e)).

*3.2. Isolation and Validation of Exosomes from Hep3B and SK-Hep1 Cells.* As presented in Figure 2(a), the exosomes were successfully isolated and characterized. The size of exosome particles was detected by NTA (Figure 2(b)). Meanwhile, the protein levels of exosome markers Alix, TSG101, and CD63 were found in exosomes extracted from Hep3B and SK-Hep1 cells (Figure 2(c)). Moreover, we found that exosomes extracted from HepG3 and SK-Hep1 cells labeled with PHK67 could be absorbed by HUVECs using confocal microscopy (Figure 2(d)).

*3.3. HCC Cell-Derived Exosomes Promote the Proliferation, Invasion, and Angiogenesis of HUVECs.* Then, we examined the function of HCC cell-derived exosomes. We found that different concentrations of exosomes extracted from HCC cells promote the proliferation of HUVECs (Figure 3(a)). Similar to the effect on proliferation, cancer cell-derived exosomes exerted a significant effect on HUVECs' invasion (Figures 3(b) and 3(d)). In addition, HUVEC angiogenesis was significantly increased by treatment with HCC-derived exosomes (Figures 3(c) and 3(e)). Moreover, HCC-derived exosomes markedly reduced E-cadherin but promoted N-cadherin expression in HUVECs (Figure 3(f)). In summary, these data suggest that HCC-derived exosomes promote cancer progression.

*3.4. AS-IV Inhibits the Function of HCC Cells in Regulating Cell Proliferation, Invasion, and Angiogenesis of HUVECs.* To identify whether AS-IV may influence the effects of HCC on HUVECs, Hep3B and SK-Hep1 cells were treated with increasing doses of AS-IV and the cell proliferation, invasion, and angiogenesis of HUVECs were assessed. Proliferation of HUVECs was significantly decreased by different concentrations of AS-IV (Figures 4(a) and 4(b)). In addition, the Transwell and tube formation assays confirmed that the invasion and angiogenesis of HUVECs induced by HCC cells were effectively reduced by AS-IV therapy (Figures 4(c)–4(f)). Moreover, the concentration of Hep3B

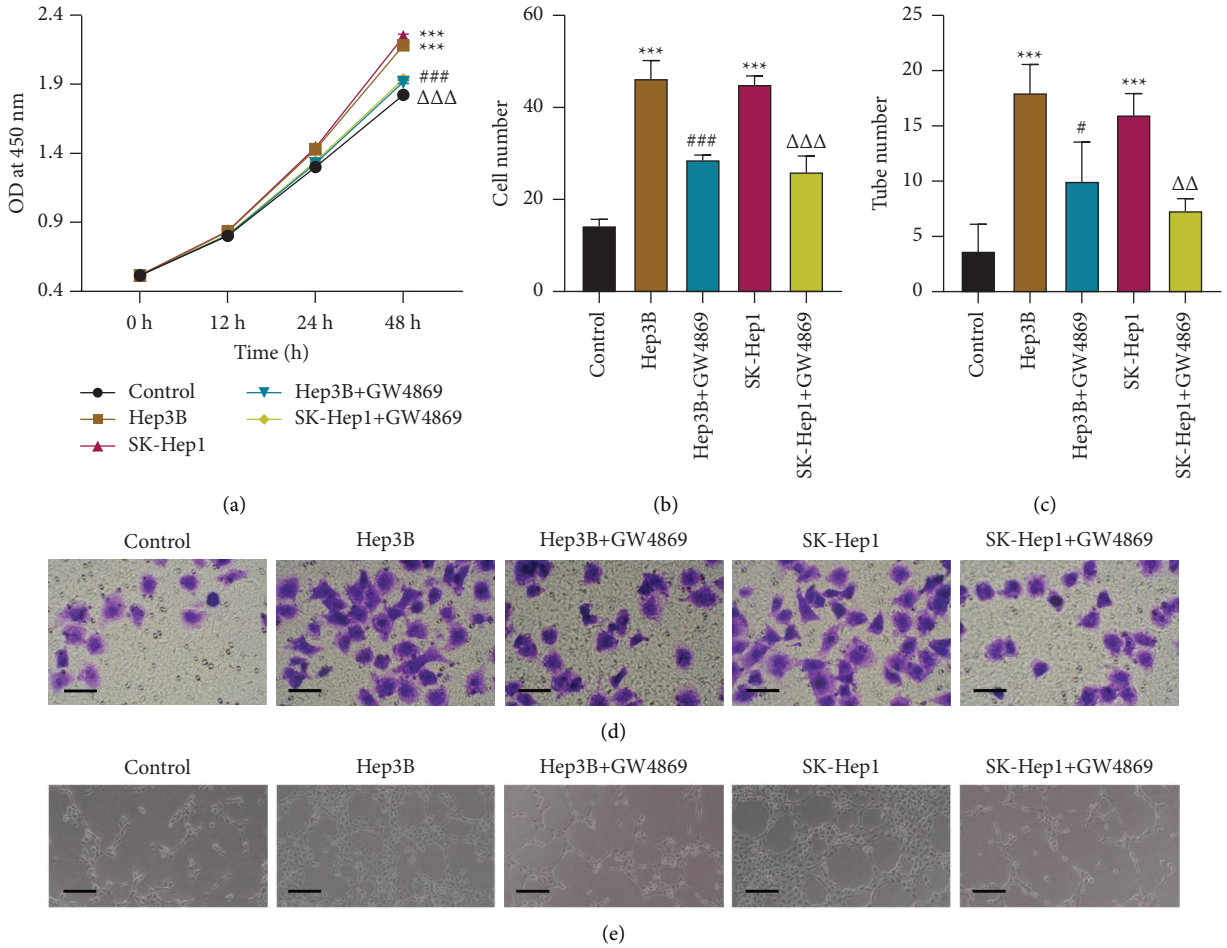



Figure 1: Effects of Hep3B and SK-Hep1 cells on the proliferation, invasion, and angiogenesis of HUVECs. (a) Proliferation, (b, d) invasion, and (c, e) angiogenesis of HUVECs cocultured with Hep3B or SK-Hep1 cells treated with GW4869. Scale bar: 100 $\mu$m. All data are expressed as the mean ± SD, $n = 3$. $^{***}P < 0.001$ vs. control; $^{\#}P < 0.05$, $^{\#\#\#}P < 0.001$ vs. Hep3B; $^{\Delta\Delta}P < 0.01$, $^{\Delta\Delta\Delta}P < 0.001$ vs. SK-Hep1.

and SK-Hep1 cell-derived exosomes was markedly reduced following the AS-IV therapy compared with the control (Figures 4(g) and 4(h)). These results indicate that AS-IV may suppress the effects of HCC cells on HUVECs by controlling biogenesis, sorting, and secretion of exosomes.

*3.5. AS-IV Inhibits the Effects of HCC Cells on HUVECs by Inhibiting Rab27a.* Generation and release of exosomes involve several Rab GTPase proteins, such as Rab11, Rab27, and Rab35 [17]. To test whether AS-IV inhibits the effect of HCC cells on exosomes, the expression levels of these proteins in HCC cells were examined after AS-IV treatment. The mRNA level of Rab27a was downregulated in the AS-IV-treated group (Figures 5(a) and 5(b)). Moreover, the mRNA level of Rab27a was higher in SK-Hep1 cells than in Hep3B cells (Figure 5(c)). Then, we successfully overexpressed the *Rab27a* gene (Figures 5(c) and 5(d)) and analyzed the proliferation, invasion, and angiogenesis of HUVECs cocultured with Hep3B cells under the AS-IV therapy. Our data show that AS-IV suppressed the proliferation, invasion, angiogenesis, and N-cadherin expression and promoted E-cadherin expression in HUVECs cocultured with Hep3B cells, which were reversed by Rab27a overexpression (Figures 5(f)–5(k)).

*3.6. AS-IV Inhibits the Effects of Hep3B Cell-Derived Exosomes on Tumor Growth In Vivo.* To confirm the function of HCC cell-derived exosomes on tumor growth in vivo, nude mice were injected with Hep3B cell-derived exosomes or AS-IV. As shown in Figures 6(a)–6(c), the Hep3B-derived exosome-injected mice presented larger tumor size and weight on mouse xenografts and this upregulation was inhibited by the addition of AS-IV. In addition, immunofluorescence analysis revealed that highly expressed protein levels of Ki-67 in the exosome group were downregulated by the AS-IV therapy (Figures 6(d) and 6(e)). AS-IV also substantially decreased the protein level of CD34 in xenograft tumors (Figures 6(f) and 6(g)). Moreover, the decreased E-cadherin and increased expression levels of N-cadherin induced by Hep3B-derived exosomes were attenuated by AS-IV (Figure 6(h)). Collectively, our data indicate that AS-IV suppressed Rab27a expression and exosome secretion of Hep3B and exerted an inhibitory function on HCC progression.

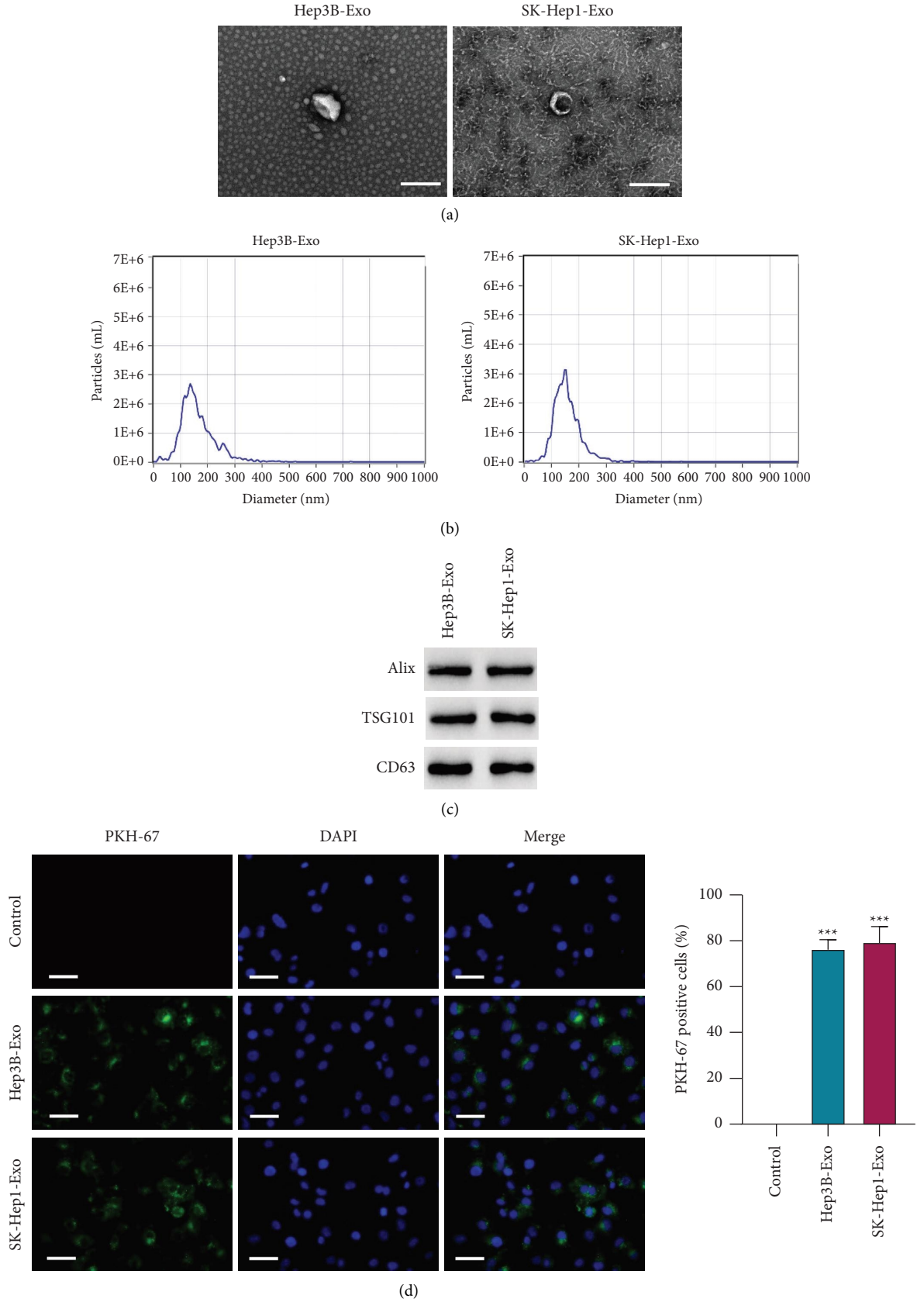



Figure 2: Characterization of exosomes from Hep3B and SK-Hep1 cells. (a) Transmission electron microscopy observation of Hep3B and SK-Hep1 cell-derived exosomes (scale bar, 200 nm). (b) Size range of exosomes was measured by NTA. (c) Expression of Alix, TSG101, and CD63 in Hep3B and SK-Hep1 cell-derived exosomes. (d) Exosome uptake by HUVECs (scale bar: 50 $\mu$m).

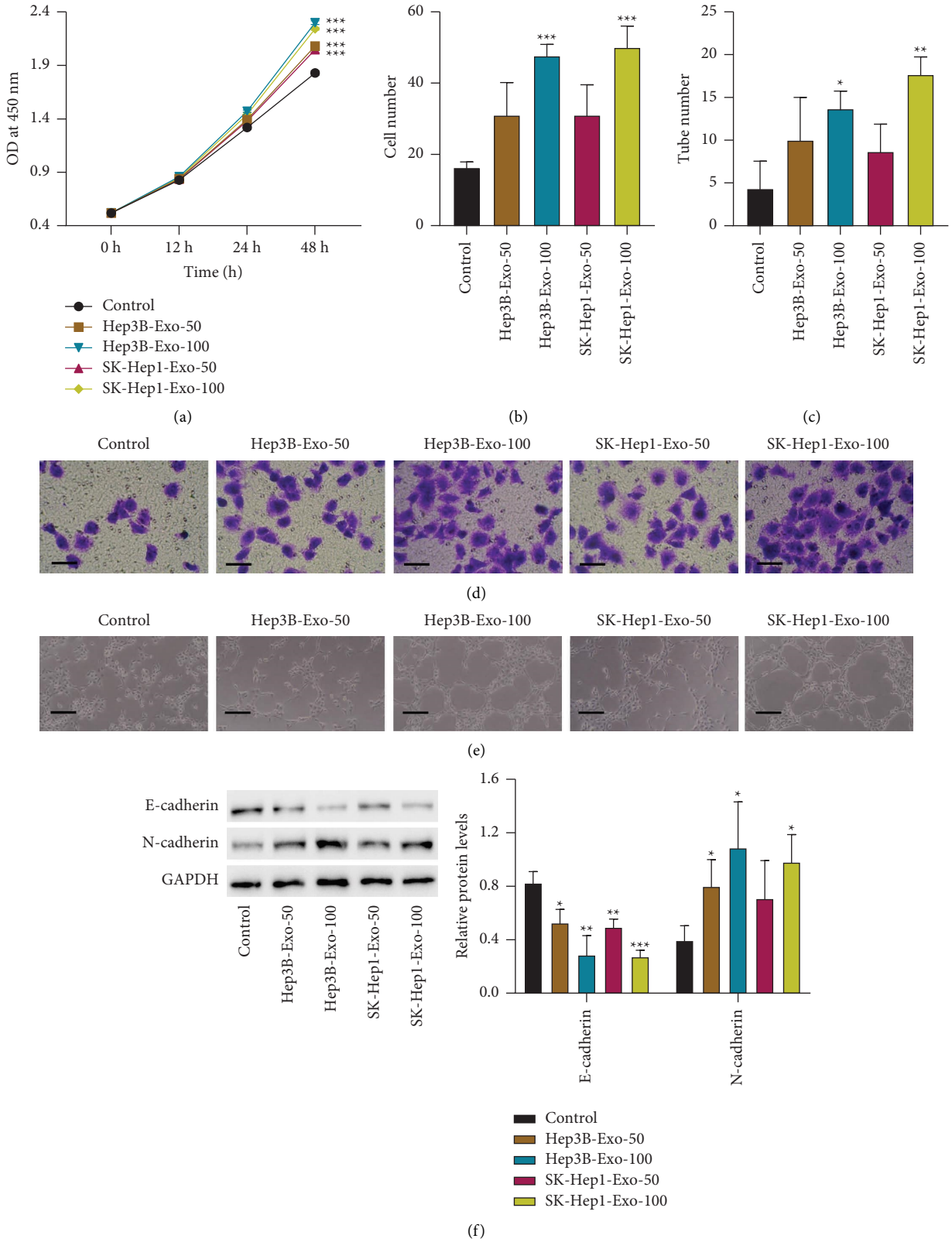
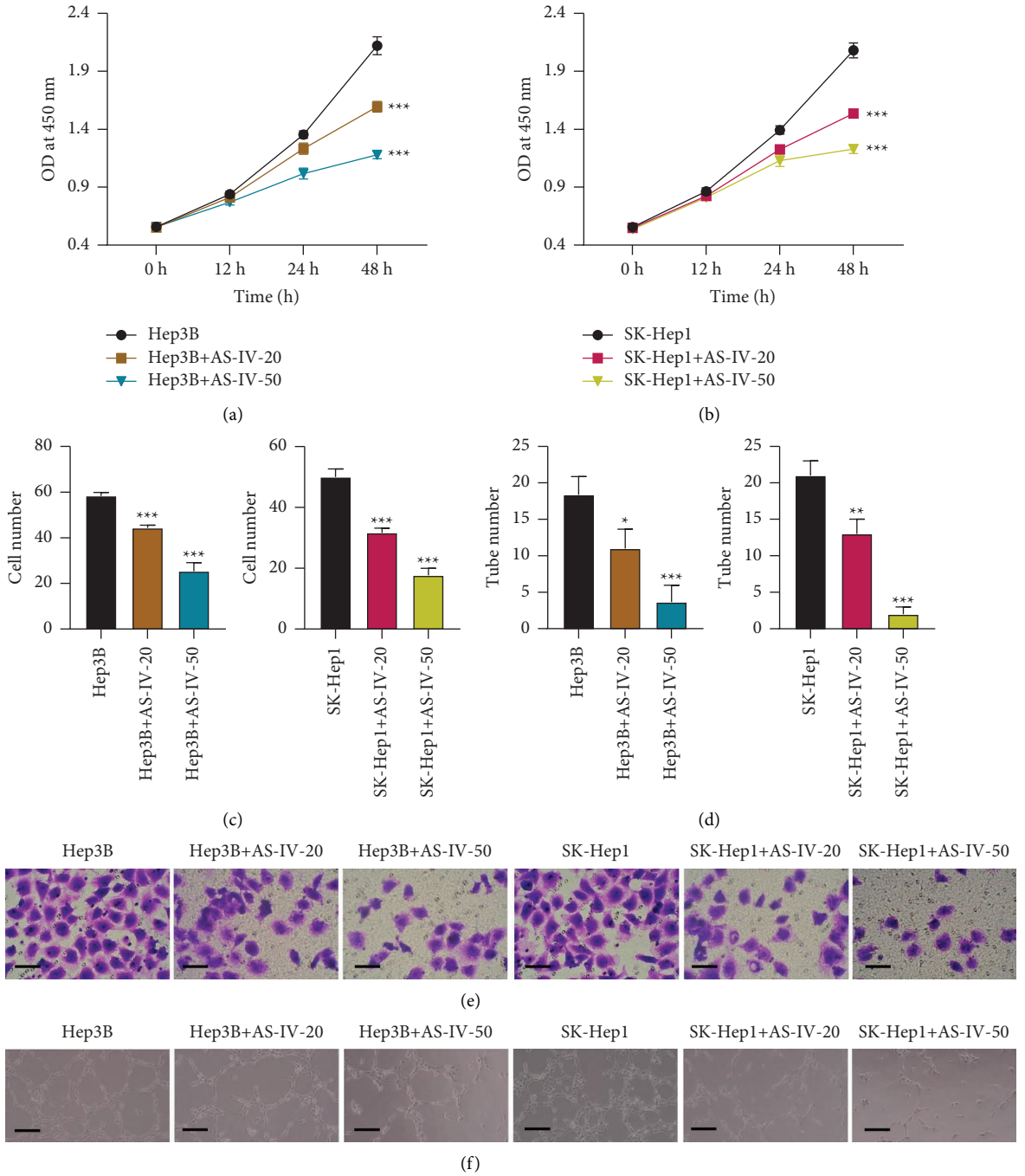



Figure 3: Exosomes derived from Hep3B and SK-Hep1 cells promote proliferation, angiogenesis, and invasion of HUVECs. (a) Proliferation, (b, d) invasion and (c, e) angiogenesis, and (f) E-cadherin and N-cadherin expression in HUVECs treated with Hep3B or SK-Hep1 cell-derived exosomes (50 and 100 $\mu$g/mL). Scale bar: 100 $\mu$m. All data are expressed as the mean ± SD, $n = 3$. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. control.

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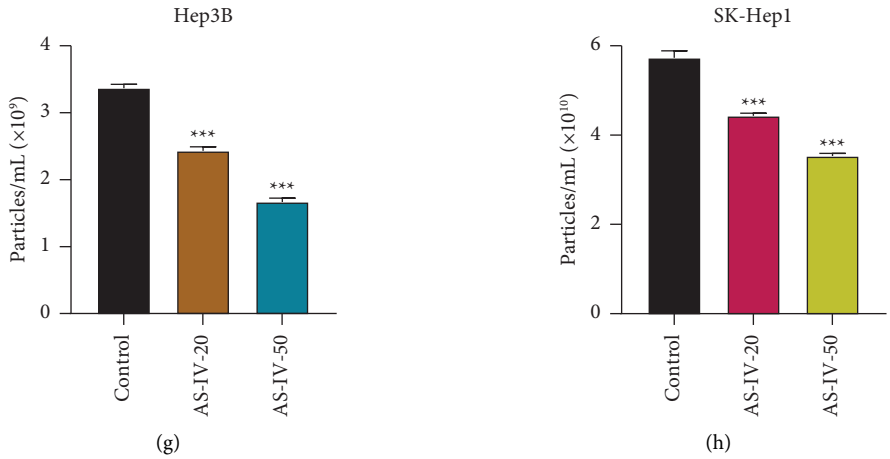
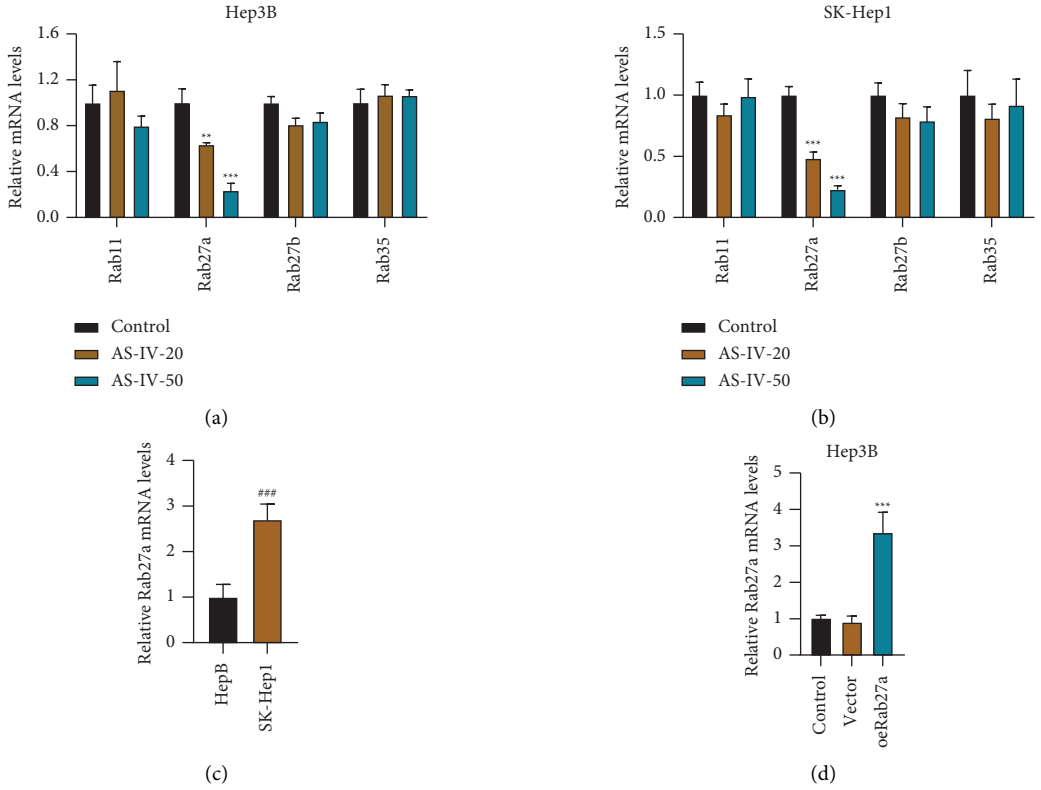



Figure 4: AS-IV inhibits the effects of Hep3B and SK-Hep1 cells on the proliferation, invasion, and angiogenesis of HUVECs. (a, b) Proliferation, (c, e) invasion, and (d, f) angiogenesis of HUVECs cocultured with Hep3B or SK-Hep1 cells pretreated with 20 and 50 $\mu$m AS-IV. (g, h) Concentration of Hep3B or SK-Hep1 cells-derived exosomes was measured by NTA. Scale bar: 100 $\mu$m. All data are expressed as the mean ± SD, $n = 3$. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. Hep3B, SK-Hep1, or control.

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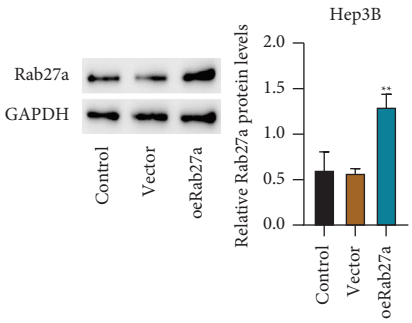
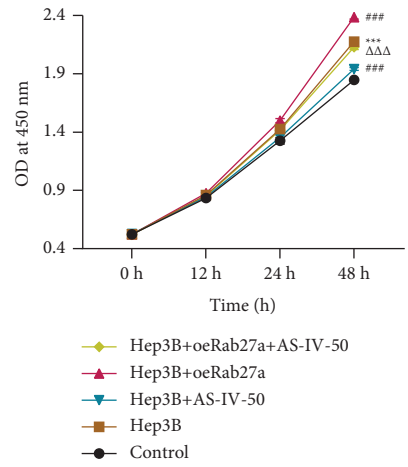
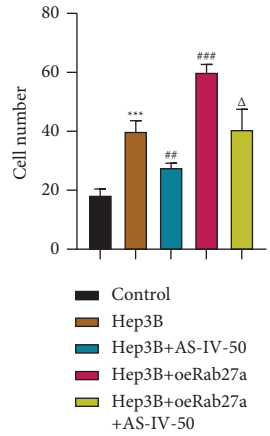


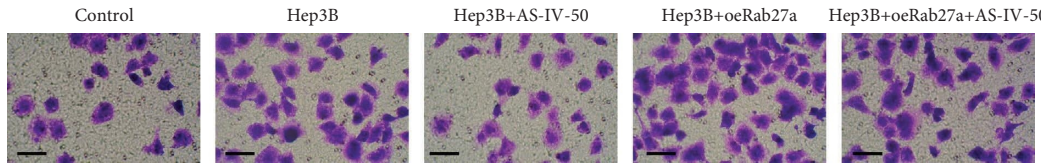

(e)



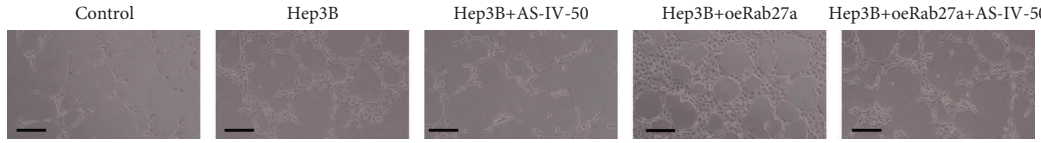

(f)




(g)




(h)

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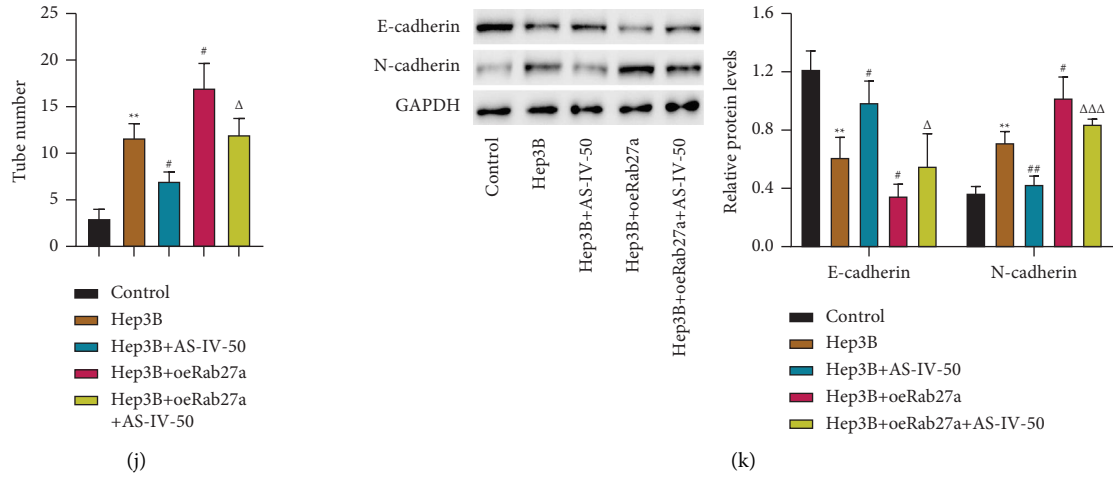
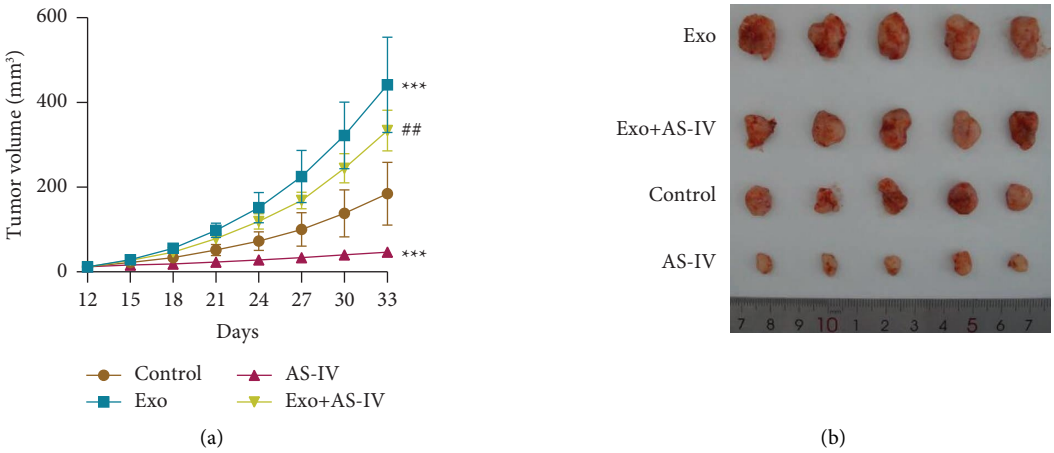



Figure 5: AS-IV inhibits the effects of Hep3B and SK-Hep1 cells on HUVECs by inhibiting Rab27a. (a, b) Expression of Rab11, Rab27a, Rab27b, and Rab35 in Hep3B and SK-Hep1 cells. (c) Expression of Rab27a in Hep3B and SK-Hep1 cells. (d, e) Expression of Rab27a in Hep3B cells. (f) Proliferation, (g, h) invasion and (i, j) angiogenesis, and (k) expression of E-cadherin and N-cadherin in HUVECs cocultured with Hep3B or SK-Hep1 cells transduced with Rab27a expression vector before treatment with 50 $\mu$m AS-IV. Scale bar: 100 $\mu$m. All data are expressed as the mean ± SD, $n = 3$. $^{**}P < 0.01$, $^{***}P < 0.001$ vs. control; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. Hep3B; $^{\Delta}P < 0.05$, $^{\Delta\Delta\Delta}P < 0.001$ vs. Hep3B + AS-IV-50.

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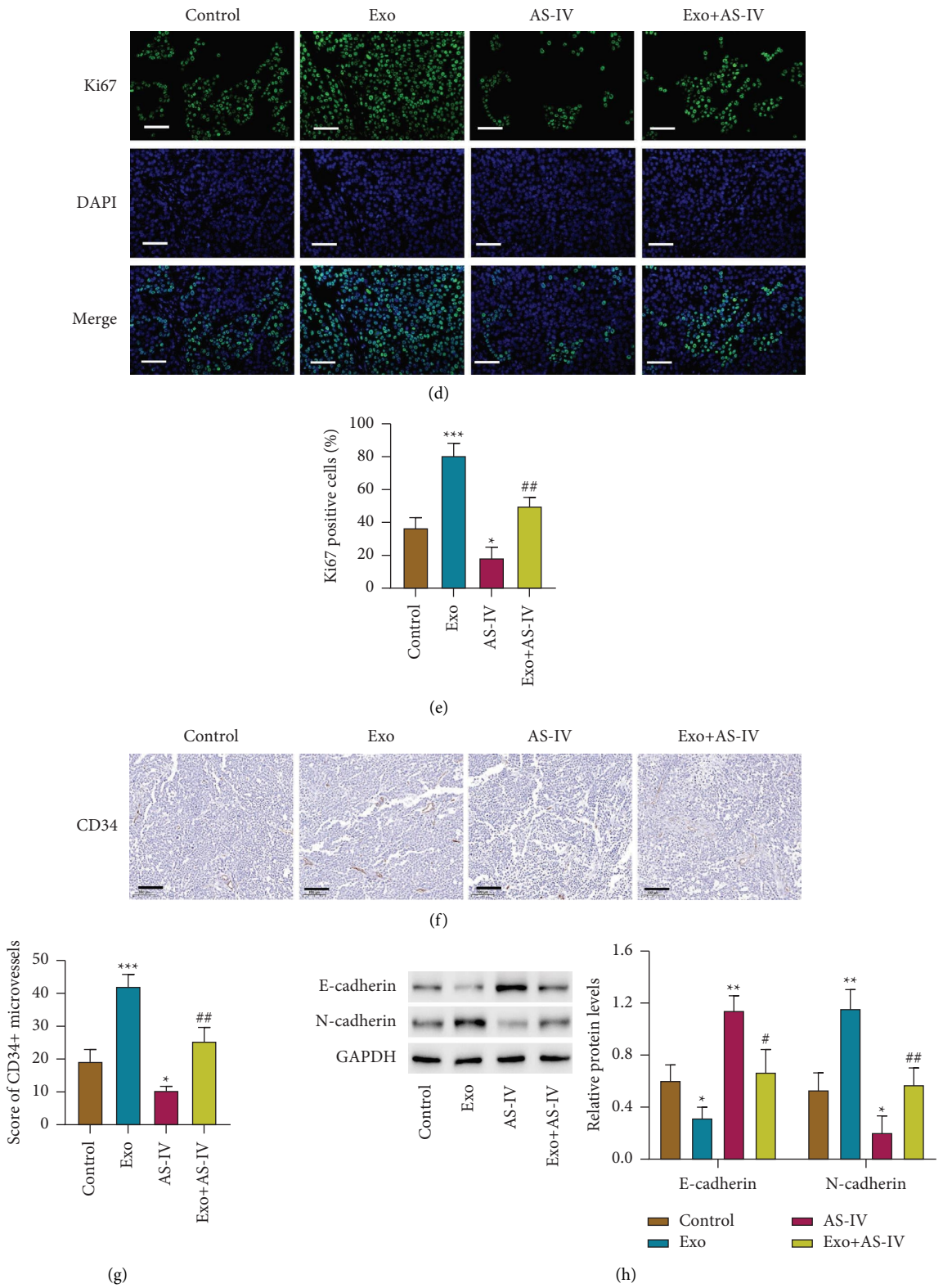


FIGURE 6: AS-IV inhibits the effects of Hep3B cell-derived exosomes on tumor growth in vivo. (a) Tumor volume, (b, c) tumor weight, (d, e) Ki67 immunofluorescence staining, (f, g) CD34-labeled microvessels, and (h) E-cadherin and N-cadherin expression in xenograft tumors from various groups. Scale bar: 100 $\mu$m. All data are expressed as the mean ± SD, $n = 3$ or 5. $^{*}P < 0.05$, $^{*}P < 0.01$, $^{***}P < 0.001$ vs. control; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. Exo.

## 4. Discussion

Exosomes have been widely investigated in recent years. Recent evidence shows that cancer-secreted exosomes affect exosome donor cells and support cancer growth and metastasis [29, 30]. For example, exosomal miR-200b could directly inhibit p27 and RND3, resulting in the proliferation of colorectal cancer cells [30]. Exosomal circRNA_101093 interacted with and increased fatty acid-binding protein 3 and led to arachidonic acid transport into the plasma [31]. Moreover, ovarian cancer cell-secreted exosomal miR-205 significantly promoted in vitro angiogenesis and tumor growth [32]. In this study, HCC-derived exosomes regulate the crosstalk between cancer cells and the tumor microenvironment. In addition, AS-IV downregulated Rab27a in Hep3B cells and inhibited the function of HCC cells in regulating cell proliferation, invasion, and angiogenesis of HUVECs. Evidence shows that the microenvironments play an important role in tumor growth, angiogenesis, and metastasis. In particular, the proliferation and movement of endothelial cells are necessary for the formation of microvascular buds and angiogenesis, which are related to cancer metastasis. The interactions among tumor, endothelial cells, and other components of the microenvironments may interact with angiogenesis, cancer cell proliferation, and cell proliferation [33]. At present, little research has been conducted on the role of endothelial cells in the development of liver cancer.

AS-IV is a natural nonsynthetic, active ingredient extracted from *Astragalus membranaceus*, which has been widely used in traditional Chinese medicine to treat diseases. AS-IV has a certain inhibitory effect on various diseases, including cancer [34]. AS-IV was reported to inhibit fibrosis development and delays the occurrence of primary HCC by regulating Smad3C/3L and Nrf2/HO-1 pathways [35]. By inducing MRP2 overexpression in liver cancer cells, AS-IV can promote the apoptosis of liver cancer cells, slow down tumor growth, enhance chemotherapy sensitivity, and further inhibit tumor progression [36]. In addition, AS-IV plays a leading role in regulating HUVECs. Zhang et al. reported AS-IV stimulating the proliferation and migration of HUVECs. AS-IV also heightened HUVEC invasion and angiogenesis. These results indicate that AS-IV has a potential role in promoting angiogenesis [37]. Coincidentally, our data showed that AS-IV inhibited the effect of liver cancer cells on the proliferation, invasion, and angiogenesis of HUVECs. The abovementioned results indicate that AS-IV has a certain inhibitory effect on liver diseases.

As a membrane of small G proteins of the Ras superfamily, Rab27a regulates vesicular transport and endocytic pathways [38]. A high Rab27a expression is positively correlated with the progression of non-small cell lung cancer [39]. Otherwise, Rab27a regulates the intracellular exosome secretion pathway in RAW 264.7 macrophages [40]. Our results revealed AS-IV suppressing proliferation, angiogenesis, and invasion of HUVECs under the Hep3B coculture. Meanwhile, the expression of Rab27a was inhibited by AS-IV. This can be a new mechanism to explain how AS-IV regulates HCC communication with HUVECs to inhibit tumor growth.

In conclusion, our study suggested that Hep3B and SK-Hep1-derived exosomes positively regulate HUVEC proliferation, invasion, and angiogenesis and that Rab27a expression was downregulated by the AS-IV therapy. Furthermore, this study sheds light on the importance of AS-IV-induced decreased Rab27a in suppressing HUVEC proliferation and angiogenesis by inhibiting the positive functions of HCC.

## Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## Conflicts of Interest

The authors declare that they have no conflicts of interest.

## Authors' Contributions

Liang Min was responsible for conceptualization of the study, Liang Min and Haiqiao Wang were responsible for data curation, Haiqiao Wang and Hong Qi conducted the formal analysis, Liang Min wrote the original draft, and Haiqiao Wang was responsible for writing the review and editing.

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