Collagen-binding vascular endothelial growth factor (CBD-VEGF) promotes liver regeneration in murine partial hepatectomy

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Abstract. Liver regeneration is a complex process that needs orchestration of multiple nonparenchymal cells including sinusoid endothelial cells. Vascular endothelial growth factor (VEGF) serves a crucial role in angiogenesis and liver regeneration. However, the lack of an high-efficiency delivery system target to the injured site reduces the local therapeutic efficacy of VEGF. In our previous study, collagen binding VEGF (CBD-VEGF) was established by fusing collagen binding domain (CBD) into the N-terminal of native VEGF and improved cardiac function after myocardial infraction. The present study investigated the therapeutic effect of CBD-VEGF on liver regeneration by a mouse model of partial hepatectomy. After injection through portal vein following 2/3 hepatectomy, CBD-VEGF was largely retained in the hepatic extracellular matrix for 48 h. Furthermore, CBD-VEGF application significantly promoted sinusoidal regeneration and remodeling in remanent liver tissue 48 h after hepatectomy. In addition, CBD-VEGF treatment significantly enhanced the proliferation of hepatocytes at 2 and 3 days post-surgery compared with native VEGF, concomitant with attenuated liver injury. In conclusion, these results demonstrated that CBD-VEGF could be a promising therapeutic strategy for liver regeneration.

Introduction

One of the exceptional features of the liver is its remarkable regenerative capacity following resection or other injuries (1). Liver transplantation remains the only cure for many end-stage hepatic diseases (2). However, one key limiting factor in the application of liver surgery or transplantation is defective regeneration of small-for-size and partial grafts (3). Therefore, there is impetus to investigate novel therapeutic strategies to enhance liver regeneration after surgical injury (4).

Liver regeneration is a highly organized and complex progress that occurs initially through the proliferation of hepatocytes and then through the proliferation of nonparenchymal cells, including bile duct epithelial cells, hepatic stellate cells and sinusoidal endothelial cells (5-7). After partial hepatectomy (PHx), the intrahepatic angiogenesis, characterized by the increased sinusoidal cell proliferation and microvascular architecture remodeling, is essential for liver regeneration, as it serves a pivotal role in the supply of blood to the newly replicating hepatocytes (8). Vascular endothelial growth factor A (VEGF-A), a 45-kilodalton dimeric heparin-binding glycoprotein, is the main growth factor in angiogenesis and vasculogenesis (9). The effects of VEGF-A are mediated through three distinct high-affinity cell membrane receptors tyrosine kinases VEGFR1, VEGFR2 and VEGFR3 (10), of which the VEGF-A/VEGFR2 pathway contributes to the majority of VEGF-regulated proangiogenic effects (11). Previous studies demonstrate that VEGF-A and VEGFR2 expression is increased during liver regeneration (12,13), whereas blockage of VEGF significantly delays liver tissue repair, indicating VEGF as a promising target for liver regeneration (14). Systemic administration of exogenous VEGF or adenovirus-mediated gene transfer of VEGF promote functional hepatic recovery following hepatectomy (15,16), as a result of neovascularization. However, due to the short half-life and rapid diffusion, it is hard to retain an effective local concentration of VEGF in the remnant liver tissue (17,18). Furthermore, high doses treatment may induce undesirable side effects (19,20). Therefore, developing an effective local VEGF delivery system may offer an optimal therapy for liver regeneration.

Our previous study constructed a fusion protein that consisted of VEGF and collagen binding domain (CBD), a short peptide with 7 amino acids (TKKTLRT) that could specifically bind to type I collagen (21). Another previous study had shown that CBD-VEGF can promote tissue regeneration and

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improve neo-urethra function in a beagle extensive urethral defect model (22). It was shown that CBD-VEGF could attenuate left ventricular remodeling, reduce infarct size and promote cardio-angiogenesis in a porcine chronic myocardial infraction model (23). The present study investigated the effect of CBD-VEGF on liver regeneration using a mice partial hepatectomy model. The results demonstrated that CBD-VEGF could significantly promote the proliferation of hepatocytes, enhance the reconstruction of vascularization and promote liver regeneration.

Materials and methods

Preparation of CBD-VEGF. CBD-VEGF was prepared as previously described (22,23). Briefly, full length complementary DNA of human VEGF165 was constructed and amplified linking a sequence that encodes the collagen binding domain (TKKTLRT). Then, the encoding gene of CBDVEGF was inserted into plasmid pET28a, followed by transfer into BL21-strain *Escherichia coli*. Selected single clones were inoculated into 4 ml Luria-Bertani (LB) medium as primary culture, followed by inoculation 4 ml into 200 ml fresh LB medium and culture at 37˚C for ~4 h at 200 rpm. When A600 reached 0.6-0.8, 1 mM isopropyl β-D-thiogalactopyranoside was used to induce protein expression for 5 h. After the inclusion body was refolded, the protein containing 6X-His tag was purified by nickel chelate chromatography and ion exchange chromatography on a prepacked HiTrap heparin HP columns (Cytiva).

Animals. A total of 60 C57BL/6 male mice (22-24 g body weight) 8-10 weeks, were purchased from Jinan Pengyue Experimental Animal Breeding Co. Ltd. All animal experiments were approved by The Ethics Committee Medical College of Qingdao University (approval no. QDU-AEC-2021166) and were conducted according to the Guide for the Care and Use of Laboratory Animals published by the National Academy of Sciences and the National Institutes of Health (24). The mice were housed in a standard room with controlled humidity (55-60%) and temperature (23-25˚C) under a12-h light/dark cycle with free access to water and food.

PHx model. Mice were subjected to two-thirds PHx as previously described (25,26). Briefly, after anesthetization by isoflurane (4% for induction, 2% for maintenance; RWD Life Science Co., Ltd.), the median lobe plus gall bladder and the left lateral lobe were removed. Immediately after PHx, native VEGF (500 ng/dose), or CBD-VEGF (500 ng/dose) was injected via portal vein., with PBS as a vehicle control (n=5 per group). The dose of CBD-VEGF/VEGF was chosen based on our previous studies and preliminary experiments (21,27). The sham operation group was conducted midline laparotomy incision without resection of the liver lobe. The mice were euthanized by cervical dislocation after deep anesthesia with isoflurane (4% for induction) at various time points after surgery. The serum and liver tissue were collected for subsequent experiments. Levels of alanine transaminase (ALT), aspartate transaminase (AST) in serum were measured by an automatic biochemical analyzer (Chemray 800; Rayto Life and Analytical Sciences) according to the manufacturer's instructions (Rayto Life and Analytical Sciences).

ELISA assay. Frozen liver tissue from VEGF or CBD-VEGF group at 24 h were homogenized and the levels of VEGF in the liver tissue of PHx mice were detected using an ELISA kit (mlBio; cat. no. ml064255) according to manufacturer's instructions.

Immunofluorescence (IF) analysis. IF analysis was performed as previously described (28). Briefly, The liver tissues of experimental and control group mice were embedded in OCT and stored at -80˚C. Next, the liver tissue samples sections (8 μm thick) were fixed with 4% paraformaldehyde for 30 min and blocked with 5% bovine serum albumin (BSA; Wuhan Servicebio Technology Co., Ltd.; cat. no. G5001) both for 1 h at room temperature. For bromodeoxyuracil (BrdU) staining, BrdU (50 mg/kg body weight) was injected intraperitoneally 4 h before liver tissue were harvested. Subsequently, the primary antibody including anti-VEGFR2 (ABclonal Biotech Co., Ltd.; 1:200; cat. no. A1484), anti-BrdU (ABclonal Biotech Co., Ltd.;, 1:200; cat. no. A1482) anti-Ki67 (Abcam; 1:300; cat. no. ab16667) , anti-CD31 (BD Pharmingen; BD Biosciences; 1:300; cat. no. 553373) and β-catenin (CTNNB1) antibody (ABclonal Biotech Co., Ltd.; 1:200; cat. no. A19657) were incubated overnight at 4˚C, followed by incubating with the corresponding fluorescein-conjugated secondary antibodies. 4',6-diamidino-2-phenylindole (DAPI; Wuhan Servicebio Technology Co., Ltd.) was used to stain cell nuclei for 5 min at room temperature. The sections were visualized using a fluorescence microscope (Olympus BX50; Olympus Corporation). Then 5-8 images were captured for each sample and the percentage of positive nuclei was analyzed using ImageJ software (version 1.43; National Institutes of Health).

Immunohistochemistry staining. The formalin-fixed liver tissue was dehydrated through graded alcohols and embedded in paraffin wax (4% phosphate-buffered and formalin-fixed for 24 h at room temperature; 5 μm thick). The sections were blocked with 5% BSA for 1 h and then incubated with anti-proliferating cell nuclear antigen (PCNA; Affinity Biosciences; 1:200; cat. no. AF0239) overnight at 4˚C. The slides were washed with PBS, then incubated with the HRP conjugated secondary antibody (ABclonal Biotech Co., Ltd.; 1:1,000; cat. no. AS014) for 1 h at room temperature. Staining was developed with diaminobenzidine substrate solution (DAB; Wuhan Servicebio Technology Co., Ltd.) and the sections were counterstained with hematoxylin for 1 min at room temperature , then they were dehydrated through graded alcohols, mounted and visualized under a light microscope. Data were expressed as the percentage of PCNA positive nuclei analyzed using ImageJ software (version 1.43; National Institutes of Health).

Hematoxylin and eosin staining. The liver tissue was fixed with phosphate-buffered and formalin (Wuhan Servicebio Technology Co., Ltd.) for 24 h at room temperature. After dehydration through graded alcohols, the tissue was embedded in paraffin wax and sectioned at 5 μm. The sections were stained with hematoxylin-eosin for 5 min at room temperature and observed under an Olympus light microscope (magnification, x200; Olympus Corporation), and three fields of view were examined per section.

Protein-dye conjugation and in vivo distribution. CBD-VEGF was labeled with 775-B2 NHS ester according to the manufacturer's instructions. CBD-VEGF conjugated with the 775-B2 NHS ester was injected into the portal vein of PHx mice. Bioluminescence images were acquired and processed using an IVIS Lumina XRMS III *in vivo* imaging system (PerkinElmer, Inc.) at 6, 24, 48 and 72 h after administration. Then, the livers were harvested for frozen sections (8 μm). The sections were incubated with DAPI for 5 min at room temperature and the distribution of fluorescein labeled protein was examined by fluorescence microscopy (magnification, x200; Olympus Corporation), and three fields of view were examined per section.

Western blot analysis. Total protein was extracted from liver tissues using RIPA lysis buffer (Beyotime Institute of Biotechnology). The protein concentration was confirmed using a BCA Protein Assay kit (Epizyme; cat. no. ZJ102) and protein samples (40 μg) were collected and subjected to SDSPAGE (6% separation gel and 10% concentration gel) and then transferred to a polyvinylidene fluoride membrane (MilliporeSigma). Next, the membranes were blocked with skimmed milk (PPLYGEN; cat. no. P1622) for 1 h at room temperature and followed by incubating with primary antibodies overnight at 4˚C: native VEGF (R & D Systems; 1:1,000; cat. no. 293-VE/CF), VEGFR2 (Abcam; 1:1,000; cat. no. AB39256), PCNA (Affinity Biosciences; 1:1,000), β-actin (ABclonal Biotech Co., Ltd.; 1:1,000; cat. no. AC028) and GAPDH (Aksomics Inc.; 1:3,000; cat. no. KC-5G5) and HRP conjugated secondary antibody (ABclonal Biotech Co., Ltd.; 1:1,000; cat. no. AS014) at room temperature for 1 h. The protein band were detected ECL regents, imaged using Tanno imaging system (Tanon 5200; Tanon Science & Technology) and the protein bands analyzed with ImageJ (version 1.43; National Institutes of Health).

Reverse transcription-quantitative PCR (RT-qPCR). Total RNA was extracted using the GeneJet RNA Purification Kit (Thermo Fisher Scientific, Inc.) from the liver tissue. Then, cDNA was synthesized from the purified RNA (200 ng/sample) using a transcription kit (Takara Biotechnology Co., Ltd.), qPCR were performed in triplicate using a SYBR Premix Ex Taq (Vazyme Biotech Co., Ltd.). The following thermo cycling conditions were used: Pre-incubation at 95˚C for 30 sec; amplification at 95˚C for 15 sec, 57˚C for 15 sec and 72˚C for 1 min, for 55 cycles; melting curve at 95˚C for 15 sec, 60˚C for 1 min and 95˚C for 15 sec. The following primers were used in the current study: HGF forward 5'-CACTCCCGAGAACTTCAAATGC-3' reverse 5'-TGTCCACTTGACACGTCACACTT-3'. GAPDH forward 5'-CAGTTACTTCCCCAGCAA-3' and reverse 5'-CACGACTCATACAGCACCT-3'. GAPDH was regarded as the internal control. The target gene expression was quantified using the $22^{-\Delta\Delta Cq}$ method (29).

Statistical analysis. All data were presented as mean ± standard deviation and all experiments were performed at ≤3 times. Differences between two groups were analyzed using unpaired Student's ttest and the multiple groups were performed using oneway ANOVA followed by Tukey's post hoc test. Data were analyzed using the GraphPad Prism program version 8.0 (GraphPad Software, Inc.). P<0.05 was considered to indicate a statistically significant difference.

Results

Bio-distribution of CBD-VEGF in PHx mice. CBD-VEGF was used to modify VEGF to enhance its binding affinity with extracellular matrix (Fig. 1A). Purified CBD-VEGF and native VEGF were analyzed by SDS-PAGE (Fig. 1B). As described previously, the *in vivo* biodistribution of CBD-VEGF was evaluated by injecting 775-B2 NHS ester conjugated CBD-VEGF via the hepatic portal vein (30). The biodistribution of CBD-VEGF was measured at 6, 24, 48 and 72 h after injection. As shown in Fig. 1D, the CBD-VEGF infused via the portal vein initially accumulated in the liver of PHx mice. At 24 and 48 h post injection, fluorescence signals were detected in the liver, intestines and kidney, possibly due to the metabolism of CBD-VEGF in the body. However, at 72 h after injection, no visible fluorescence signal were detected in the liver. In addition, ELISA results showed that the concentration of CBD-VEGF in liver was significantly increased at 24 and 48 h after PHx compared with native VEGF group (24 h: 36.39±2.03 pg/ml vs. 22.53±1.62 pg/ml, P<0.01; 48 h: 26.20±1.22 pg/ml vs. 7.72±1.04 pg/ml, P<0.01) (Fig. 1C), indicating that CBD-VEGF could be retained at the injury site with less diffusion than that of native VEGF.

CBD-VEGF can promote the reconstruction of vascularization. Previous reports has identified CD31 as a cell marker for liver sinusoidal endothelial cells (LSECs) and VEGFR2 and serving an important role in regulating LSECs biological function during liver regeneration (31,32). The extent of vascular remodeling in regenerated liver was measured by immunostaining. As shown in Fig. 2A, the neovascularization in CBD-VEGF group was markedly enhanced compared with native VEGF group. In addition, the quantitative analysis of VEGFR2 and CD31 revealed a more positive area in the CBD-VEGF group (Fig. 2B and C; VEGFR2: 20.25±2.31% vs. 11.5±1.34%, P<0.01; CD31: 18.75±1.32% vs. 12.75±0.74%, P<0.01). Moreover, western blotting further verified that VEGFR2 expression was significantly upregulated in CBD-VEGF group (Fig. 2D and E). Immunostaining results further demonstrated that CBD-VEGF could improve proliferation of LSECs (Fig. 2F and G). Taken together, these results indicated that CBD-VEGF promoted angiogenesis in remnant liver during liver regeneration.

CBD-VEGF alleviates PHx-induced liver injury. The therapeutic effect of CBD-VEGF on liver regeneration was further verified via PHx mice model. No significant discrepancy was detected in hematoxylin and eosin-stained liver sections (Fig. 3A and Fig. S1. However, serum levels of ALT and AST (two liver injury markers), were significantly decreased in CBD-VEGF treated group compared with those of native VEGF group, at 2 days and 3 days after PHx, respectively (Fig. 3B and C). These results suggested that CBDVEGF application alleviate PHx-induced liver injury.

CBD-VEGF promotes liver regeneration following PHx. The present study further investigated the effect of CBD-VEGF

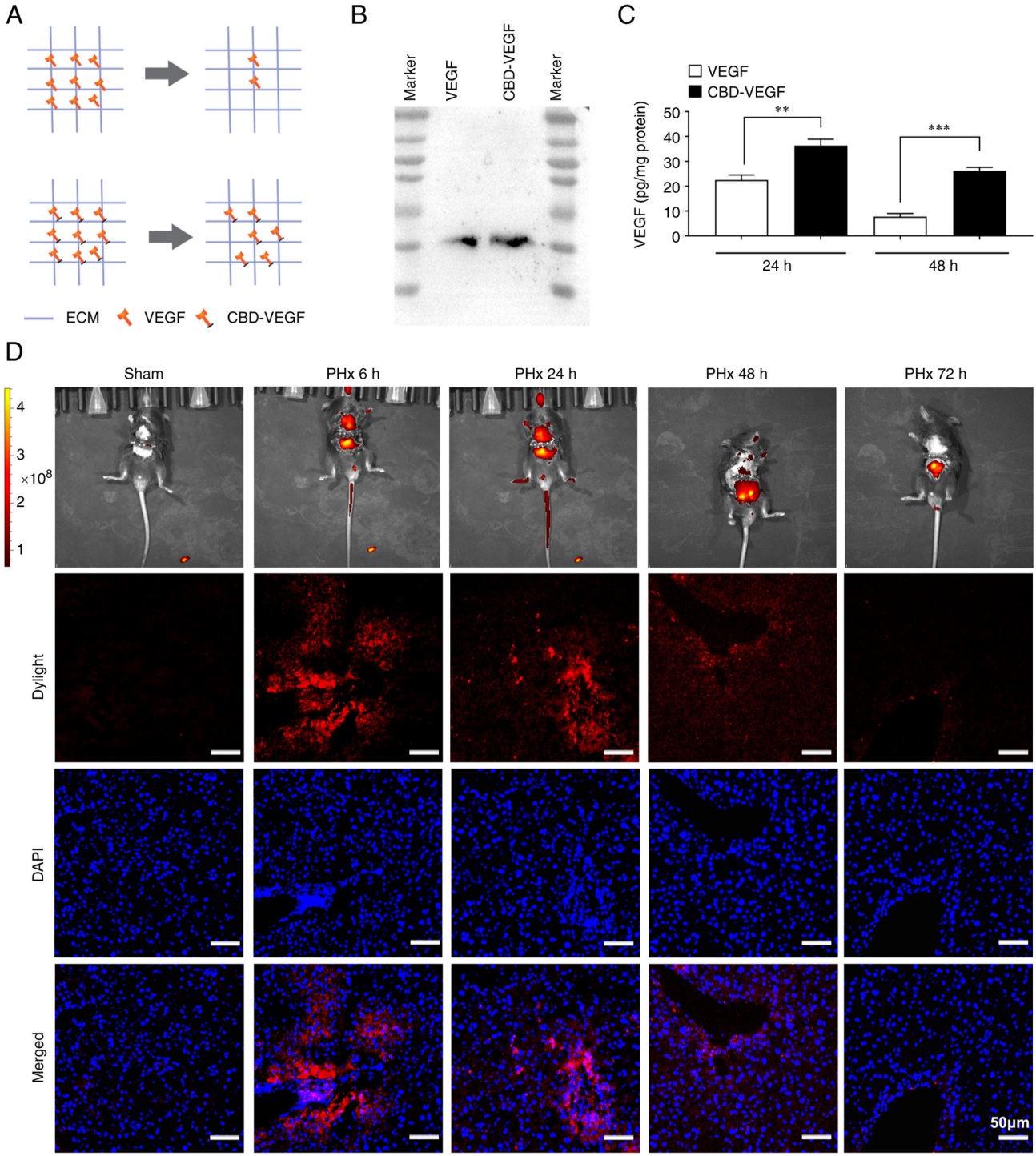

Figure. 1. CBD-VEGF collects and is retained in liver tissue following portal vein injection. PHx mouse model was established with VEGF or CBD-VEGF treatment for 6, 24 or 48 h. Sham group representatives only performed open surgery. (A) Pattern diagram of CBD-VEGF delivery system (B) Dimer of native VEGF and CBD-VEGF in SDS-PAGE gel without reduction. (C) At 24 and 48 h after PHx, the protein contents of VEGF in liver tissue were detected by ELISA (n=3). (D) *In vivo* representative IVIS images obtained 6, 24, 48 and 72 h following CBD-VEGF injection in mice and stained with DAPI (scale bar, 50 μm). (n=3) All data are expressed as the means ± standard deviation. **P<0.01, ***P<0.001. CBD-VEGF, collagen-binding vascular endothelial growth factor; PHx, partial hepatectomy; Dylight, CBD-VEGF conjugated with 775-B2 NHS ester.

on hepatocyte proliferation and liver regeneration following PHx. The results showed that the liver-to-body weight ratio of CBD-VEGF group was significantly restored compared with that of native VEGF group, at 2 and 3 days post-PHx, respectively. Liver weight could be restored almost to the original level at 7 days following surgery and no significant difference was found between groups at 7 days following surgery, indicating that the CBD-VEGF treated group could regenerate faster at the early phase of liver regeneration (Fig. 4A and B). Furthermore, no significant difference in the body weight loss and liver cell size was noted between groups following CBD-VEGF treatment (Fig. 4C-E). To further confirm the effect of CBD-VEGF on hepatocyte proliferation, expression of Ki67 and BrdU were evaluated at 2 and 3 days after PHx

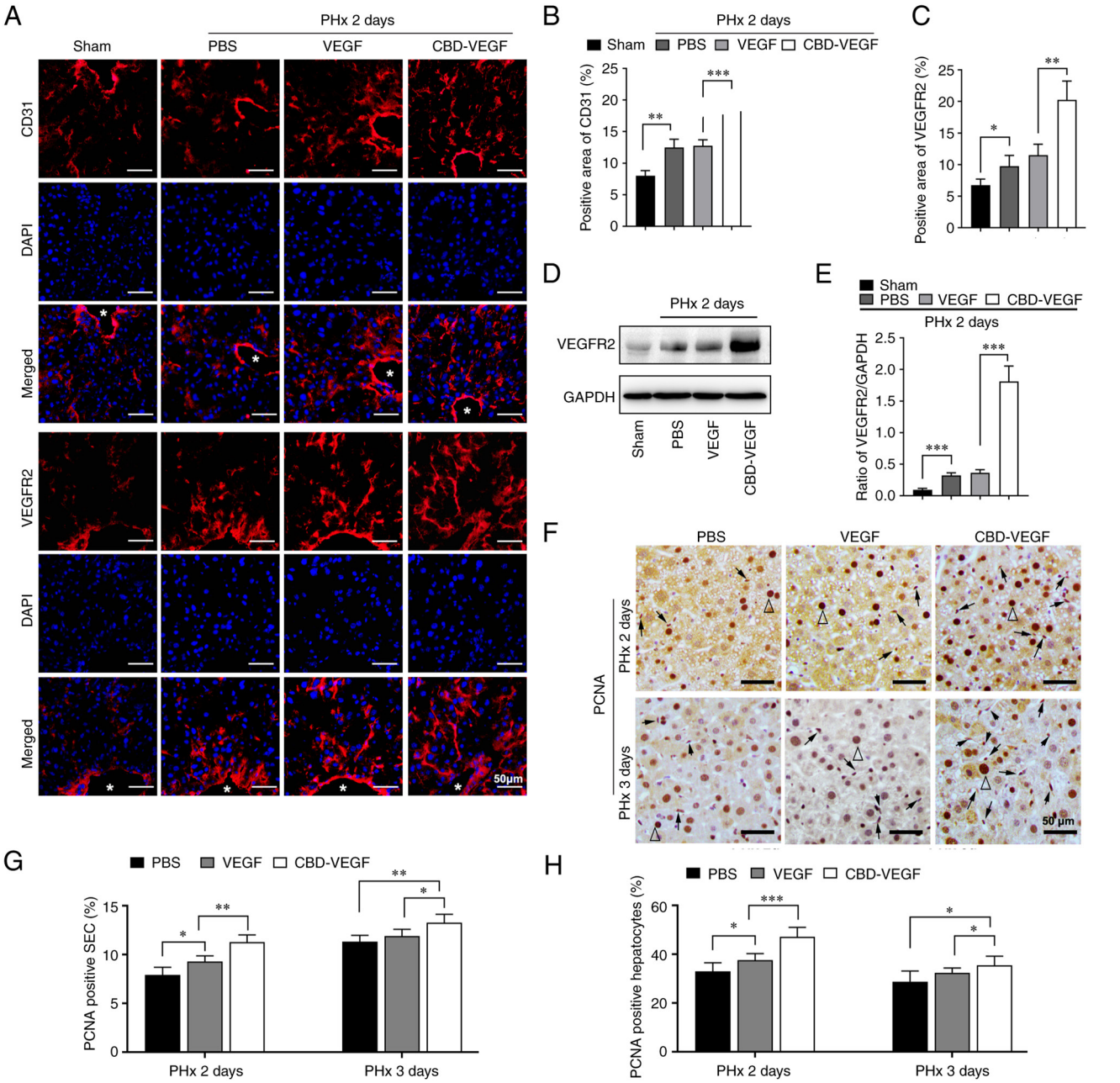

Figure 2. CBD-VEGF treatment promoted hepatic vascular remodeling during liver regeneration. The liver sections of each group were stained with CD31 and VEGFR2 and the formation of neovascularization was detected 2 days after PHx. (A) Representative images of CD31 and VEGFR2 staining. Quantification of (B) CD31 and (C) VEGFR2-positive area (n=5). (D) The expression of VEGFR2 was detected by western blotting. (E) The statistics of VEGFR2 were analyzed via ImageJ. (F) Immunohistochemical staining for PCNA days 2 and 3 after 70% hepatectomy. Quantification of PCNA positive nuclei from (G) SEC and (H) hepatocytes (n=5). The asterisk (*) represented the central vein, the arrows (→) represented positive SEC, the triangle (△) represented positive hepatocytes. A total of three independent experiments were pooled in the statistical analysis. All data are expressed as the means ± standard deviation. *P<0.05, **P<0.01, ***P<0.001. CBD-VEGF, collagen-binding vascular endothelial growth factor; PHx, partial hepatectomy; PCNA, proliferating cell nuclear antigen; SECs, sinusoidal endothelial cells.

(Figs. 5A and C and 6A and C). CBDVEGF administration could significantly increase the number of Ki67^{+} cells when compared with the native VEGF (Figs. 5B and 6B). (day 2: 20.8±2.135% vs. 13.2±1.166%, P<0.01; day 3 9.8±1.470% vs. 5.8±0.748%, P<0.01;) Consistently, compared with native VEGF group, the ratio of BrdU incorporated hepatocyte was significantly increased in CBD-VEGF group (Figs. 5D and 6D; day 2: 16.4±1.85% vs. 10.4±1.85%, P<0.01; day 3: 8.60±0.93% vs. 4.40±0.93%, P<0.01). The levels of PCNA expression were also verified by IHC staining and western blot analysis, which further confirmed that the ability of CBD-VEGF on hepatocyte proliferation (Fig. 2F and H; Fig. S2; Figs. 5E and F and 6E and F). Taken together, the results indicated that CBD-VEGF treatment could significantly promote hepatocyte proliferation and liver regeneration following PHx.

Discussion

Despite the robust regenerative capacity of liver, liver failure remains one of the significant clinical challenge with a high

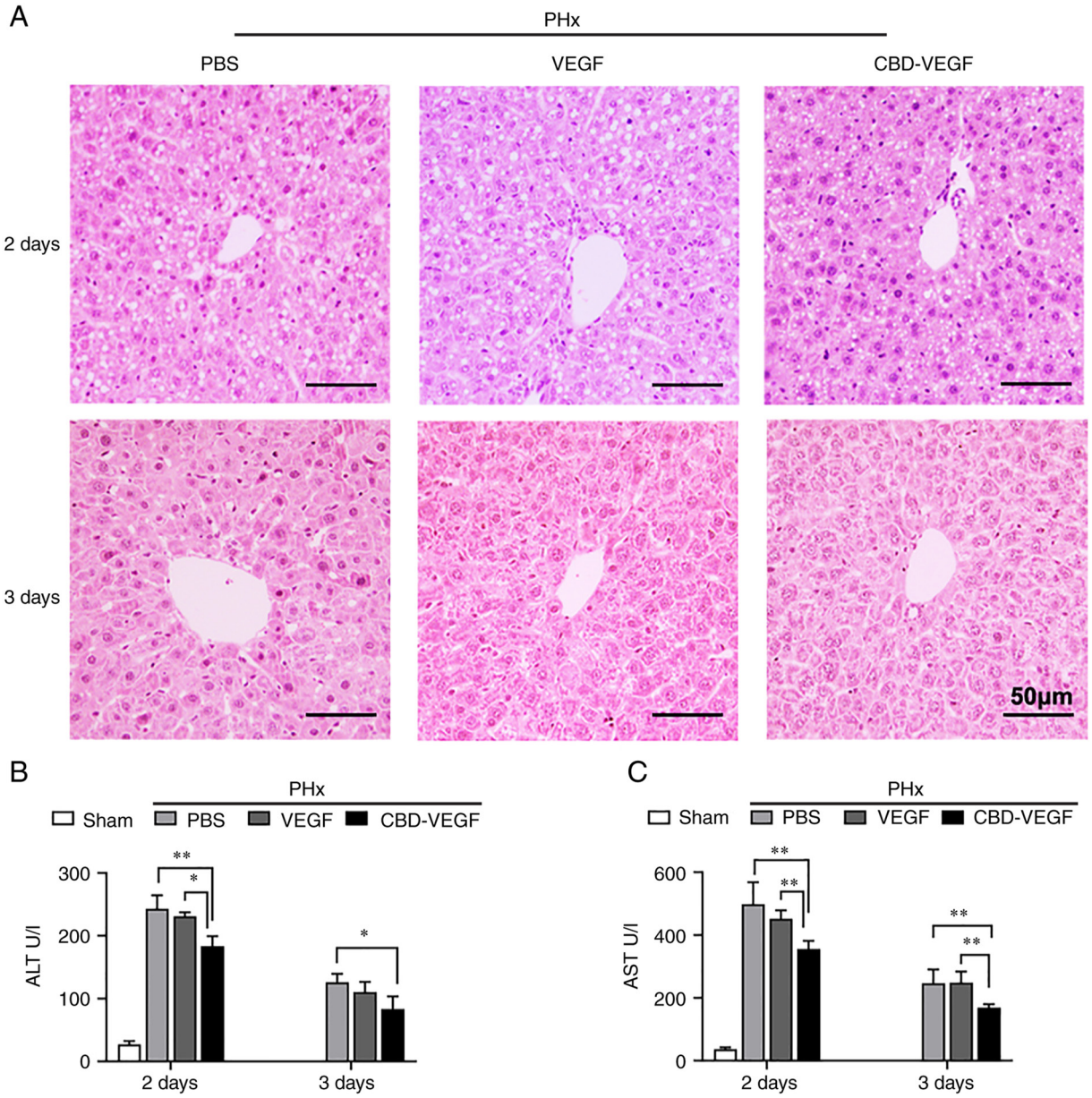

Figure 3. CBD-VEGF treatment attenuates liver injury in the PHx mice. The PHx mouse model was established and mice were treated with PBS, VEGF, or CBD-VEGF. (A) Hematoxylin-eosin staining of liver tissue. Serum (B) ALT and (C) AST levels following PHx (n=5). A total of three independent experiments were pooled in the statistical analysis. All data are expressed as the means ± standard deviation. *P<0.05, **P<0.01. CBD-VEGF, collagen-binding vascular endothelial growth factor; PHx, partial hepatectomy; ALT, alanine transaminase; AST, aspartate transaminase.

mortality rate (33). The ability of liver to restore the cell mass loss is an important component of hepatic functional recovery following resection surgery (34). In the present study, CBD-VEGF, which could specifically bind to the hepatic extracellular matrix to retain VEGF in remnant liver mass, was used in the regenerative therapy for 2/3 PHx. The findings demonstrated that CBD-VEGF treatment significantly attenuate liver injury, promote hepatocyte replication and enhance sinusoidal regeneration after hepatectomy.

Research groups are working on localizing and sustaining VEGF protein at the sites of injury tissue. Injectable alginate and nanofiber have been used as vehicles to deliver VEGF (17,35). A previous study by Yu *et al* (35) reported a local delivery of VEGF to regenerating liver tissue by using biodegradable nanofiber meshes, which provided a sustained release of VEGF and increased the proliferation of hepatocytes. As the main components of hepatic extracellular matrix are type I and type III collagens, collagen I could be a potential target for VEGF to be enriched and control released in remnant liver tissue (36). In a previous study, de Souza *et al* (37) reported that a heptapeptide (TKKTLRT) could bind specifically to the type I collagen. With this domain, growth factors showed a remarkable collagen binding ability and remained excellent biological activity. Indeed, several growth factors, such as basic fibroblast growth factor, brain-derived neurotrophic factor and VEGF were fused with CBD (38,39) Previous preclinical studies demonstrated that the recombinant proteins could retain the effective concentration at the injury site and exhibit enhanced tissue regeneration. Consistently, the present study

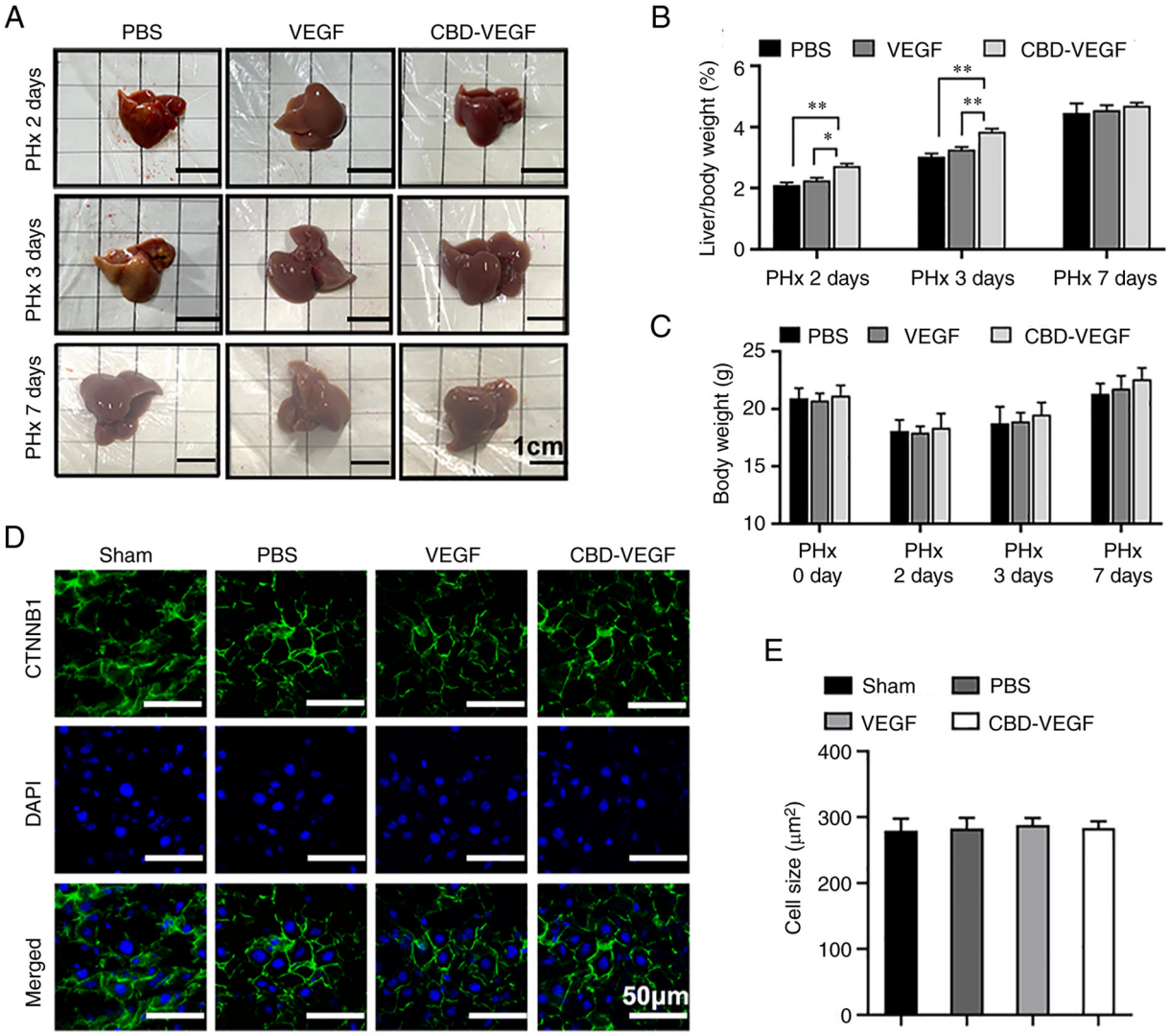

Figure 4. CBD-VEGF treatment promotes liver regeneration in PHx mice. The PHx mouse model was established and mice were injected with PBS, VEGF, or CBD-VEGF through hepatic portal vein. (A) The liver weight to body weight ratio was counted at 2, 3 and 7 days following PHx (n=5). (B) The liver weight to body weight ratio was counted at 2, 3 and 7 days following PHx (n=5). (C) Body weights following surgery (n=5). (D) Representative images of CTNNB1 staining. (E) Quantification of cell size (n=5). A total of three independent experiments were pooled in the statistical analysis All data are expressed as the means ± standard deviation. *P<0.05, **P<0.01. CBD-VEGF, collagen-binding vascular endothelial growth factor; PHx, partial hepatectomy; CTNNB1, catenin β.

demonstrated that CBD-VEGF injection through portal vein could collect and be retained with a higher concentration in liver tissue compared with native VEGF. Additionally, CBD-VEGF treatment promoted hepatocyte replication and enhanced liver mass restoration.

Angiogenesis is essential for liver regeneration and repair, as it serves a vital role in the supply of blood to the newly replicating hepatocytes. LSEC is a specialized endothelial cell that constituting ~10% of the liver cellular mass (40). LSECs have been noted to contribute to liver regeneration following liver injury. During the early phase of liver regeneration, the transition from quiescent to replicative hepatocytes increase after 12 h and reaches a peak ~48 h after PHx, while the proliferation of LSECs is delayed in comparison to hepatocytes (40-43). Previous studies have identified VEGF as a powerful mitogen of LSECs via upregulating VEGF receptors during liver regeneration (44,45). It has been demonstrated that endogenous VEGF expression in the remnant liver is increased from 24 h after hepatectomy, with a peak at 72 h (46). Ding *et al* (32) revealed that inducible genetic ablation of VEGFR2 in LSECs impairs the first wave of hepatocyte proliferation and subsequent hepatovascular regeneration, suggesting VEGFR2 as the main mediator of VEGF signaling in LSECs. Thus, a synchronized proliferation of hepatocytes and LSECs is a crucial requirement for proper liver regeneration. CD31, also known as PECAM, is considered as golden standard marker of LSECs (46). The present study showed that CD31 and VEGFR2 expression in the remnant liver tissue were significantly upregulated in the CBD-VEGF treatment group, indicating that CBD-VEGF promoted liver regeneration possibly through regulating sinusoidal regeneration and revascularization following PHx. It is reported that hepatocyte growth factor (HGF), one of the mitogenic growth factors, is involved in regulating liver regeneration (47). The expression of HGF was measured and the results of the present study revealed that CBD-VEGF treatment could promote liver HGF expression at 2 days after surgery (Fig. S3).

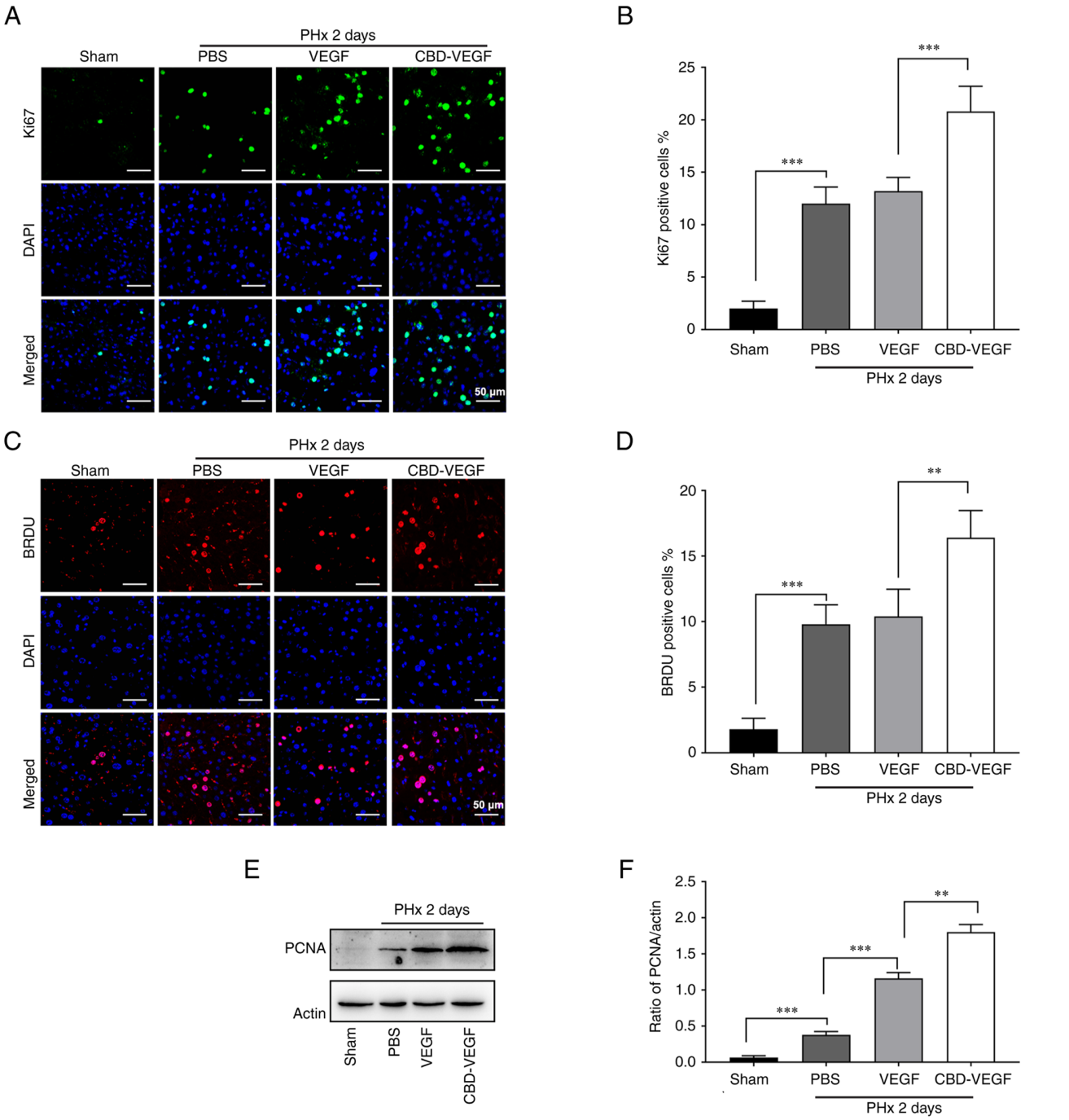

Figure 5. CBD-VEGF treatment enhances the proliferation of hepatocytes at 2 days after PHx. (A) Representative images of Ki67 staining and (B) quantification of Ki67 positive cells (n=5). (C) Representative images of BrdU staining and (D) quantification of BrdU-positive cells (n=5). (E) The expression of PCNA was detected by western blotting at 2 days after PHx. (F) The statistics of PCNA were analyzed via ImageJ. A total of three independent experiments were pooled in the statistical analysis All data are expressed as the means ± standard deviation. **P<0.01, ***P<0.001. CBD-VEGF, collagen-binding vascular endothelial growth factor; PHx, partial hepatectomy; BrdU, bromodeoxyuracil; PCNA, proliferating cell nuclear antigen.

In brief, the present study indicated that injection of CBD-VEGF could significantly reduce the liver injury after partial hepatectomy, enhance the reconstruction of neovascularization and promote liver regeneration. These results suggested that CBD-VEGF may be a potential clinical candidate for liver regeneration.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article

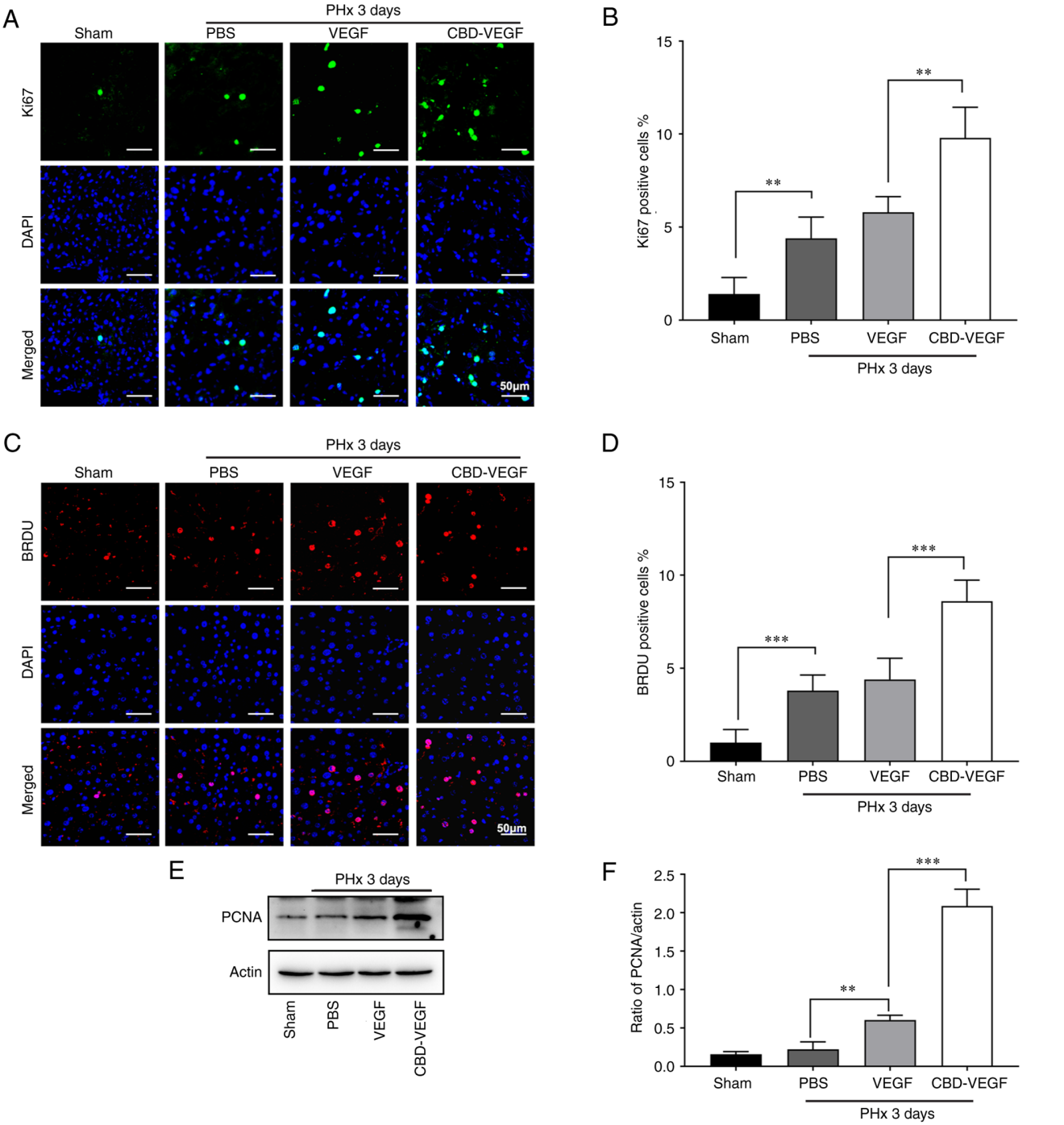

Figure 6. CBD-VEGF treatment enhances the proliferation of hepatocytes at 3 days after PHx. (A) Representative images of Ki67 staining and (B) quantification of Ki67 positive cells (n=5). (C) Representative images of BrdU staining and (D) quantification of BrdU-positive cells (n=5) (E) The expression of PCNA was detected by western blotting at 3 days after PHx. (F) The statistics of PCNA were analyzed via ImageJ. A total of three independent experiments were pooled in the statistical analysis All data are expressed as the means ± standard deviation. **P<0.01, ***P<0.001. CBD-VEGF, collagen-binding vascular endothelial growth factor; PHx, partial hepatectomy; BrdU, bromodeoxyuracil; PCNA, proliferating cell nuclear antigen.

Authors' contributions

SW, GD and CS contributed to sample testing, data analysis and study design; ZL contributed to sample testing and data analysis; QS, XL, XY and YL contributed to sample preparation; CG, XW contributed to study design; GD and CS directed the project, contributed to the discussion, reviewed and edited the manuscript. GD had full access to all the data in the study and had final responsibility for the decision to submit for publication. GD and SW confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by The Ethics Committee Medical College of Qingdao University (approval no. QDU-AEC-2021166).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests

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