

Cancer Exosome-derived Integrin $\alpha 6$ and Integrin $\beta 4$ Promote Lung Metastasis of Colorectal Cancer

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Abstract

Background: Colorectal cancer (CRC) metastasis remains the major cause of the CRC mortality, while the underlying mechanisms remain to be fully understood. In this study we investigated the role of cancer exosomes in CRC lung metastasis *in vivo* and *in vitro*.

Methods: Expressions of Integrin $\alpha 6$ and Integrin $\beta 4$ were examined in CRC cells as well as released exosomes. Co-culture assay with vascular endothelial cells was also analyzed.

Results: We found that Integrin $\alpha 6$ and Integrin $\beta 4$ are overexpressed in highly tumorigenic and metastatic CRC cell lines HCT116 and SW620 and their secreted exosomes, compared to the low tumorigenic and non-metastatic CRC cell lines. Disruption of ITGA6 and ITGB4 expression in CRC decreased the proliferation and tubulogenic capacities of vascular endothelial cells significantly, while ectopic expression of ITGA6 and ITGB4 gave rise to opposite effects. Further more, we demonstrated that exosomal ITGA6 and ITGB4 promoted the lung metastasis of CRCs *in vivo*.

Conclusions: Our study provides new insight into the molecular mechanism of CRC metastasis by which CRC-derived exosomal ITGA6 and ITGB4 induce organotropism to the lung, leading to increased tubulogenic capacity and metastasis. It also reveals a biomarker-based prediction for CRC metastasis and a novel potential therapeutic targets for CRC.

Introduction

Colorectal cancer (CRC) is one of the leading causes of cancer-related deaths worldwide[1], and its mortality has been steadily increasing over the past decades[2]. Metastasis is one of the most lethal causes contributing to the poor outcome of colorectal cancers[3]. The five-year survival rate of CRC patients diagnosed with localized CRC is 90.1%. However, it drops to 11.7% when tumors spread to adjacent organs or distant tissues[4]. The lung is one of the main target organs for CRC metastasis, which accounts for 10–20% of the diagnosed patient population and which contribute to a worse outcome[5]. However, the underlying mechanisms underlying CRC lung metastasis remains to be elusive.

Exosomes are small vesicles ranging in size between 30 and 150 nm, composed by a lipid bilayer containing membrane proteins that surrounds a lumen comprising proteins and nucleic acids, that vary according to cell type and mechanism of biogenesis[6]. Exosomes functions to transport the bioactive molecules between cells, such as mRNA and miRNA to induce the genetic or epigenetic change in the target cells[7, 8]. Cancer-derived exosomes have been shown to promote tumor growth or metastasis by suppressing antitumor immune responses, increasing neoangiogenesis or migration to other cells[9].

Integrin $\alpha 6$ (ITGA6), also known as CD49f, is a member of Integrin family transmembrane proteins[10]. Integrin functions as a cell surface receptor that mediates the transfer of extracellular mechanical and chemical signals into the cell interior, and then modulates the different signal transduction cascades[11, 12]. Integrin also coordinates cell survival, apoptosis, proliferation and motility[13]. ITGA6 is also found to

be critical for the migration of human thymic epithelial cells[14] and the progression of various types of malignant tumors[10].

Integrin $\beta 4$ (ITGB4) also is the structural component of hemidesmosomes that maintains epithelial architecture and acts as a signaling adaptor driving tumor cell proliferation and movement, and metastasis[15–17]. Tang and his colleagues have indicated that ITGB4 taken part in regulating the Chromobox homolog 8 (CBX8)-mediated proliferation and metastasis of CRC[18]. ITG $\alpha 6\beta 4$, a heterodimer of ITG $\alpha 6$ with ITG $\beta 4$ [19] showed increased expression in murine Lewis lung carcinoma variants with high metastatic potential[20]. In addition, tumor-derived exosomal integrins have a role in determining the organotropic metastasis[21]. However, these exosomal integrins have not been explored in colorectal cancer.

In this study, we have demonstrated a role of intracellular and extracellular ITGA6 and ITGB4 in CRC lung metastasis. We found that CRC-derived exosomal ITGA6 and ITGB4 is associated with CRC metastasis and is able to induce the proliferation and tubulogenic capacity of vascular endothelial cells. Furthermore, we analyzed the biodistribution and functions of high metastatic CRCs-secreted exosomes *in vivo* and demonstrated that exosomal ITGA6 and ITGB4 directed organ-specific colonization which might help promote the metastasis of CRC to the lung. Our findings could pave the way for the development of an effective method to predict organ-specific metastasis and therapies to halt the metastatic spread.

Materials And Methods

Patients and plasma samples

The study was approved by the Guangxi Medical University Cancer Hospital Ethics Committees, and all patients were enrolled by written informed consent. This study was conducted under the principles of the Helsinki Declaration. Five patients with histologically confirmed CRC with or without lung metastasis only at Department of colorectal and anal surgery, Guangxi Medical University Cancer Hospital at 2016 were recruited for this study. Their diagnoses were independently re-reviewed by two pathologists and classified by WHO criteria. Plasms were collected from these patients.

Cell culture

Human CRC cell lines HCT116, SW620, SW480, and Caco2 from American Type Culture Collection (ATCC) were authenticated by short tandem repeat profiling and were passaged for less than six months. The vascular endothelial cells were obtained from the Laboratory of Tumor Immunopathology (Nanjing, China). Cells were maintained as a monolayer culture in Dulbecco's modified Eagle medium (DMEM) (Gibco, Grand Island, NY) containing 10% fetal bovine serum (FBS) and were incubated at 37 °C in a humidified incubator with 5% CO₂.

Western blot

Western blot analysis was conducted as previously described[22]. Primary antibodies were listed as follows: rabbit anti-human β -actin mAb (#5125, 1:1000, Cell Signaling Technology, Danvers, MA), rabbit anti-human CD9 mAb (ab92726, Abcam, Cambridge, UK), rabbit anti-human CD63 mAb (ab134045, Abcam), rabbit anti-human ITGA6 mAb (ab181551, Abcam), rabbit anti-human ITGB4 mAb (ab182120, Abcam). Secondary antibodies were HRP-labeled goat anti-rabbit IgG (H + L) (A0208, 1:5000, Beyotime, Shanghai, China).

ELISA assay

The ITGA6 and ITGB4 concentration in the supernatant of cultured CRC and serum of patients were measured by human ITGA6 and ITGB4 ELISA kit (Boster, Shanghai, China) according to the manufacturer's instructions. Briefly, CRC cells cultured in DMEM medium without for 24 h. The cells and supernatants or the serum from patients were collected for cell counting and ELISA assay, respectively.

Exosomes Isolation and characterization

Isolation of exosomes from the cell culture medium using ExoQuick-TC Exosome Precipitation kit (EXOTC50A-1, SBI, Palo Alto, CA). The conditioned medium was harvested from cells cultured in advanced DMEM medium (Life Technologies, Carlsbad, CA, USA). Briefly, 1×10^6 cells were seeded on 100-mm dish, and the conditioned medium was collected two days later. The supernatant was centrifuged at 3,000 g for 15 min to remove debris. Added 3.3 ml ExoQuick-TC volume in 10 ml culture media, and then, resuspended the pellets with the mixture. Incubation of the mixture at 4°C for 12 hours. Next, the supernatant was centrifuged at 1500 g for 30 min to remove supernatant, then the pelleted resuspended in PBS and stored at -80°C for using. Characterization was obtained via transmission electron microscopy (TEM) according to the protocol described by Zaharie *et al*[23].

Quantitative real-time PCR (qRT-PCR)

qRT-PCR was performed as previously described[24]. The sequences of the specific primer sets for ITGA6, ITGB4 and ACTN were listed in Supplementary Table S1. The level of ACTN was used as internal control.

Tumor cell proliferation assay

Cell proliferation was examined using Cell Counting Kit-8 (Beyotime, Shanghai, China), for five days. Briefly, cells (1000 cells) were seeded into 96-well plates. At each interval, added ten μ l CCK-8 solution to each well and incubated for 2 hours at 37 °C. The absorbance value was measured at 450 nm using a microtiter plate reader. The experiment was conducted for in triplicate.

Lentiviral infection

The human ITGA6 and ITGB4-specific short hairpin RNA (shRNA) vectors, the non-targeting shRNA (shNT) vector, the ITGA6- and ITGB4-expressing vector and control vector were purchased from Hanyin Bio-technology Corp.,Ltd. (Shanghai, China). Tumor cells stably expressing ITGA6, ITGB4 or shRNAs were enriched by puromycin treatment for selecting positive clones.

Tubule formation experiment

In vitro tubule formation experiment was conducted on growth factor-reduced Matrigel (BD Biosciences). Vascular endothelial cells (3×10^4) were seeded onto Matrigel-coated wells in RPMI containing 10% FBS with exosome isolate from different cells. Cells were visualized under high magnification ($\times 200$) 24 hours later. The total tube area was quantified as the mean pixel density obtained from image analysis of five random microscopic fields. This experiment was conducted in duplicates and repeated two times. Data are expressed as the number of branched points per well under each culture condition.

Xenograft experiments

Six-week-old male nude mice were tail vein injected with 150 μ g of exosome or vehicle control (saline solution) post CRC cells inoculation. The tumors formed in distant organs quantified by bioluminescence imaging using In Vivo Imaging System (IVIS, PerkinElmer) Spectrum (PerkinElmer) and Living Image Software for IVIS at 24 hours after injection. The animal experiments were approved by the Institutional Animal Care and Use Committee of the Guangxi Medical University Cancer Hospital, in accordance with the Guide for the Care and Use of Laboratory Animals.

Statistical analysis

All statistical analyses were performed using SPSS 18.0 software. The unpaired Student's t-test was used to determine the statistical difference between two groups. Data were presented as the mean \pm SD. $p < 0.05$, $p < 0.01$ and $p < 0.001$ was considered with statistical significance.

Results

Upregulation of ITGA6 and ITGB4 expression in highly metastatic CRC cells

ITGA6 and ITGB4, members of integrin, have been reported to be involved in tumor metastasis[25, 26]. To explore a possible role of ITGA6 and ITGB4 expression in CRC metastasis, we first evaluated their expression in metastatic vs. non-metastatic CRC cell lines. As shown by Western blot in Fig. 1A, ITGA6 and ITGB4 were highly expressed in high-metastatic-potential HCT116 and SW620 cell lines[27], compared to low tumorigenic and metastatic SW480 and Caco2 cell lines[28, 29]. A similar result was also obtained in RT-PCR analysis (Fig. 1B). Further immunofluorescent analyses confirmed that ITGA6 and ITGB4 were located in the cytoplasm, and their expressions were higher in HCT116 and SW620 cells compared to SW480 and Caco2 cells (Fig. 1C-D). These results revealed a possible association of ITGA6 and ITGB4 with CRC metastasis.

Upregulation of extracellular ITGA6 and ITGB4 protein in metastatic CRC patients and cell lines

Previous research found that exosomal integrins could be used to predict organ-specific metastasis in clinical[30]. We next used the ELISA assay to examine the abundance of ITGA6 and ITGB4 protein in the

blood plasma CRC patients. The result showed that ITGA6 and ITGB4 protein levels were expressed in higher levels in patients with distant metastasis compared to those without distant metastasis (Fig. 2A). Furthermore, ITGA6 and ITGB4 proteins levels were also found in higher levels in supernatants of HCT116 and SW620 cell culture compared to that of SW480 and Caco2 cells (Fig. 2B). Thus, based on the data obtained from both patient's blood samples and cancer cell culture supernatant, we conclude that ITGA6 and ITGB4 proteins are highly abundant in metastatic CRC compared to low-metastatic ones, which suggest a possible role of CRC-secreted ITGA6 and ITGB4 in metastasis.

Upregulation of exosomal ITGA6 and ITGB4 proteins in metastatic CRC cells

A mount of studies has demonstrated that exosomes released from CRCs can be taken by various types of cells[31]. Given that a previous study has shown that ITGB4 acts as a cargo carried by exosome to exchange in tumor cells[32], we next explored whether ITGA6 and ITGB4 can be secreted via exosomes. We incubated the CRC cell lines in an exosome-free medium made from exosome-free FBS and then isolated the CRC-secreted exosomes using ExoQuick-TC Exosome Precipitation kit. Transmission electron microscope (TEM) was used to characterize the morphology and relative purity of isolated exosomes. As shown in Fig. 2E, the exosomes secreted in the CRC cell culture supernatant were a double-layer membrane with a uniformly cup-shaped morphology; ranged from 40 nm to 100 nm. To further explore the characteristics of those exosomes and to quantify them in the cell culture supernatants, we examined CD63 and CD9, exosome-specific markers[33, 34]. The western blot analysis showed that exosomal ITGA6 and ITGB4 expression were higher in the supernatant of HCT116 and SW620 cells culture compared to SW480 and Caco2 cells (Fig. 2F). Together, these results demonstrate that the vesicles isolated from the conditional media were the exosomes and exosomal ITGA6, ITGB4 were highly expressed in metastatic CRC cells.

The exosomal ITGA6 and ITGB4 prompt the proliferation and tube formation capacity of endothelial cells.

Vascular endothelial cells, which line tumor blood vessel, are well known to be required for a tumor to metastasize. To evaluate the functional role of exosomal ITGA6 and ITGB4 in promoting metastasis, we labeled the exosome with PKH67, a green fluorescence protein dye, and then co-cultured with vascular endothelial cells. The results shown by fluorescence microscope indicated that vascular endothelial cells could effectively acquire the exosome from CRC cells (Fig. 3A). Meanwhile, we investigated the effect of exosomal ITGA6 and ITGB4 on the proliferation in vascular endothelial cells *in vitro*. The results showed that the exosomes derived from HCT116 and SW620 cells could dramatically promote the proliferation of vascular endothelial cells compared to the exosome from Caco2 and SW480 (Fig. 3B). The tubulogenic capacity is a critical feature of vascular endothelial cells, which is benefit to tumor growth. We further assessed the effect of ITGA6 and ITGB4-containing exosomes on the Tubulogenesis of vascular endothelial cells. The result showed that HCT116 and SW620-derived exosomes, when co-cultured with

vascular endothelial cells significantly increased the tubulogenic capacity as compared to that Caco2 and SW480-derived exosomes (Fig. 3C).

To further demonstrate the role of exosomal ITGA6 and ITGB4, we silenced the expressions of ITGA6 and ITGB4 in HCT116 and SW620 cells using different, non-overlapping small hairpin RNA (shRNA) as confirmed by qRT-PCR (Supplementary Fig. S1A) and Western blot (Supplementary Fig. S1B). Knockdown of ITGA6 and ITGB4 in CRC cells also resulted in downregulation of ITGA6 and ITGB4 in exosome collected from SW620 and HCT116 cells, respectively (Fig. 4A). Accordingly, exosomes from SW620-shITGA6/ITGB4 and HCT116-shITGA6/ITGB4 showed markedly reduced ability to promote the proliferation of vascular endothelial cells (Fig. 4B). Furthermore, when co-cultured with vascular endothelial cells, the tubulogenic capacity was markedly decreased upon ITGA6/ITGB4 knockdown (Fig. 4C).

The above results suggested that exosomal ITGA6 and ITGB4 directly regulate the proliferation and tubulogenesis of vascular endothelial cells. To further confirm this conclusion, we over-expressed the ITGA6 and ITGB4 in SW480 and Caco2 cells (Supplementary Fig. S2A-B), which leads to increased expression of ITGA6 and ITGB4 in exosomes (Fig. 5A). Consistently, the ITGA6/ITGA4-enriched exomes from SW480 and Caco2 promoted the proliferation of vascular endothelial cells significantly (Fig. 5B). Moreover, the tubulogenic capacity was markedly increased when co-cultured with ITGA6/ ITGB4-overexpressing SW480 and Caco-2 cells (Fig. 5C). Taken together, our results showed that exosomal ITGA6 and ITGB4 could significantly promote the proliferation and the tubulogenesis of vascular endothelial cells.

The exosomal ITGA6 and ITGB4 promote the lung metastasis of CRC.

Tumor spread to the distant organ is an obstacle for cancer treatment[35]. Meanwhile, a different type of metastatic cancer cells has a characteristic metastatic pattern, which defined as organ tropism. The lung is one of a common target organ for CRC metastasis[36]. To further explore the biological effects of exosomal ITGA6 and ITGB4 on CRC metastasis *in vivo*, we used a xenograft mouse model. The CRC cells were injected through the caudal vein to establish an animal model for colon cancer metastases to the lung. The exosomes derived from HCT116 and SW620 cells were also injected through the caudal vein (25 µg/ml/100 µl; 3 injections per week). As shown in Fig. 6A, there is no obvious lung metastasis in the mice injected with SW480 cells alone. However, the exosomes derived from highly metastatic counterpart SW620 could significantly increase the lung metastasis of SW480 cells (Fig. 6A-B). The SW620-xenograft gave rise to obvious metastasis in lung; but treatment with GW4869, an inhibitor of exosome biogenesis/release[37] significantly inhibited the CRC cells metastasize to the lung (Fig. 6C-D). Moreover, exosome derived from SW620-shITGA6/ITGB4 has reduced the ability to promote lung metastasis (Fig. 6E-F). Taken together, those data has validated a role of exosomal ITGA6 and B4 in underlying the lung metastasis of CRC.

Discussion

Exosomes are small extracellular membrane vesicles secreted by most cultured cells. They are involved in regulating intercellular communication, providing an opportunity for the exchange of DNA, mRNAs, microRNAs (miRNAs), proteins, and other molecules between donor cells and recipient cells and acting to regulate their function[38–40]. The previous study showed that exosomes take part in mediating the tumor invasion and metastasis[41]. In this study, we demonstrated a close correlation of intracellular and extracellular ITGA4 and ITGB6 with CRC metastasis through both clinical sample and CRC cell line analysis. We further provided functional validation of exosomal ITGA4 and ITGB6 in vitro and in vivo. Our work demonstrates that CRCs secrete ITGA4 and ITGB6-carrying exosomes, to promote lung metastasis of CRC.

Our finding is consistent with the previous reporting indicating a role of ITGA6 or ITGB4 in cancer invasion, metastasis and poor prognosis in human gallbladder carcinoma[26]. Kwon *et al.* have shown that ITGA6 was highly expressed in esophageal squamous cell carcinoma and plays a vital role in the progression of cancer cells by regulating the proliferation and invasiveness of these cells[42]. ITGB4 has been reported to participate in regulating the invasive and metastatic behavior in cancer cells[43]. Chen *et al.* showed that ITGB4 is involved in regulating migration and invasion of ovarian cancer cells[44].

Angiogenesis plays a vital role in survival, growth, and development of solid tumors, and it has become clear that the establishment of new microvessels may provide nutrition for cancer cells proliferation[45–47]. It has been shown that cancer-associated exosomes take part in the establishment of a pre-metastatic microenvironment which contributes to metastasis formation[48]. In this study, we demonstrated that exosomal ITGA6/ITGB4 could promote the proliferation and tubulogenic capacities of endothelial cells, which may organize a pre-metastatic microenvironment and contribute to CRC metastasize to the lung.

Conclusions

In conclusion, our findings might imply that circulating tumor-derived exosomes perform distinct roles in completing pre-metastatic niche evolution. Our findings demonstrate an important role for exosomes in dictating distant organ metastasis, thus providing a basis for deciphering the mystery of organotropism and developing the novel therapeutic strategy for CRCs.

Abbreviations

CRC: Colorectal cancer; ITGA6: Integrin $\alpha 6$; ITGB4: Integrin $\beta 4$; CBX8: Chromobox homolog 8; ATCC: American Type Culture Collection; DMEM: Dulbecco's modified Eagle medium; FBS: Fetal bovine serum; TEM: transmission electron microscopy; qRT-PCR: Quantitative real-time PCR; miRNA: microRNA.

Declarations

Acknowledgements

Not applicable.

Authors' contributions

WT and JL conceived and designed the present study; TL, JH, CW, QH and GQ performed the experiments; HZ and YL analyzed and interpreted the data. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Informed consent forms have been signed by all patients before this study. The research protocol has been approved by the Ethics Committee of the Guangxi Medical University Cancer Hospital and is based on the ethical principles of medical research involving human subjects in the Helsinki Declaration.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

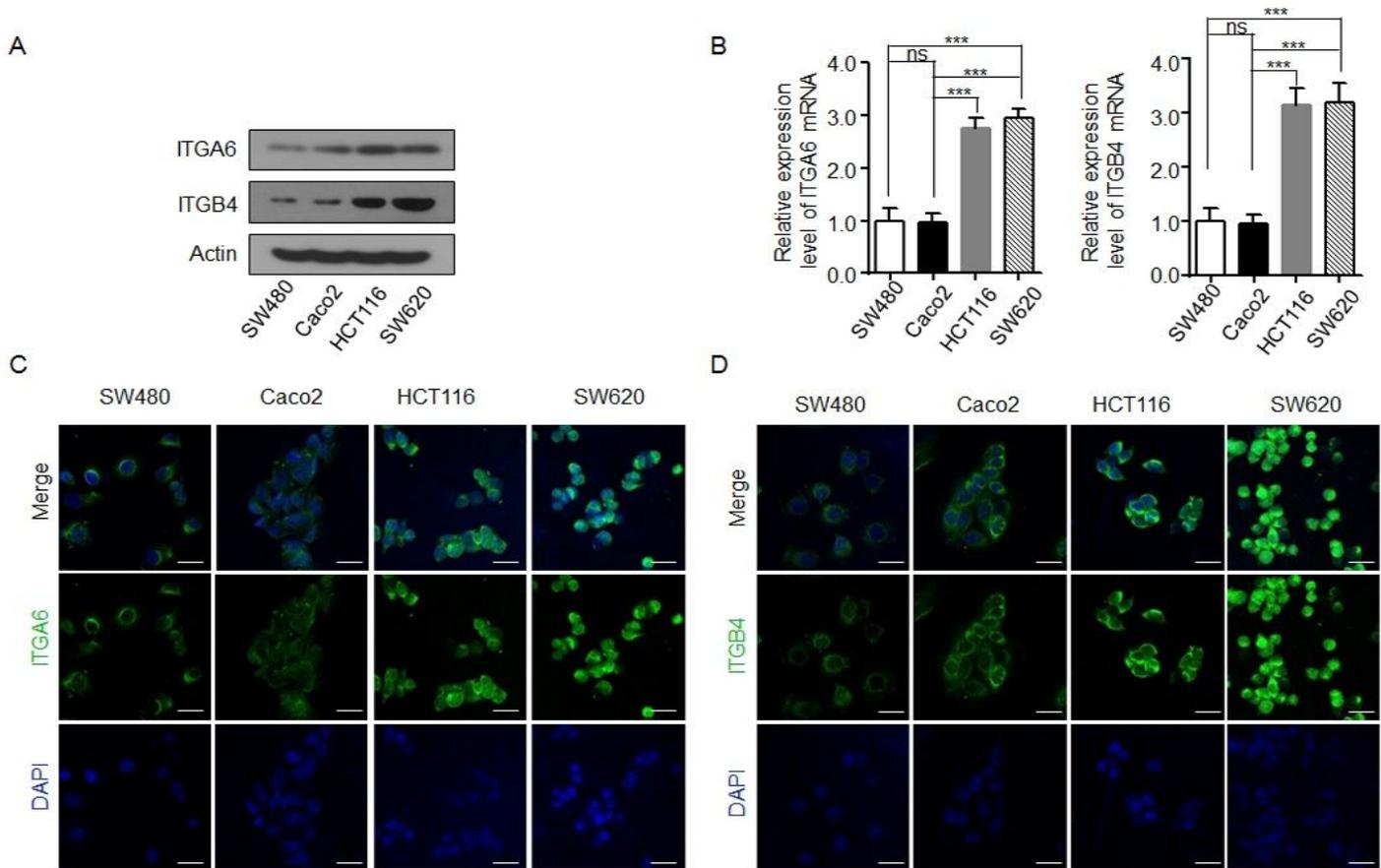


Figure 1

ITGA6 and ITGB4 are highly expressed in SW620 and HCT116 compared to SW480 and Caco2. (A) Western blot analysis showed higher ITGA6 and ITG4 expression in SW620 and HCT116 compared to SW480 and Caco2. (B) qRT-PCR analysis confirmed the Western blot results at mRNA level. (C) Immunofluorescence analysis showed that ITGA6 is increased in SW620 and HCT116 compared to SW480 and Caco2. Scale bar = 100 μ m. (D) Immunofluorescence analysis showed that ITGB4 is increased in SW620 and HCT116 compared to SW480 and Caco2. Scale bar = 20 μ m. Data was presented as the mean \pm SD from three independent experiments. ***, $p < 0.001$; ns, no significance.

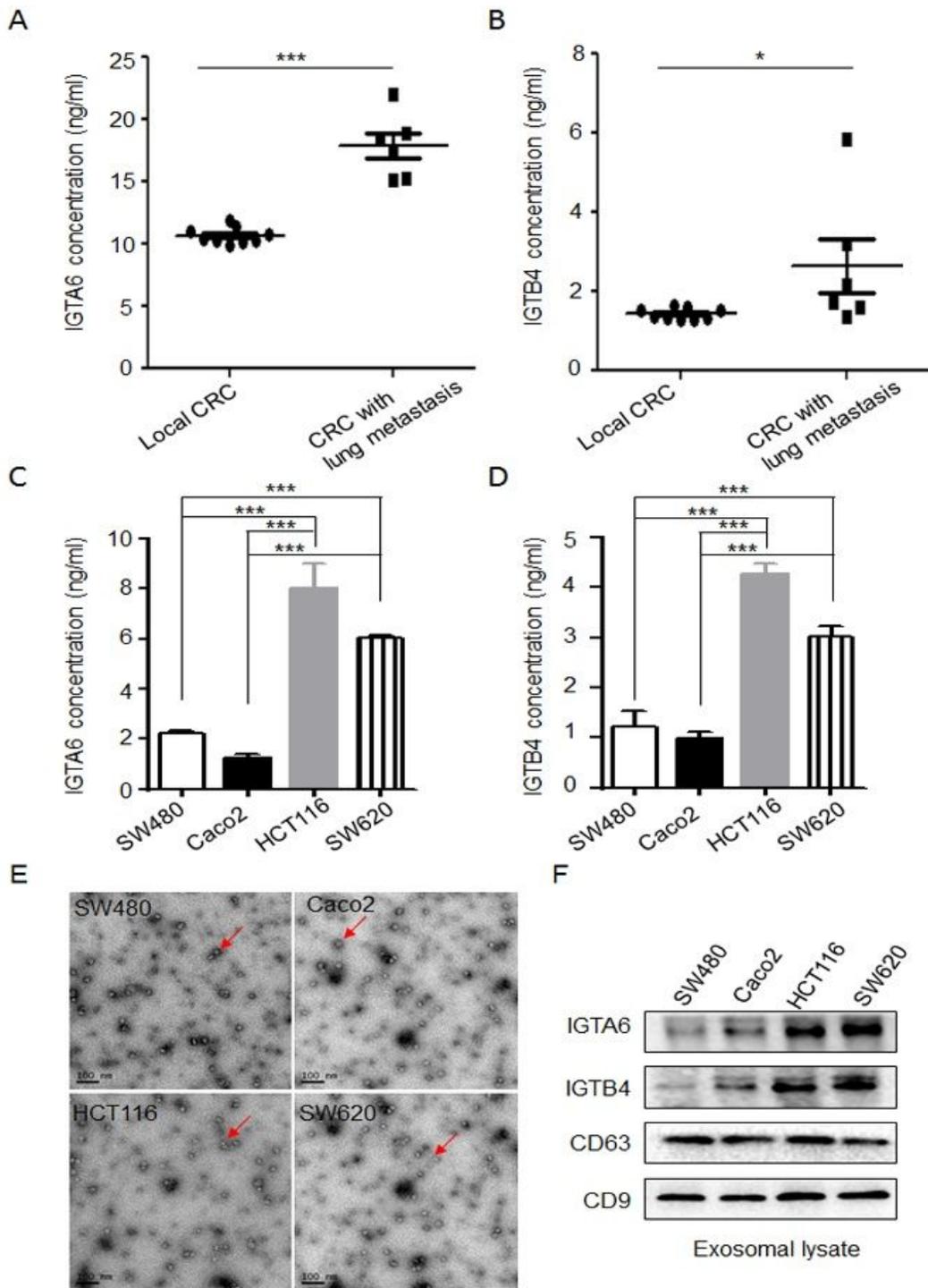


Figure 2

ITGA6 and ITGB4 are highly secreted in high-metastatic-potential CRCs compared to low-metastatic-potential ones. (A-B) ELISA assay revealed that ITGA6 (A) and ITGB4 (B) concentration in the serum from the patients with distant metastasis (Case#4 and Case#5) compared to patients without distant metastasis (Case#1, Case#2 and Case#3). (C-D) ELISA assay revealed that ITGA6 (C) and ITGB4 (D) concentration in the supernatants from the culture of SW620 and HCT116 were significantly higher than

that from the culture of SW480 and Caco2. (E) Transmission electron microscope (TEM) images of CRC-derived exosomes isolated from supernatants of CRC cells. Scale bar = 100 nm. (F) Western blot analysis showed higher exosomal ITGA6 and ITG4 expression in SW620 and HCT116 compared to SW480 and Caco2. Data was presented as the mean \pm SD from three independent experiments. ***, $p < 0.001$

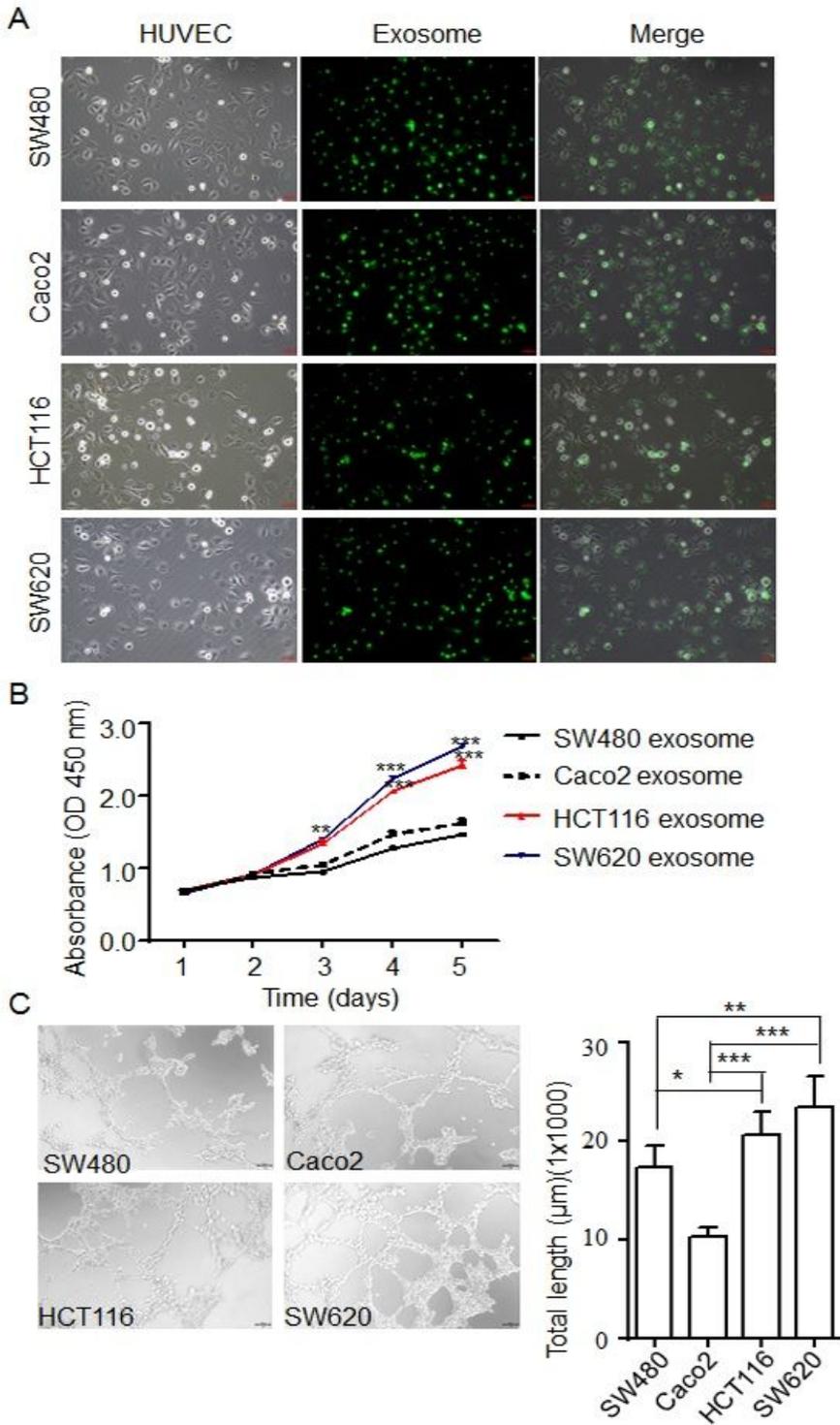


Figure 3

Exosomal ITGA6 and ITGB4 regulated the proliferation and tube formation of vascular endothelial cells. (A) Representative images of PKH67-labelled (green) CRC-derived exosomes were co-cultured with vascular endothelial cells (HUVEC). The uptake of CRC-derived exosomes by HUVEC was observed under a confocal microscope. (B) In vitro proliferative analysis of HUVEC cells co-cultured with CRC-derived exosomes. (C) The representative images and quantification of tube formation of HUVEC cells co-cultured with CRC-derived exosomes. Inspection under a phase contrast fluorescent microscope ($\times 100$). Data was presented as the mean \pm SD from three independent experiments. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

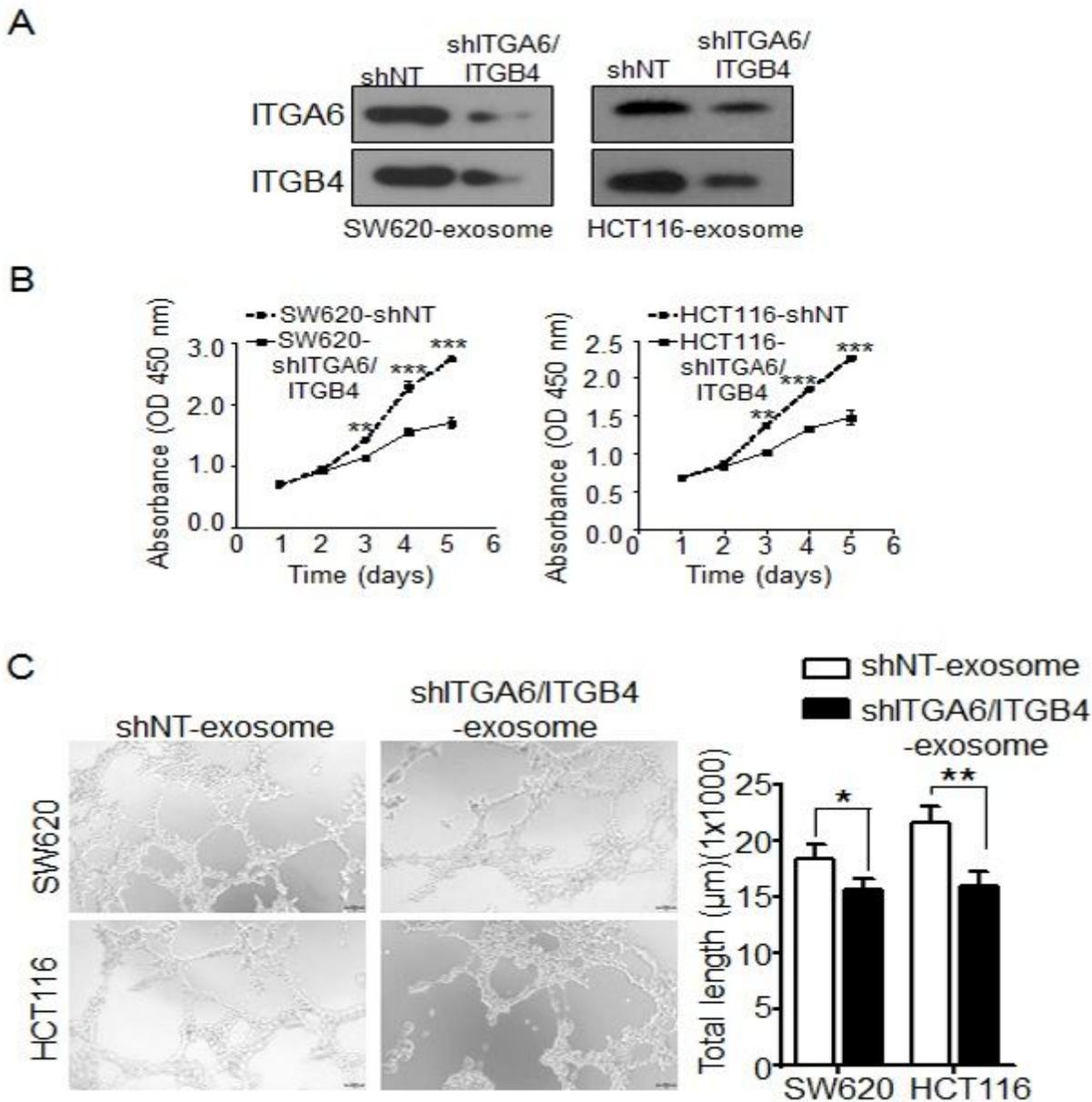


Figure 4

Disrupting ITGA6 and ITGB4 in CRC cells decreased the proliferation and tube formation of vascular endothelial cells. (A) Western blot analyses of the expression of ITGA6 and ITGB4 in SW620- and HCT116- derived exosomes expressing shITGA6/ITGB4 or shNT. (B) In vitro proliferative analysis of HUVEC cells co-cultured with exosomes derived from SW620 and HCT116 expressing shITGA6 /ITGB4 or shNT. (C) The representative images and quantification of tube formation of HUVEC cells co-cultured exosomes derived from SW620 and HCT116 expressing shITGA6 /ITGB4 or shNT. Inspection under a phase contrast fluorescent microscope ($\times 100$). Data was presented as the mean \pm SD from three independent experiments. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

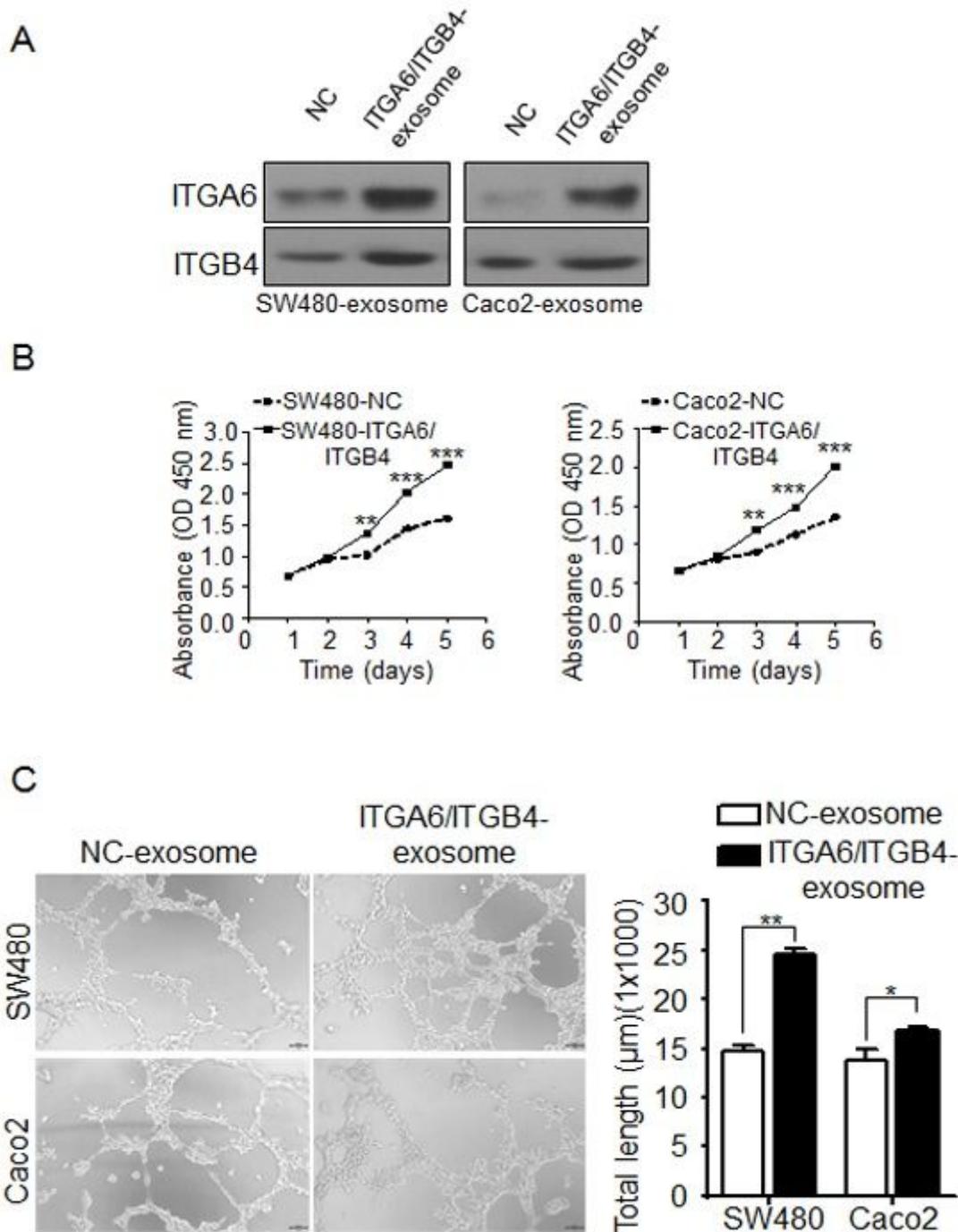


Figure 5

Over-expression of ITGA6 and ITGB4 enhances the proliferation and tube formation of vascular endothelial cells. (A) Western blot analyses of the expression of ITGA6 and ITGB4 in exosomes derived from SW480 and Caco2 expressing ITGA6/ITGB4 or control. (B) In vitro proliferative analysis of HUVEC cells co-cultured with exosomes derived from SW480 and Caco2 expressing ITGA6/ITGB4 or control. (C) The representative images and quantification of tube formation of HUVEC cells co-cultured exosomes

derived from SW480 and Caco2 expressing ITGA6/ITGB4 or control. Inspection under a phase contrast fluorescent microscope ($\times 100$). Data was presented as the mean \pm SD from three independent experiments. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

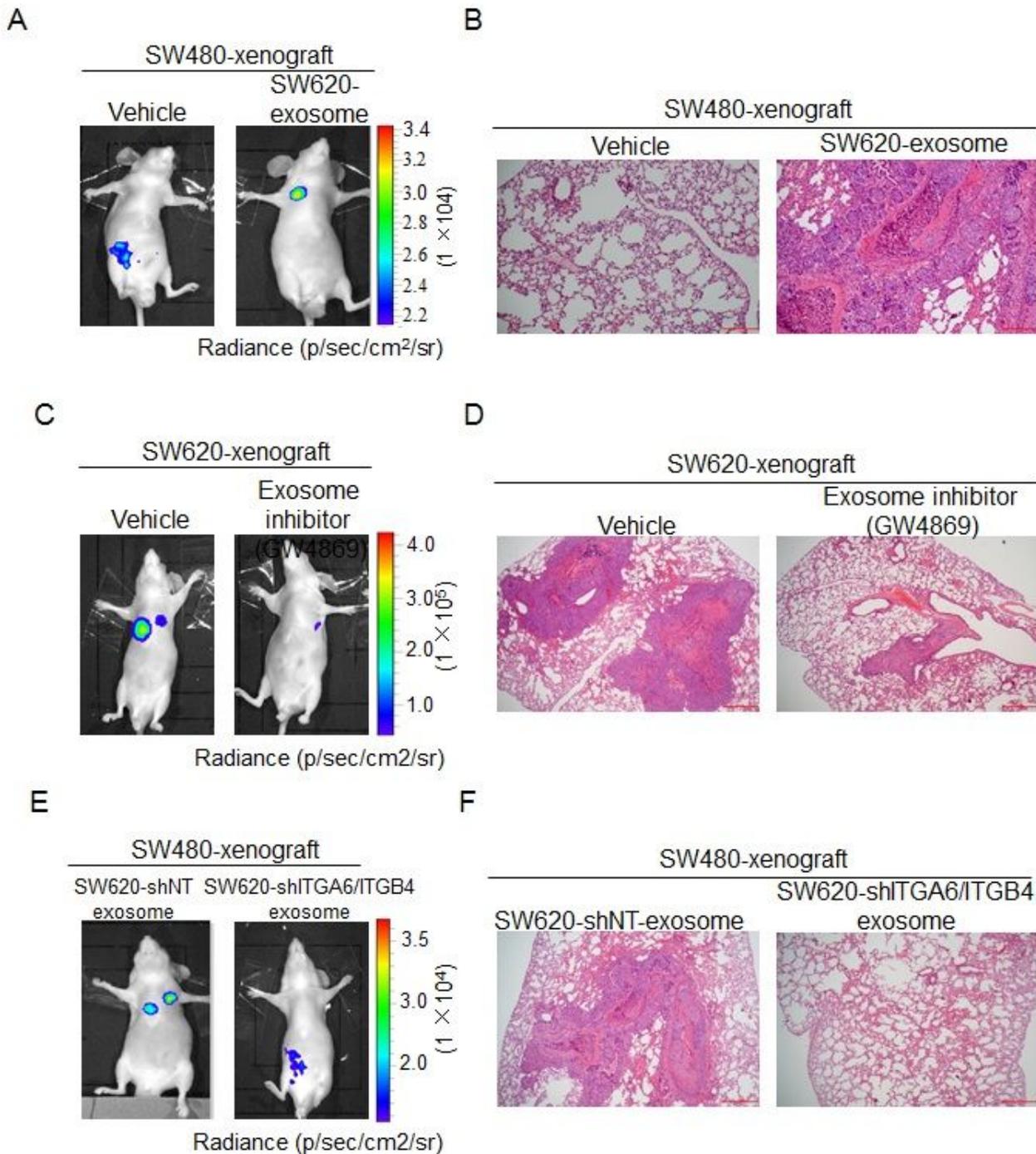


Figure 6

Exosomal ITGA6 and ITGB4 promote the lung metastasis of CRC. (A) Representative bioluminescent images of xenografts derived from SW480 treat without or with SW620-derived exosomes. Abbreviation:

p, photons; sec, second; sr, steradian. (B) Representative images of H&E staining in xenograft specimens without or with SW620-derived exosomes. (C) Representative bioluminescent images of xenografts derived from SW620 treat without or with GW4869, an inhibitor of exosome biogenesis/release. Abbreviation: p, photons; sec, second; sr, steradian. (D) Representative images of H&E staining in xenograft specimens derived from SW620 treat without or with GW4869, an inhibitor of exosome biogenesis/release. (E) Representative bioluminescent images of xenografts derived from SW480 treat without or with SW620-derived exosomes expressing shITGA6/ITGB4 or shNT. .Abbreviation: p, photons; sec, second; sr, steradian. (F) Representative images of H&E staining in xenograft specimens derived from xenografts of SW480 treat without or with SW620-derived exosomes expressing shITGA6 /ITGB4 or shNT. Data was presented as the mean \pm SD from three independent experiments.

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