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CELLULAR AND MOLECULAR BIOLOGY

Inhibiting CD164 Expression in Colon Cancer Cell Line HCT116 Leads to Reduced Cancer Cell Proliferation, Mobility, and Metastasis *in vitro* and *in vivo*

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Background: CD164 (Endolyn) is a sialomucin, which has been found to play roles in regulating proliferation, adhesion, and differentiation of hematopoietic stem cells. Possible association of CD164 with solid cancer development remains unknown.
Methods and Results: We first studied CD164 expression in biopsies from colorectal cancer, breast, and ovary cancer patients by semi-quantitative immunohistochemistry, and found that CD164 was strongly expressed in all the colorectal cancer samples compared to the matching normal colon tissues. The possible roles of CD164 in colon cancer development were further investigated using a well-established human colon cancer cell line HCT116. We found that knockdown of CD164 expression in HCT116 cells significantly inhibited cell proliferation, mobility, and metastasis *in vitro* and *in vivo*. The knockdown of CD164 expression was associated with decreased chemokine receptor CXCR4 expression HCT116 cell surface and immunoprecipitation studies showed that CD164 formed complexes with CXCR4.
Conclusions: CD164 is highly expressed in the colon cancer sites, and it promotes HCT116 colon cancer cell proliferation and metastasis both *in vitro* and *in vivo*, and the effects may act through regulating CXCR4 signaling pathway. Therefore, CD164 may be a new target for diagnosis and treatment for colon cancer.

Keywords CD164; Colon cancer; CXCR4; Metastasis.

INTRODUCTION

CD164 (MGC-24v or endolyn) is a member of the sialomucin family a mucin containing sialic acid, which is highly conserved in human and other species (1–4). CD164 was first identified in CD34+ human hematopoietic progenitor cells and bone marrow stromal reticular cells (2, 5, 6). CD164 has been implicated in adhesion, proliferation, and differentiation of hematopoietic stem and progenitor cells (5, 7), it was suggested to mediate the adhesion of $CD34^+$ hematopoietic progenitor cells to bone marrow stromal cells and SDF-1 induced binding to bone marrow endothelial cell (2, 8.9). CD164 are thought to regulate hematopoiesis by facilitating the adhesion of human CD34+ cells to bone marrow stroma (10). Knocking down CD164 expression in Drosophila S2 cells increased the cell apoptosis rate (1). Little is known about the relationship between CD164 and solid tumor development till Havens *et al.* reported that CD164 may participate in mediating prostate cancer bone metastasis in 2006 (11). Continuous exposure to an environment of high Zn^{2+} can lead to the upregulation of CD164 in prostate cancer cells, CD164 is considered to be a cancer promoting gene (12). Recently, CD164 has also been recognized as a potential diagnostic marker for acute lymphoblastic leukemia and allergy (13, 14).

Cancer metastasis is a complex process in which malignant cells break away from primary tumor, attach to the degraded proteins from the surrounding extracellular matrix and migrate to other locations via the bloodstream or the lymphatic system. The tumor cell proliferation, adhesion, and migration are involved and tightly regulated during tumor metastasis process. The relationship of CD164 and cancer development especially metastasis remains unexplored. In this study, we have investigated the expression of CD164 in human colon cancers and used a well-established human colon cancer cell line HCT116 to evaluation the roles of

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CD164 in cancer cell proliferation and migration (metastasis) *in vitro* and *in vivo*. We also explored the possible underlining mechanisms of CD164 in colon cancer development.

MATERIALS AND METHODS

Immunohistochemistry on human colon and other cancer specimens

Twenty (20) biopsies from colorectal cancer patients, 5 samples from breast cancer patients and 5 samples from ovary cancer patients were obtained with patient's consent following excision of the cancers. The specimens were coded and fixed in 10% buffered formalin pH 7.0 for 24 hr and then embedded in paraffin wax. The 5-μm thick sections were cut using a rotary microtome (Microm HM315, Walldorf, Germany) and the sections were placed on the poly-L-lysine (Sigma Chemical Co., Poole, UK) coated slides. For immunohistochemistry, sections were dewaxed in xylene and immersed in graded ethanol and distilled water. Endogenous peroxidase activity was inhibited by immersing the tissue sections in 0.3% hydrogen peroxidase for 20 min at room temperature. The sections were subsequently rinsed in phosphate buffer solution (PBS). The sections were then incubated with purified rabbit anti-human CD164 antibody (1:100 ratio in PBS) for 45 min at room temperature. Subsequently, the primary antibody was blotted and the tissues were rinsed with PBS. The secondary purified goat anti-rabbit biotinglated antibody (DAKO, Dorset, UK, 1:100 in PBS) was applied to the sections, and incubated for 30 min at room temperature. Sections were rinsed in PBS and were then incubated with Streptavidin-peroxidase (1:50 in PBS, DAKO, Dorset, UK) for 30 min at room temperature. Diaminobenzidine chromagen solution (0.5 mg/mL 2,4,2′,4′–tetrabiphenyl hydrochloride, Sigma Chemical Co., Poole, UK) in 0.1 m imidazole in PBS, containing 0.3% hydrogen peroxidase was added to the sections, and incubated at room temperature for 5 min. Following a brief rinse in distilled water the tissue sections were counterstained in Gill's No. 3 hematoxylin (Sigma Chemical Co., Poole, UK) at a dilution of 1:3 for 5 min, rinsed in distilled water and dehydrated through a series of graded ethanol solutions to xylene, mounted in DPX and examined by light microscopy. Primary antibody was omitted as a negative control for the immunostaining method.

Plasmids

The lentivirus packaging plasmids pLP1, pLP2, and pLP/VSVG were bought from Invitrogen, USA. All expression vectors were constructed with conventional cloning and PCR techniques. To construct a GFP and Luciferase vector for lentivirus production, luciferase and eGFP cassette was cloning by PCR methods from plasmid pCDNA3-Luciferase-eGFP as described previously (15). Lenti-Luciferase-eGFP-TOPO construct was selected and identified through restriction digestions and DNA sequencing.

Cell culture

HCT116 cell line was obtained from the European Collection of Cell Cultures. CD164 knockdown HCT116 (HCT116-CD164-shRNA), knockdown control clone (HCT116-shRNA control) were established by short hairpin RNA (shRNA) transduction method (see below). These cells were cultured in DMEM medium supplemented with penicillin G (100 U/mL Sigam, USA), streptomycin (100 mg/mL), and 10% fetal calf serum. Cells were grown at 37°C in a humidified atmosphere of 5% CO_2 and were routinely sub-cultured using 0.25% (w/v) trypsin-EDTA solution.

Lentiviral vector and cell transduction

ViraPower™ lentiviral expression system (Invitrogen, Paisley, UK) was used to introduce Lenti-Luciferase-eGFP-TOPO construct into HCT116 cells. The ViraPower™ packaging mix and Lenti-Luciferase-eGFP-TOPO construct were cotransfected using a gene carrier kit (Epoch-Biolabs, USA) into the 293T cell line to produce a lentiviral stock. At 48-hr post transfection, the virus-containing supernatant was harvested by collecting the medium. Viral particles were purified by ultracentrifugation through a 20% sucrose cushion. For infecting HCT116 cells, cells were cultured in 24 well plates till 80% confluence, the lentivirus was added to the culture dishes and incubated for 48 hr, the medium was then replaced by the selection medium containing 10 μg/mL Blasticidin.

CD164 knockdown HCT116 (HCT116-CD164-shRNA) was established by short hairpin RNA (shRNA) transduction. A set of MISSION shRNA lentiviral transduction particles specific to human CD164 gene was purchased from Sigma Aldrich (Poole, UK). The set included five clones targeting different parts of CD164 cDNA sequences. For transduction, HCT116 cells were plated in 96-well plate the day to allow 50% confluence, 100 μL medium containing 15 μL of thawed MISSION shRNA lentiviral particles (MOI 30∼40) as well as 8 μg/mL Polybrene were added to the cells for 48 hr. The medium was then changed to media containing 2.5 μg/mL Puromysin for stable clone selection. The mock knockdown clone was used as control (HCT116-shRNA control).

RT-PCR

The RNA samples and cDNA samples were prepared using Trizol reagent (Invitrogen, Paisley, UK) and Qiagen QuantiTect™ reverse transcription kit (Qiagen, West Sussex, UK). The PCR was performed using PCR master mix (Promega, Southampton, UK). Briefly, a master mix was prepared by mixing 2× PCR master mix (10 μL), primers mix (2 μL), RNase-free water (6 μL), and cDNA sample (2 μL; 200 ng/μL). Amplification was performed as follows: 30 cycles of 30S at 94°C for denaturation; 30S at 57°C for annealing and then 45S at 72°C for amplification. PCR of 18S is used as internal control. The PCR products were evaluated by electrophoresis in 1.0% Agarose gels. The primers used are shown as follows:

CD164-forward: 5′-GTGCTGTCCGCGGACAAGAAC-3′
CD164-reverse: 5′- TGTGAACAATAGCTCTCATC -3′
18S-forward: 5′-GTAACCCGTTGAACCCCATT-3′

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18S-reverse: 5′-CCATCCAATCGGTAGTAGCG-3′

Immunoprecipitation and Western blot

For immunoprecipitation, resuspended protein A-sepharose beads (Amersham Biosciences, UK) in 1 mL of mRIPA lysis buffer, 200 ug of cell total protein sample and 4 ug of rabbit-anti-human CD164 were added to each reaction tube, and the tubes were rotated at room temperature for 2 hr. The immuneprecipitates were then washed, suspended in sample buffer, and boiled for 5 min to dissociate protein with beads.

For Western blotting, protein was extracted with mRIPA buffer containing protease inhibitors (mRIPA, 50 mM Tris, pH 7.4, 100 mM NaCl, 1% Nonidet P-40, 0.5% deoxycholic acid, 0.1% SDS, Aprotinin 10 μg/mL, Leupeptin 10 μg/mL, PMSF 1 mM) and quantified using BCA™ protein assay kit (Pierce, Northumberland, UK). Rabbit-anti-human CD164 antibody (Abcam, Cambridge, UK) was prepared in 3% blocking buffer at a dilution of 1:1000, incubated with the membrane at 4°C overnight followed by a brief wash and incubation with secondary antibody for 1 hr at room temperature. Finally, peroxide and luminol solutions 1:1 (Pierce Company, UK) were added to cover the blot surface for 5 min at room temperature and the membrane was placed in a developing cassette.

MTT and clonogenic assays

Cells were seeded at a density of 5×10^3 cells/well cells in 96 well plates and MTT assays were performed according to manufacturer's instructions. For clonogenic assay, 200 cells per well were plated in 6-well plates and incubated in DMEM medium for 14 days. Medium was then removed, plates were washed by PBS, stained with 1 mL of 0.4% crystal violet in 70% methanol for 20 min and the colony numbers were counted.

Wound closure assay and transwell migration assay

Cells were grown to 90% confluence in a 6-well plate and a wound was created using a sterile 1000-μL pipette tip. Then, cells were cultured in serum-free media, migration at the corresponding wound site was documented using a digital camera and the wound gap distance was measured. For transwell migration assay, 24-well transwell plates (8-μm pores; Corning Costar, USA) were coated with 0.1% fibronectin (Sigma, UK) for 1 hr at 37°C before cell seeding. Cells were cultured in serum-free media for 24 hr before seeded in triplicate at 0.5×10^5 cells per well in Transwell. After 12 hr, the media from both the chamber and the Transwell were removed, the chamber was gently wiped with a cotton swab. Migrated cells were fixed in 100% methanol for 1 min air dried and the cell numbers on lower suface of the membrane were quantified.

In vivo tumor animal model and *in vivo* imaging examination

Female BALB/c athymic nude mice were used. All animal experiments were carried out in accordance with the Institution's ethical and animal welfare guidance. Xenotransplanted tumor was set up by subcutaneously injecting 4×10^5 HCT116 WT, HCT116-luci-GFP (control) or HCT116-CD164-shRNA cells into the rear dorsum at separate sites. Each group had four mice and the tumor nodules were measured using calipers and calculated using the equation $(1/2)\times(L\times W\times H)$ and volumes recorded from day 4 to day 15.

For metastasis tumor model, 2×10^5 HCT116-luci-GFP (control) and HCT116-CD164-shRNA-luci cells were injected intravenously into tail veins of 10-week old female BALB/c nude mice. D-Luciferin (150 mg/kg) was given to each mouse via i.p. before examination by *in vivo* imaging (IVIS 200, USA). Bioluminescence values were calculated by measuring photons/s/cm2/sr in the region of interest. The gradient number of HCT116-luci-GFP control cells and HCT116-CD164-shRNA-luci cells were used to generate a standard curve.

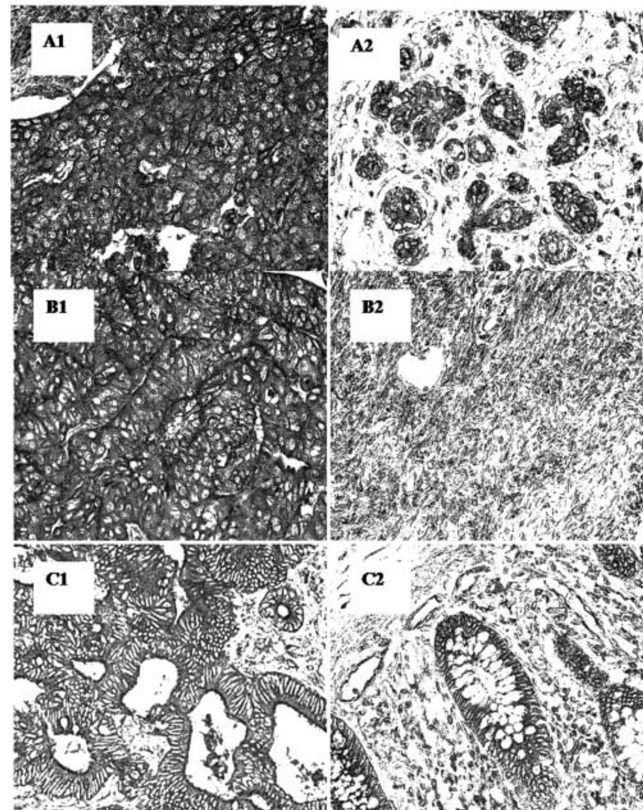

Figure 1. CD164 is overexpressed in human colon cancer as well as breast and ovary cancer. Immunostaining results of CD164 in tumour and matching normal tissues with semi-quantitative scoring (range, +1–+3) based on the staining intensity (brown color) are shown, with +1 being the intensity detected in normal colon, +2 being strong, and +3 being the strongest staining. (A1-A2) In breast cancer, tissue CD164 immunostaining in the infiltrating ductal carcinoma region was very strong (+3), whereas CD164 staining in non-neoplastic of breast tissues was weak (+1); (B1-B2) In serous (ovary) adenocarcinoma, immunostaining of CD164 was strong (+3) and in the non-neoplastic of ovary tissues was weaker (+2); **C1-C2**: Primary colon adenocarcinoma had strong CD164 expression (+3) and the immunostaining of CD164 in non-neoplastic of colon tissue was weak (+1); magnification ×200.

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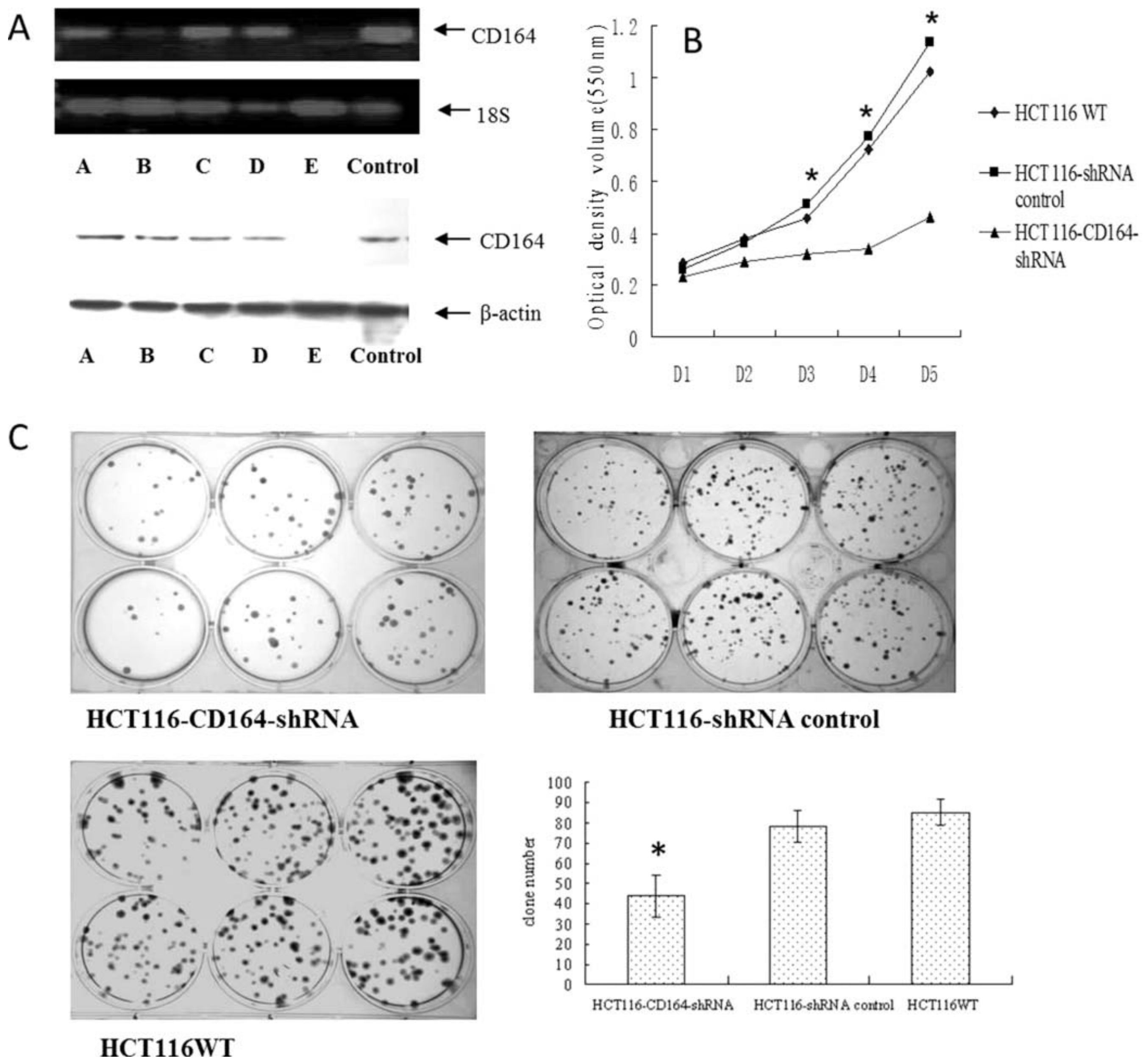

Figure 2. Knocking down CD164 inhibited tumor cell proliferation *in vitro*. (A) Differential expression of CD164 in different HCT116-CD164-shRNA cell clones; expression of CD164 was measured by RT-PCR and western blot in HCT116 cells infected with MISSION shRNA lentiviral transduction particles shRNA A-E, with β-actin as an internal control. shRNA E (SHVRSC-TRCN0000067737) was the most effective sequence to block the CD164 expression, whereas shRNA C has little effect and was used as a methodological control for future study. (B) MTT assay demonstrated that the growth rate of HCT116-CD164-shRNA was significantly reduced than that of the control cell line HCT116-shRNA control and HCT116 WT from day 3 to day 5 ($^*p < .05$, Student's t-test; $n = 6$). (C) The clone formation numbers of the HCT116-CD164-shRNA cells were significantly reduced than those of the HCT116-shRNA control cells and HCT116 WT cells ($^*p < 0.01$, Student's t-test).

Dunn chamber chemotaxis assay

Chemotaxis was measured using Dunn chemotaxis chambers (Hawksley Technology, Lancing, UK). Glass cover-slips were plated with 1×10^5 of HCT116 cells. Cells were starved in serum free DMEM for 24 hr before chemotaxis analysis. Gradients of SDF-1 were formed by placing serum free DMEM in the inner well and 400 ng/mL of SDF-1 in DMEM in the outer well of the Dunn chamber slides. The cover slip was inverted on to the assay slide and the edges sealed with wax. The assay slide was then placed on the heated (37°C) stage of an inverted Nikon microscope equipped with $\times 100$ phase contrast objective linked to a CCD camera. The process was recorded at a time-lapse interval of 5 min over a 10-hr period. The data were analyzed using MATHEMATIC 3.0 (Wolfram Research, Illinois, USA) and AQM 2001 software (Kinetic Imaging Ltd., Manchester, UK).

Statistical analysis

Statistical analysis was performed using Excel (Microsoft) and GraphPad Prism 5.0 (GraphPad Software, USA).

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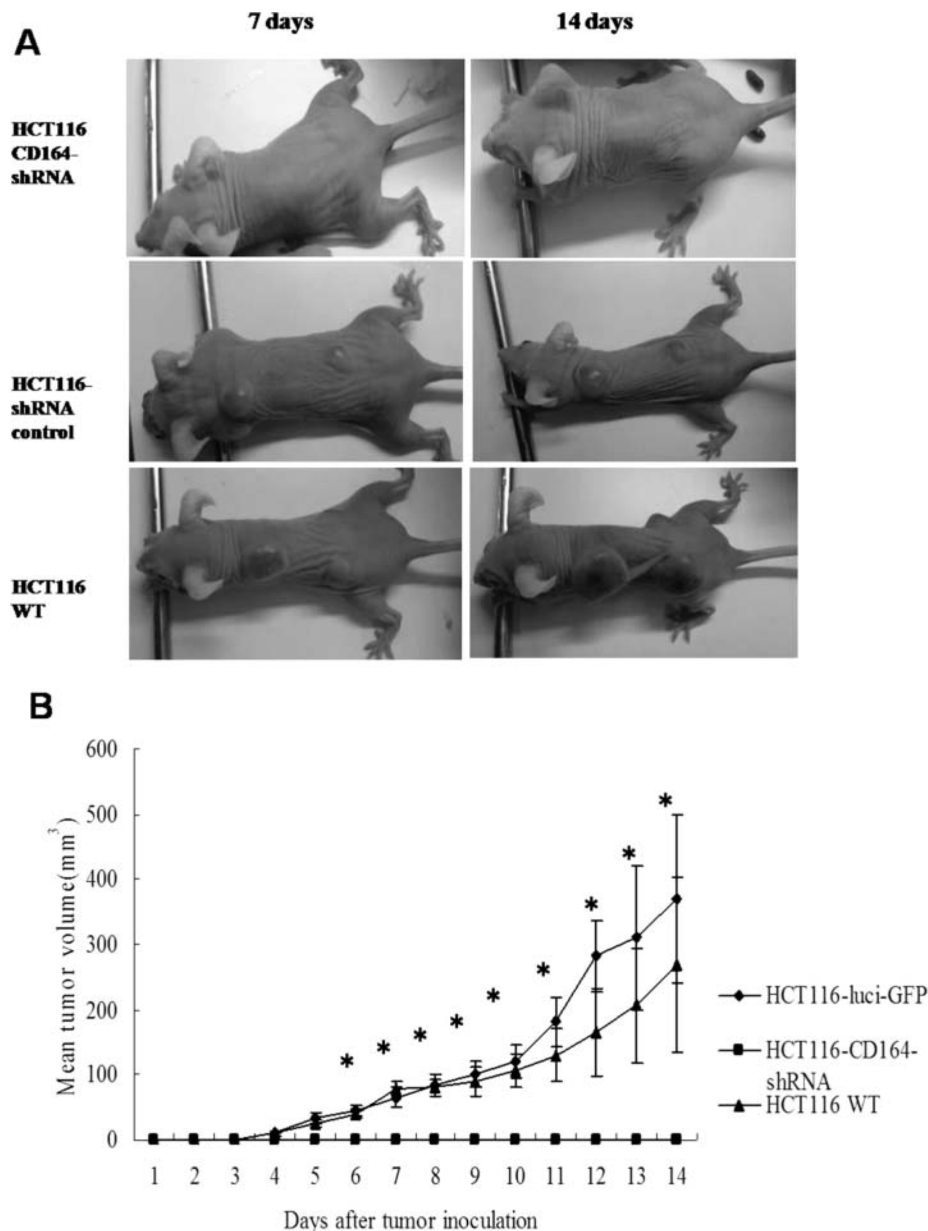

Figure 3. (A) Knocking down CD164 has significantly inhibited HCT116 tumor growth *in vivo*. In Nude mice with subcutaneous injection of HCT116 WT and HCT116-shRNA, control cells had significant tumor growth on day 7 and day 14; whereas the tumor growth in the HCT116-CD164-ShRNA group was negligible at both day 7 and day 14. (B) Knocking down CD164 showed significant retardation in tumor size comparing to control groups from day 6 to day 14, mean ± SD ($n = 4$/group, $p < .001$, Student's t-test).

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Student'st-test and ANOVA were used for comparison; $p < .05$ was considered as significant difference.

RESULTS

CD164 is overexpressed in human colon cancer tissues

Strong expression of CD164 was observed in samples of colon adenocarcinoma, metastatic ovary serous adenocarcinoma and breast infiltrating ductal carcinoma, while only weak staining was detected in the matching normal tissues (Figure 1). On the basis of the relative intensity of staining, the immunostaining was graded on a scale of +1 to +3, with +1 being the intensity detected in normal colon, +2 being strong, and +3 being the strongest staining. As shown in Figure 1, majority of colon cancer showed strong (+2) to very strong (+3) staining and (+1) staining was shown in normal matching colon tissues.

Knockdown of CD164 inhibited tumor cell growth *in vitro* and *in vivo*

Five shRNA clones (shRNA A–E) that specifically block different CD164 gene sites were tested respectively for the knockdown efficiency, and shRNA E (SHVRSC-TRCN0000067737) was shown to be the most effective sequence to knockdown CD164 expression (Figure 2A). The shRNA E cell clone was therefore used as HCT116-CD164-

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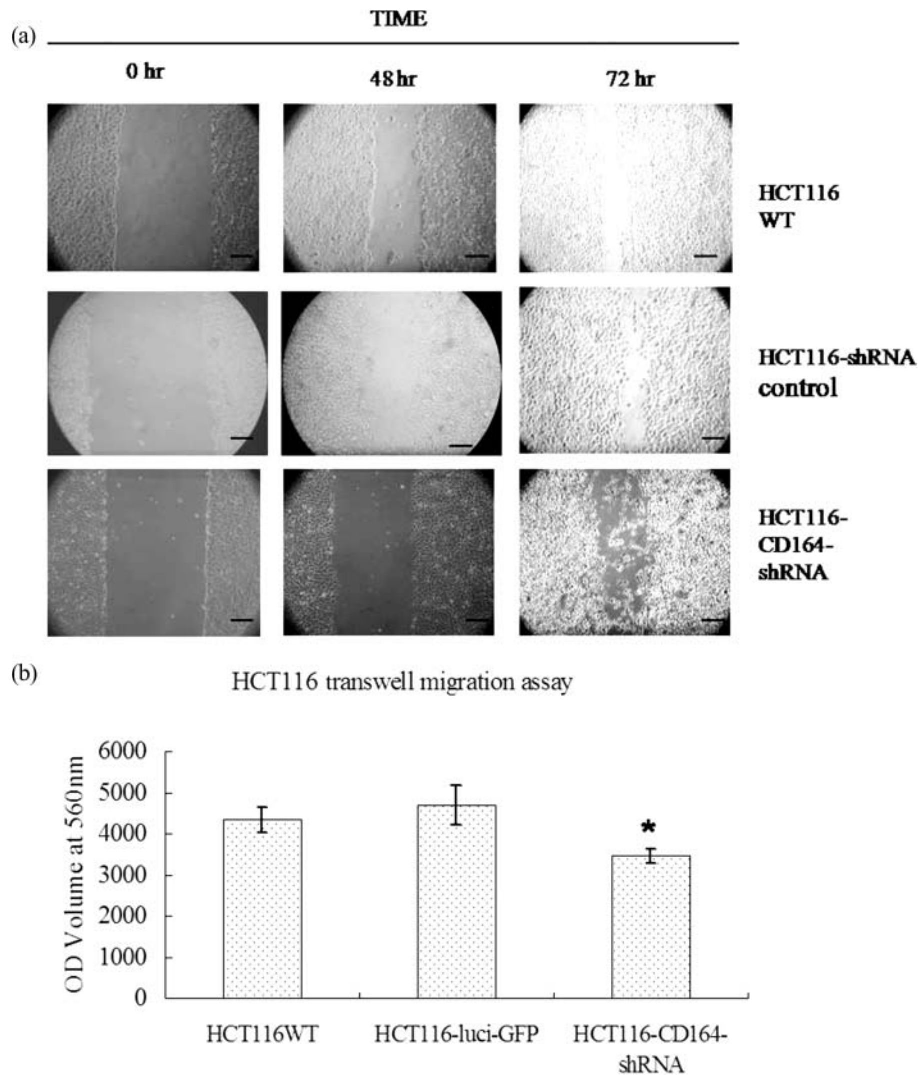

Figure 4. Downregulating CD164 attenuated cell migration ability of HCT116 cells. (A) Photomicrographs of the wound assay experiments showed that the wound gap was significantly wider in the HCT116-CD164-shRNA group than that of the HCT116-shRNA control and HCT116 WT cells groups at 48 and 72 hr. (B) The transwell cell migration assay confirmed that the motility of HCT116-CD164-shRNA cells was significantly reduced compared to the HCT116-luci-GFP and HCT116WT cells (*$p < .05$, Student's t-test).

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shRNA cell line in the future experiments. The shRNA C cell clone that showed weak silence effect was used as a control cell line named HCT116-shRNA control.

MTT assays revealed that the growth rate of HCT116-CD164-shRNA cells was significantly lower than that of the control cell line HCT116-shRNA control and HCT116 WT from day 3 ($p<0.05$, Student's t-test, Figure 2B). The clone numbers of HCT116-CD164-shRNA control and the wild type HCT116 cells were 78.5 ± 8.09 and 85.2 ± 6.24 respectively and there was no statistical significance between these two groups ($p = .247$, Student's t-test), but the clone number in the two groups was significantly higher than that of HCT116-CD164-shRNA, which was 43.8 ± 10.5 ($p < .01$, Student's t-test, Figure 2C).

Subcutaneous injection of HCT116-shRNA control cells and HCT116 WT cells resulted in tumor formation in all nude mice (tumor forming rate was 100%, Figure 3A) and the tumors grew to measurable size 4 days after implantation, whereas in HCT116-CD164-shRNA group, there was only one tumor visible at day 14 but it was too small to be measured (tumor forming rate was 8.3%, Figure 3A). At 14 days following implantation, the average tumor size in the HCT116 WT group (268.5 ± 134.2 mm^3) and HCT116-CD164-shRNA control (370.4 ± 129.9 mm^3) group was similar ($p > .05$, Student's t-test, Figure 3B); but knocking down CD164 significantly inhibited tumor growth at day 14 ($p < .001$, Student's t-test, Figure 3B).

Knockdown CD164 expression inhibited HCT116 cell motility and metastasis

Knocking down CD164 delayed the wound gap closure in a time-dependent manner (Figure 4A). The wound gap was wider in HCT116-CD164 shRNA cells than that of the control cells ($p < .05$. Student's t-test). The transwell migration assay confirmed that the motility of HCT116-CD164-shRNA cells was significantly reduced compared to

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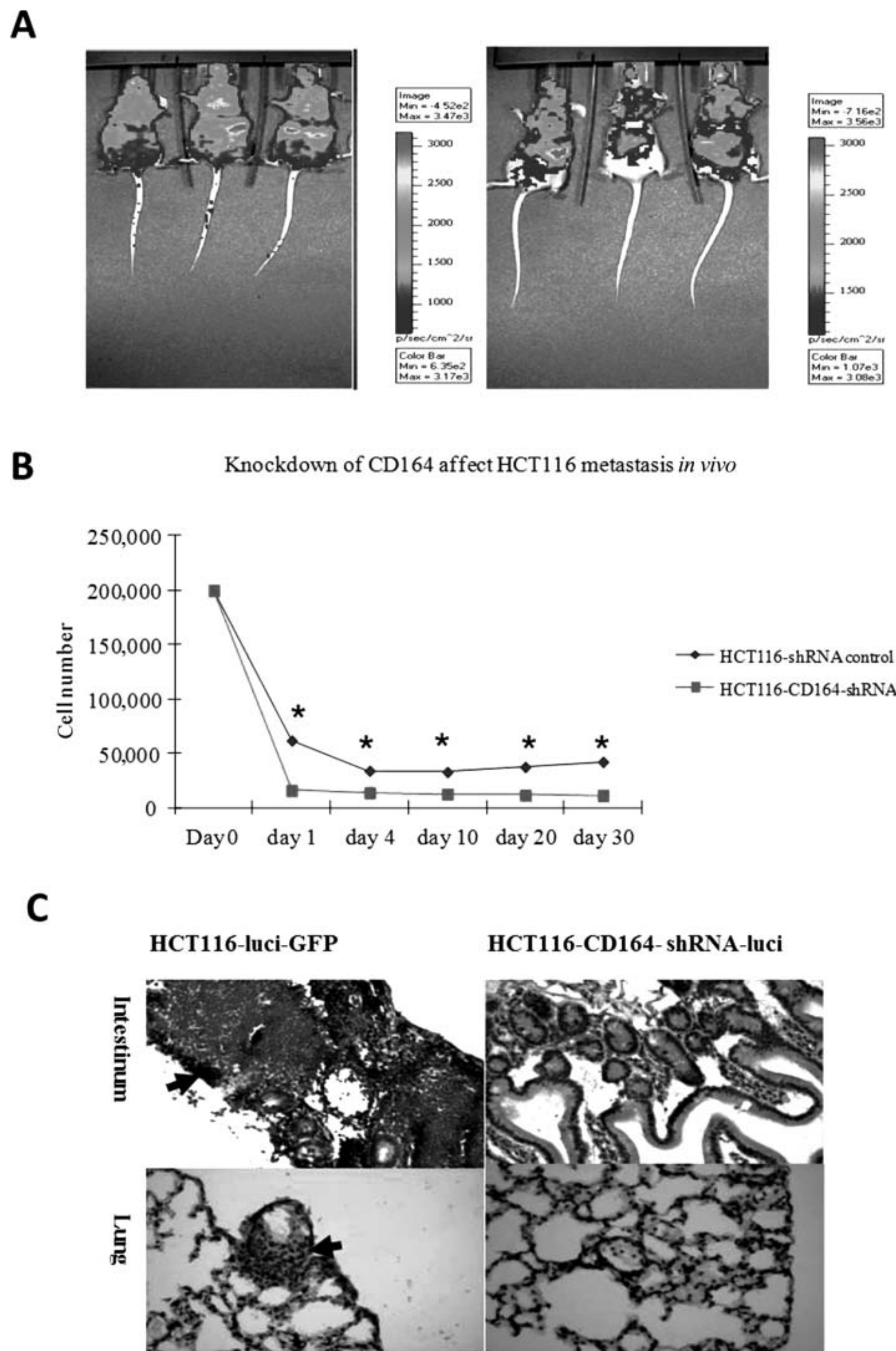

Figure 5. (A) Representative of luciferase *in vivo* images of HCT116-luci-GFP cells (left panel) and HCT116-CD164-shRNA-luci cells (right panel) systemic distribution in nude mice following tail vein injection at day 14. (B) There were significantly more luciferase-labelled tumor cells detected in the HCT116-Luci-GFP group comparing to the HCT116-CD164-shRNA-luci from day 1 to day 30 following injection ($^*p < .05$, Student's *t*-test). The color bar indicates the luciferase signal intensity and the mean cell numbers in both groups were calculated from the standard curves, means ± SD ($n = 3$/group, $p < .05$, Student's *t*-test). (C) The metastatic nodules, irregular cells and nucleic heteromorphism were detected in the HCT116-luci-GFP control group (arrows), whereas no visible metastatic nodule was found in the HCT116- CD164-shRNA-luci group, H&E staining, ×200.

the HCT116-luci-GFP and HCT116WT cells (Figure 4B). In the Nude mice received systemic injection of cancer cells, the time course of cell survival and proliferation were showed in Figure 5A–B using *in vivo* imaging system, which demonstrating that the cell survival ability and proliferation in nude mice were obviously attenuated in HCT116-CD164-shRNA-luci group compared to HCT116-CD164-shRNA control group (Figure 5B). The metastasis nodules, irregular cells, and nucleic heteromorphism in lung and intestinal tracts were frequently detected in the HCT116-shRNA control

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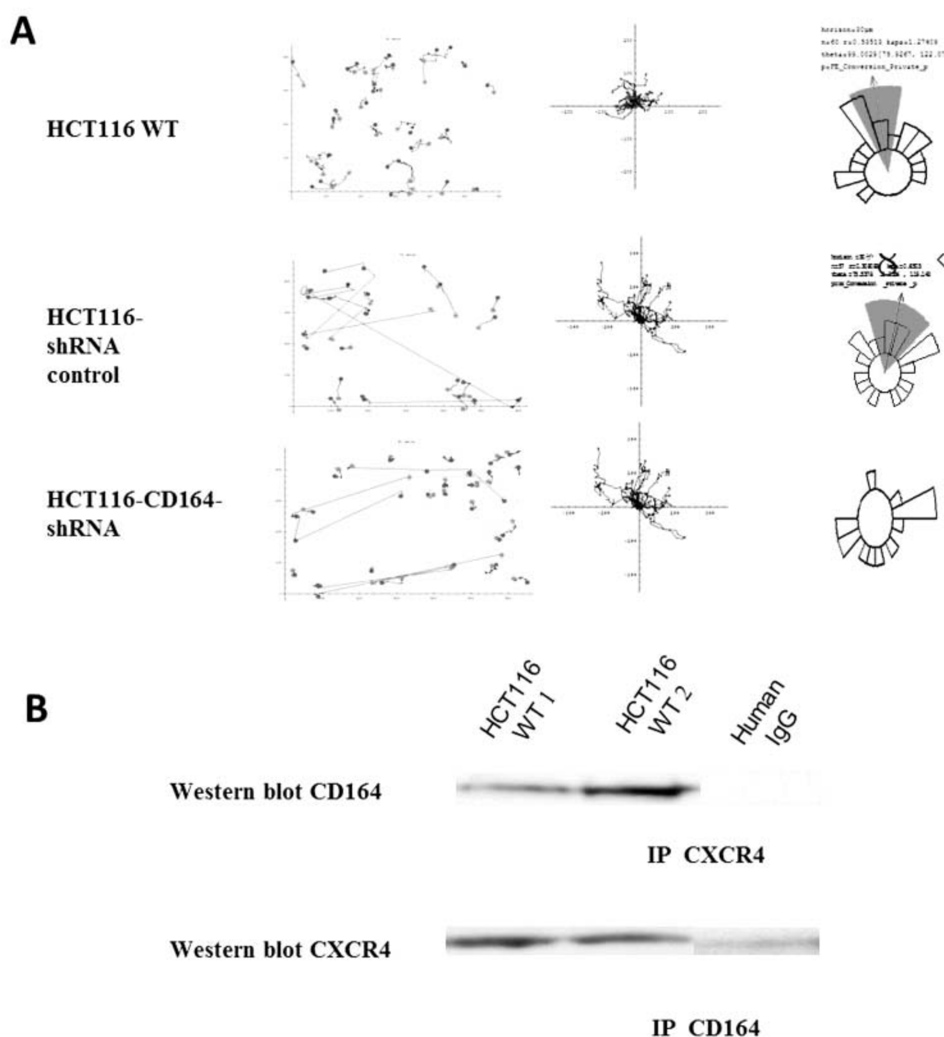

Figure 6. (A) The directional migration of HCT116 WT, HCT116-shRNA control, and HCT116-CD164-shRNA cells toward SDF-1 (400 ng/mL) were assayed using a Dunn chemotaxis chamber system. The HCT116 WT and HCT116-shRNA control cells showed significant directional movement toward to SDF-1 gradient, whereas the migration of HCT116-CD164-shRNA cells was random. Scatter graphs depicted trajectories and endpoints (red spots) of the cell migrations and circular histograms represented the proportion and moving direction of the cells; the arrows and the green sector represented the mean significant direction of migration and 95% confidence interval ($p < .01$, Rayleigh test). (B) Co-immunoprecipitation of CD164 and CXCR4 confirmed that CD164 and CXCR4 form complexes. HCT116WT cell lysates were first immunoprecipiated with CXCR4 (or CD164) antibody, then analyzed by Western blot with CD164 (or CXCR4) antibody. Lane1-2: HCT116 WT cells; Lane 3: human IgG as negative control.

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group, but there was no visible nodule seen macroscopically and microscopically in the HCT116-CD164-shRNA group (Figure 5C).

CD164 regulates HCT116 cell migration through SDF-1/CXCR4 axis

HCT116 WT and HCT116-shRNA control cells had significant directional migration toward SDF-1 (400 ng/mL; $p < .01$, Rayleigh test), but the cell migration was random towards SDF-1 in the HCT116-CD164-shRNA cells (Figure 6A). When HCT116 cell lysates were immuneprecipitated with an anti-CXCR4 antibody, CD164 was detected in the products, and *vice versa*, suggesting that CD164 forms complexes with CXCR4 in HCT116 cells (Figure 6B).

DISCUSSION

Previous studies in both Drosophila S2 cells and human $CD34^{+}CD38^{-}$ hematopoietic stem/progenitor cells showed that inhibition of cell proliferation when CD164 expression was knocked down (1, 3, 6). In this study, the relationship between CD164 expression and cancer cell proliferation was explored by shRNA technology that specifically knocked down CD164 expression in HCT116 cells, demonstrating that knocking down CD164 attenuated HCT116 cell growth both *in vitro* and *in vivo* compared to the control or wild type cells.

There is a general agreement that tumor cell migration is a pivotal step in tumor metastasis. Whereas several groups have confirmed the dramatic effect on growth inhibition with knockdown of CD164 in various cell lines, little is known

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about the effect of knockdown of CD164 on tumor cell migration and/or metastasis. In this study, it has been found that knockdown of CD164 in HCT116 cells significantly inhibited the ability of HCT116 cells to close a wound scratch in culture flasks, the decreased cell migration ability of CD164 knockdown HCT116 cells was also confirmed by a transwell test. The *in vivo* IVIS imaging showed the metastasis inhibition of CD164 knockdown HCT116 cells in the tumor bearing animal models. CD164 belongs to the mucin glycoprotein family and knocking down other tumor associated glycoproteins that belong to the mucin family, such as MUC1 has also been found to inhibit cell migration through promoting E-cadherin/catenin complex formation in cancer cells (16).

Initially we have tested the CD164 expression using immunostaining in several types of cancers, including colon, breast, and ovary cancers. We have compared the expression of CD164 in the cancer sites and the adjacent "normal" tissue sites and found that the CD164 was highly upregulated in colon cancer, as well as in other cancer types. However, the focus of this study is the role of CD164 in colon cancer, but CD164 may also play equally important role in other types cancer development and metastasis, which is yet to be explored. Further studies are needed to explore the molecular mechanisms underlying the observed inhibition of proliferation and metastasis with CD164 knockdown. It is possible that endocytic process of specific receptors responsible for cell proliferation may be disrupted because of the modification of CD164 expression, as CD164 plays an important role for endocytic processes. Our data also confirmed that CD164 formed a complex with CXCR4, suggesting that CD164 might also act through SDF-1/CXCR4 pathway. SDF-1 is a chemokine has been found to enhance tumor cell proliferation (17); promote cell migration and tumor growth (18). A possible "cross-talk" between SDF-1/CXCR4 and EGFR intracellular pathways (19) or STAT5 activation (17) may be responsible for upregulated cell proliferation in cancer cells. SDF-1 treatment resulted in increased expression of proliferating cell nuclear antigen and a downregulation of cleaved caspase-3 that is responsible for cell apotosis, therefore, promoted tumor cell proliferation and tumor growth and inhibited tumor cell apoptosis. A signaling pathway involves SDF-1/CXCR4 and CD164 may also exist that influences HCT116 cell proliferation as observed in the present study. Many evidences proved that the interactions of chemokines and their receptors are involved in the homing of metastatic tumor cells (20–22). The SDF-1/CXCR4 chemokine axis was a well-characterized and important chemotactic receptor pathway that mediates cancer cells migration (23). Recently, Sinead Forde reported that CD164 modulated the CXCR4 downstream signaling pathway in UCB CD133^{+} precursors and regulated cell migration through the SDF-1/CXCR4 pathway (24). A role of CD164 in SDF-1 mediated chemotaxis of HCT116 cells was also confirmed by Dunn chamber chemotaxis analysis in this study. The formation of CD164 and CXCR4 complex was confirmed by co-immunoprecipitation in HCT116 cell. Knocking down CD164 in HCT116 resulted in the decreased surface expression of CXCR4 in HCT116 cells. CD164 was therefore involved in SDF-1/CXCR4 complex in HCT116 cells, which was also true in C2C12 cell and umbilical cord blood CD133^{+} cells (24, 25). Our data proved the hypothesis that CD164 forms a complex with CXCR4 and subsequently affects cell mobility of HCT116 cells.

This study has confirmed that CD164 promotes HCT116 colon tumor cell proliferation, survival and migration both *in vitro* and *in vivo*. The promoting effect of CD164 on colon cancer development may act through SDF-1/CXCR4 pathway. CD164 may therefore be a potential target for treatment or diagnosis of colon cancer metastasis.

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DECLARATION OF INTERESTS

The authors report no declarations of interest. The authors alone are responsible for the content and writing of the paper.

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