## ORIGINAL RESEARCH Dicer Enhances Bevacizumab-Related Inhibition of Hepatocellular Carcinoma via Blocking the Vascular Endothelial Growth Factor Pathway

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Purpose: Vascular endothelial growth factor (VEGF) family members contribute greatly to the development and angiogenesis of hypervascular hepatocellular carcinoma (HCC). We have previously shown that Dicer inhibited HCC growth. In this study, we aimed to determine the relationship between Dicer and VEGF in HCC.

Methods: Gain-of-function studies were performed to determine the effect of different treatments on the proliferation, migration, and invasion of HCC cells. Expression of VEGF-A in xenograft tumor tissues was analysed using Western blotting, and that of CD31 using immunohistochemical analysis.

Results: We found that Dicer inhibited proliferation, migration and invasion of HCC cells by suppressing VEGF-A expression. Interestingly, VEGF-A165, which is the most prominent VEGF-A isoform, counteracted Dicer-induced inhibition of HCC cells. In addition, a monoclonal anti-VEGF antibody (bevacizumab) enhanced Dicer-induced inhibition of HCC in vitro and in vivo. Further, immunohistochemical analysis of CD31 indicated bevacizumab and Dicer synergized to reduce tumor microvessel density.

Conclusion: Our data demonstrated that Dicer enhanced bevacizumab-related inhibition of HCC cell via the VEGF pathway; therefore, Dicer in coordination with bevacizumab may provide another potential approach for HCC therapy.

Keywords: hepatocellular carcinoma, vascular endothelial growth factor, Dicer, bevacizumab, microvessel density

#### Introduction

Primary liver cancer (PLC) is the sixth most common diagnosed cancer worldwide, with approximately 906,000 new cases and 830,000 deaths, rendering it the third leading cause of cancer mortality in 2020.1 In approximately 50% new PLC cases that occur in China, chronic hepatitis B virus (HBV) infection is the main risk factor.<sup>2,3</sup> Hepatocellular carcinoma (HCC) is the most common liver cancer and accounts for over 75-85% of PLC. Potentially curative surgical resection, local ablation therapy, and radiation intervention are options for patients with early-stage HCC. However, most patients cannot undergo such treatments due to intrahepatic or distant metastasis.<sup>4</sup>

As angiogenesis is mediated by the vascular endothelial growth factor (VEGF) and VEGF receptor (VEGFR) contributes to the invasion and metastasis of hypervascular HCC, antitumor angiogenesis is a potential target for HCC treatment.<sup>5</sup> Although many antitumor angiogenesis agents, including antibodies and tyrosine kinase inhibitors (TKIs) such as brivanib, sunitinib, linifanib, everolimus and axitinib have failed in

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HCC therapy,<sup>6–11</sup> sorafenib and lenvatinib have shown to improve outcomes of HCC patients and recommended by National Comprehensive Cancer Network (NCCN) as the first-line treatment for HCC.<sup>12,13</sup> Moreover, apatinib and ramucirumab have succeeded in the therapy of HCC patients those who have failed first-line treatment with sorafenib.<sup>14,15</sup> These data demonstrate that anti-VEGF/VEGFR therapy is an effective treatment for HCC. Bevacizumab, which is a monoclonal antibody against VEGF, is used for the treatment of advanced colorectal, lung, breast, and brain cancers.<sup>16</sup> Currently, bevacizumab has been used in few clinical trials in HCC as single agent. A phase II trial showed that the median progression-free survival (mPFS) was 6.9 months in 46 HCC patients who underwent bevacizumab therapy,<sup>17</sup> suggesting that bevacizumab is potentially effective treatment for advanced HCC. As antitumor angiogenesis agents can normalize tumor vessels and change the immunosuppressive environment of HCC, combination therapy with immune checkpoint inhibitors and antitumor angiogenesis agents demonstrated a synergistic antitumor effect.<sup>18</sup> The combination therapy of programmed cell death 1 ligand 1 (PD-L1) inhibitor at zolizumab with bevacizumab improved mPFS significantly, when compared with sorafenib (6.8 versus 4.3 months, HR 0.59; 95% CI: 0.47–0.76; p<0.0001).<sup>19</sup> However, atezolizumab is too expensive for the developing countries, thus bevacizumab may be HCC treatment regime.

MicroRNAs (miRNAs) are 18–25 nt short noncoding RNA sequences that bind to the 3'-untranslated region (3'UTR) of target mRNAs to modulate gene expression programs by regulating their translation and stability.<sup>20–22</sup> Dicer is a cytoplasmic RNaseIII enzyme that cleaves premicroRNAs into mature microRNAs and short interfering RNAs in the cytoplasm.<sup>23</sup> Several lines of evidence have demonstrated that Dicer downregulation was associated with poor prognosis in some human cancers, such as HCC, renal cell carcinoma (RCC), gastric cancer, breast cancer, colorectal cancer and chronic lymphocytic leukemia, thus acting as a tumor suppressor.<sup>24–29</sup> As Dicer suppressed VEGF-A, a key prototypical member of the VEGF family in RCC,<sup>30</sup> we investigated whether Dicer may control HCC progression through the VEGF pathway.

## Materials and Methods Cell Culture and Transduction

The human HCC cell line HuH-7 was purchased from Procell Life Science & Technology Co., Ltd (Wuhan, Hubei, China), and SMMC-7721 was acquired from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). Both cell lines were characterized by mycoplasma detection (Supplementary Figure S1), DNA fingerprinting, isozyme detection and cell viability. HCC cells were cultured in DMEM high-glucose medium (GibcoTMLife Technologies, NY, USA) with 10% fetal bovine serum (FBS) (GibcoTMLife Technologies) in a humidified incubator containing a 5% CO<sub>2</sub> atmosphere at 37 °C.

To overexpress *Dicer*, approximately  $1 \times 10^5$  HCC cells were incubated with a Dicer-overexpressing lentivirus (pCMV-Dicer) (GeneCopoeia, Rockville, MD, USA) tagged with green fluorescent protein (GFP) as described previously.<sup>31</sup> As a negative control, HCC cells were infected with control lentivirus (pCMV-NC) (GeneCopoeia, Rockville, MD, USA). GFP expression was observed under the microscope 72 h after infection to determine transduction efficiency. Seventy-two hours post-transduction, cells were selected with puromycin (2 µg/mL) for 2 weeks to generate stable cell lines. Successful overexpression of Dicer was confirmed with Western blotting using an anti-Dicer antibody (Abcam, Cambridge, UK).

#### Western Blot

Western blotting was performed, as previously described,<sup>32</sup> to confirm *Dicer* overexpression and the level of VEGF-A. Equal protein quantities from total cell lysates were subjected to SDS-PAGE electrophoresis and transferred to PVDF membranes. Membranes were blocked for 2 h in blocking buffer (5% non-fat dry milk in Tris-buffered saline with 0.1% Tween 20) at room temperature and incubated overnight at 4°C with the following primary antibodies: anti-Dicer (dilution 1:1000; Abcam, Cambridge, UK), anti-VEGF-A (dilution 1:1000; Abcam, Cambridge, UK), and anti- $\beta$ -actin (dilution 1:5000; Abcam, Cambridge, UK), followed by incubation with an anti-mouse IgG antibody (Abcam, Cambridge, UK) at a dilution of 1:5000. The relative intensities of protein bands were visualized using ECL (BD, San Diego, CA).

## Cell Proliferation Assay

VEGF-A165 cytokine was purchased from Meilun Biotechnology Co. Ltd. (Dalian, China), and bevacizumab was purchased from Roche pharma (South San Francisco, CA). We used Cell Counting Kit-8 (CCK-8; Dojindo, Kumamoto, Japan) to measure cell proliferation.<sup>33</sup> According to the manufacturer's protocol, approximately  $1 \times 10^4$  cells were seeded into 96-well plates with 100 µL medium per well. Cell proliferation was determined at different time points, including 0, 12, 24, 48, 72 and 96 h after a 2 h incubation with 10  $\mu$ L of CCK-8. Absorbance in each well was measured at 450 nm wavelength by a microplate reader (Bio-Rad, Hercules, CA).

## Wound Healing Assay

A wound healing assay was performed to determine the migration ability of the cells.<sup>34</sup> Briefly, cells were seeded on 6-well plates. After cells reached approximately 100% confluence, the surface of the plates was scratched linearly with a 200- $\mu$ L pipette tip. Cells were washed twice with PBS and cultured in DMEM medium with 2% FBS. Images were captured using an inverted microscopy (Nikon, Tokyo, Japan) at 0 and 24 h. Healing rates were calculated as the width of a wound at 24 h divided by the initial width.<sup>35</sup>

## Invasion Assay

Cell invasion was determined using 24-well transwell chambers with 8-µm pore size (Corning, New York, NY) precoated with 1mg/mL BD Matrigel (BD Biosciences, NJ).<sup>36</sup> Before the invasion assay, cells were cultured for 24 h in DMEM medium with 2% FBS. In the upper compartment of the chamber, approximately  $1\times10^5$  cells were added to DMEM without FBS, while 500 µL of DMEM medium with 10% FBS were added to the lower chamber. After being incubated at 3<sup>7</sup>C in a 5% CO<sub>2</sub> atmosphere for 24 h, cells invaded into the underside were then washed, fixed with 4% paraformaldehyde and stained with 0.5% crystal violet. Stained cells were counted with an inverted microscopy (Nikon, Tokyo, Japan) in five random fields for each membrane (magnification 200×).

## Mouse Xenograft Tumor Model

This study was approved by the Ethics Board of the Animal Ethics Committee of the Fourth Hospital of Hebei Medical University. Sixteen 4-week-old athymic nude BALB/c mice were purchased from Charles River Laboratories [Beijing, China; permission no. SCXK (Jing) 2016–0006]. Nude mice were housed and treated in accordance with the guidelines established by the National Institutes of Health Guide for the Care and Use of Laboratory Animals.<sup>37</sup>

Xenograft tumors were generated with subcutaneous injection of  $1 \times 10^7$  SMMC-7721 treated with pCMV-*Dicer* or pCMV-NC in 0.2 mL medium into the shoulder of nude mice.<sup>38</sup> Seven days after tumor cell injection, mice were divided into the following four groups (four mice per group): pCMV-NC (group 1), pCMV-NC plus bevacizumab (group 2), pCMV-Dicer (group 3), and pCMV-Dicer plus bevacizumab (group 4). Groups 2 and 4 were intraperitoneally injected with 20 mg/kg bevacizumab at a concentration of 2.5mg/mL once every 3 days for 3 weeks, while groups 1 and 3 were intraperitoneally injected with 8 mL/kg saline as the negative control. Tumor growth and weight were measured every 7 days, and tumor volume was calculated according to the following formula: Volume = Length  $\times$  (Width)<sup>2</sup>/2.<sup>39</sup> In vivo green fluorescent images were acquired with NightOwl Bioimager (Berthold Technologies, Bad Wildbad, Germany) at timepoint of 18 and 28 days after implantation. The fluorescent intensity was analyzed by WinLight32 software package (Berthold Technologies).

## VEGF-A Protein Expression in Xenograft Tumors

For xenograft tumors, 20 mg of tissue was added to 100 mL RIPA lysis buffer (Zomanbio, Beijing, China), homogenized, and centrifuged at 13,000 g for 15 min. Protein concentration was determined by a BCA Protein Assay Kit (Zomanbio, Beijing, China) according to the manufacturer's protocol. Then, the protein level of VEGF-A was examined with Western blotting, as described previously. For quantification analysis, the total density of each band was analyzed using Image-J software (National Institutes of Health, Bethesda, USA).

## Immunohistochemical Analysis

After mice were sacrificed, tumor samples from each group were harvested, fixed in 4% formaldehyde for 24 h, then embedded in paraffin. Five-micrometer sections were immunostained with CD31 (Abcam, Cambridge, UK). For semi-quantification of microvessel density (MVD), positive staining was defined with the Weidner method: a microvessel was counted when cells or cell clusters were stained brown with CD31 with a clear separation from the surrounding tissues. Areas of highest neovascularization were found by scanning the tumor sections at low magnification ( $100\times$ ), and then individual microvessels were counted at high magnification ( $200\times$ ).<sup>40</sup>

## miRNA Microarray Analysis

RNA samples from pCMV-*Dicer* and pCMV-NC group cells (three samples for each group) were examined with

microarray analysis at Cnkingbio Biotechnology Corporation (Beijing, China), using FlashTag Biotin HSR RNA Labeling Kit (Affymetrix, Santa Clara, CA). Labeling and hybridization were performed according to the manufacturer's instructions. Microarray for miRNAs was manufactured and processed as described.<sup>41</sup> Microarrays were scanned on GeneChip Scanner 3000 (Affymetrix), and data were analyzed using the GeneChip Command Console software (Affymetrix).

## Statistical Analysis

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS Company, version 21.0; Chicago, IL, USA). Results were presented as the mean  $\pm$  standard deviation. Two experimental groups were performed using Student's *t*-test after tested for normality. Multiple groups were compared using a one-way analysis of variance (ANOVA) first. The least significant difference *t*-test was applied, if the overall difference was statistically significant. A *p*-value≤0.05 was considered statistically significant.

## Results

# Dicer Downregulates VEGF-A Expression in HCC

Successful overexpression of Dicer was confirmed with Western Blotting (Figures 1A and 2A). We then assessed the relationship between *Dicer* and VEGF-A in the HCC cell lines SMMC-7721 and HuH-7. As shown in Figures 1A and 2A, the level of VEGF-A was dramatically decreased upon *Dicer* overexpression. These data demonstrated that *Dicer* could downregulate VEGF-A expression in HCC.

## VEGF-A165 Counteracts the Growth Inhibition Induced by Dicer in HCC Cells

We examined the Dicer-induced effect on HCC cells by comparing HCC cells infected with pCMV-*Dicer* or pCMV-NC, and found that the proliferation of pCMV-*Dicer* cells significantly decreased 48 to 96 hours after infection (Figures 1B, C, 2D and E, p<0.05); moreover, the ability of HCC cells to migrate decreased dramatically in pCMV-*Dicer* cells (Figures 1D, E, 2F and G, p<0.01), together with their invasive capacity (Figures 1F, G, 2H and I, p<0.01). Based on these data, we examined whether Dicer inhibited HCC proliferation, migration, and invasion via the VEGF pathway. As VEGF-A165 is the most prominent VEGF-A isoform involved in HCC angiogenesis,<sup>42,43</sup> we added VEGF-A165 to the medium of the pCMV-*Dicer* cell culture to investigate its effect on HCC cell growth. Proliferation of pCMV-*Dicer* cells significantly increased 48 to 96 h after addition of 50 and 100 ng/mL VEGF-A165 to the medium (Figure 2B and C, p<0.05), therefore, 50 ng/mL VEGF-A165 was used for subsequent analysis.

VEGF-A165 promoted the proliferation from 48 to 96 h (Figure 2D and E, p<0.05), migration (Figure 2F and G, p=0.001) and invasion (Figure 2H and I, p=0.01) in pCMV-NC infected SMMC-7721 cells. In addition, VEGF-A165 promoted proliferation from 72 to 96 h (Figure 2D and E, p<0.01), migration (Figure 2F and G, p=0.004) and invasion (Figure 2H and I, p=0.002) in pCMV-*Dicer* cells. The fact that VEGF-A165 counteracted the growth inhibition induced by Dicer in HCC cells implied that Dicer may inhibit HCC cell growth via the VEGF pathway.

## Bevacizumab Enhanced Dicer-Induced HCC Cell Growth Inhibition in vitro

As Dicer inhibited HCC cell growth via the VEGF pathway, we investigated the effect of a blocking anti-VEGF antibody (bevacizumab) on Dicer-induced HCC cell growth inhibition. As shown in Figure 3A, after incubation with 20 µg/mL bevacizumab, proliferation of pCMV-Dicer-infected SMMC-7721 cells significantly decreased from 48 to 72 h (Figure 3A, p < 0.01), hence we used this concentration for subsequent experiment. Bevacizumab inhibited cell proliferation from 48 to 96 h (Figure 3B and C, p < 0.01), migration (Figure 3D and E, p=0.000) and invasion (Figure 3F and G, p=0.000) of pCMV-NC infected SMMC-7721 cells. Bevacizumab resulted in additive inhibition of cell proliferation from 24 to 96 h (Figure 3B and C, p<0.01), migration (Figure 3D and E, p=0.004) and invasion (Figure 3F and G, p=0.018) in pCMV-Dicer cells. Furthermore, the ability of pCMV-Dicer plus Bevacizumab HCC cells to proliferation from 48 to 96 h (Figure 3B and C, p<0.01), migration (Figure 3D and E, p=0.000) and invasion (Figure 3F and G, p=0.000) obviously decreased compared to pCMV-NC HCC cells. Taken together, we conclude that bevacizumab suppressed proliferation, migration and invasion of HCC cells, thereby enhancing Dicer-induced HCC inhibition.



Figure I Dicer inhibits proliferation, migration and invasion of HuH-7 cells. (A) Western blot of Dicer and VEGF-A in HuH-7 cells; (B) The proliferation measurement by CCK-8 assay; (C) Quantification of results of (B); (D) The migration measurement by wound healing assay; (E) Quantification of results of (D); (F) The invasion measurement by transwell assay; (G) Quantification of results of (F). \*\* $p \le 0.01$ . Abbreviation: CCK-8, Cell Counting Kit-8.

## Bevacizumab Enhanced Dicer-Induced HCC Growth Inhibition in vivo

In vivo, the mean tumor volume was significantly smaller in pCMV-*Dicer* xenografts than in pCMV-NC xenografts at 28 days after implantation (Figure 4A–C, p=0.001). Throughout the course of treatment, the bevacizumab-related growth inhibition of pCMV-NC xenografts was achieved at 28 days, when compared to pCMV-NC xenografts (Figure 4A–C, p=0.005). Moreover, the tumor volume of pCMV-*Dicer* xenografts treated with bevacizumab was significantly reduced from 14 to 28 days, when compared with that of pCMV-*Dicer* xenografts (Figure 4A–C, p<0.05) and pCMV-NC xenografts (Figure 4A–C, p<0.05). These data demonstrated that treatment with 20 mg/kg bevacizumab every three days enhanced Dicer-induced inhibition of HCC xenografts growth.

pCMV-Dicer xenografts showed lower VEGF-A expression compared to pCMV-NC xenografts, as determined with Western blotting (Figure 4D and E, p=0.001). Bevacizumab decreased the level of VEGF-A in pCMV-NC HCC xenografts (Figure 4D and E, p=0.002). Furthermore, the level of VEGF-A in pCMV-Dicer xenografts treated with bevacizumab was significantly than that in pCMV-NC xenografts (Figure 4D and E, p=0.000). Although VEGF-A expression in pCMV-Dicer xenografts treated with bevacizumab was reduced compared with that in pCMV-Dicer xenografts, the difference was not statistically significant (Figure 4D and E, p=0.121). CD31 expression was subsequently measured to compared the MVD difference upon bevacizumab treatment. As shown in Figure 4F and G, the MVD in pCMV-Dicer xenografts treated with bevacizumab was lower than that in pCMV-



Figure 2 VEGF-A165 counteracts the inhibition induced by Dicer. (A) Western blot of *Dicer* and VEGF-A in SMMC-7721 cells; (B) Determine the concentration of VEGF-A165 by CCK-8 assay; (C) Quantification of results of (B); (D) The proliferation measurement by CCK-8 assay; (E) Quantification of results of (D); (F) The migration measurement by wound healing assay; (G) Quantification of results of (F); (H) The invasion measurement by transwell assay; (I) Quantification of results of (H). \* $p \le 0.05$ , \*\* $p \le 0.01$ .

Abbreviation: CCK-8, Cell Counting Kit-8.

*Dicer* xenografts (Figure 4F and G, p=0.046). In addition, tumor MVD was significantly decreased in pCMV-*Dicer* xenografts treated with bevacizumab compared with pCMV-NC xenografts (Figure 4F and G, p=0.040). Moreover, bevacizumab decreased the MVD in pCMV-NC xenografts at a marginal statistical level (Figure 4F and G, p=0.070). These results indicated that bevacizumab and *Dicer* had a synergistic effect on suppression tumor angiogenesis.

#### Discussion

In the present study, we found that Dicer inhibited the growth of HCC cell in vitro and in vivo, as well as downregulated VEGF-A expression. VEGF-A165 counteracted the Dicer-induced HCC cell growth inhibition, while bevacizumab enhanced Dicer-induced HCC cell growth inhibition. Furthermore, bevacizumab and Dicer had a synergistic effect on the suppression tumor angiogenesis in HCC xenografts. Our data implied that Dicer could



Figure 3 Bevacizumab enhances the inhibition induced by Dicer in vitro. (A) Determine the concentration of bevacizumab by CCK-8 assay; (B) The proliferation measurement by CCK-8 assay; (C) Quantification of results of (B); (D) The migration measurement by wound healing assay; (E) Quantification of results of (D); (F) The invasion measurement by transwell assay; (G) Quantification of results of (F). \*\* $p \le 0.01$ . Abbreviation: CCK-8, Cell Counting Kit-8.

enhance bevacizumab-induced HCC inhibition via the VEGF pathway.

HCC is a hypervascular tumor with a complex vascular network and several angiogenesis growth factors, VEGF, platelet-derived growth factor (PDGF) and basic fibroblast growth factor (bFGF), promote invasion and metastasis of HCC.<sup>5</sup> VEGF contributes greatly to HCC pathogenesis,<sup>44,45</sup> by promoting the growth of vascular endothelial cells (ECs) derived from arteries, veins and lymphatics.<sup>46</sup> It prevents apoptosis via phosphatidylinositol (PI)-3 kinase-Akt pathway and promotes monocyte chemotaxis and colony formation of granulocyte-macrophage progenitor cells.47,48 It also induces an increase in vascular leakage mediated by calcium influx,<sup>49</sup> thereby, potentially promoting tumor growth and metastasis. Bevacizumab, which is the most successful VEGF-neutralizing antibody, normalized tumor vessel growth leading to more efficient delivery of drugs in tumor microenvironment. When combined with chemotherapy, bevacizumab prolonged the survival of patients with lung, colon and breast cancer.<sup>16,50</sup> In addition to chemotherapy synergism, bevacizumab significantly enhanced the outcome

of treatment with the PD-L1 inhibitor atezolizumab in HCC patients by decreasing the activity of myeloid-derived suppressor cells and regulatory T cells as well as increasing cytotoxic T lymphocyte infiltration.<sup>19,51</sup>

Dicer, a key enzyme in the process of miRNA maturation,<sup>24–29</sup> has been reported as an inconsistent prognostics factor for several cancers. We found that Dicer suppressed HCC growth by deregulating VEGF-A expression, consistent with a previous report showing that Dicer inhibited migration, invasion of clear cell RCC through suppressing VEGF-A expression.<sup>30</sup> Because Dicer regulates miRNA expression, we performed miRNA microarray analysis to identify potential miRNAs that could affect VEGF-A expression. A total of 42 miRNAs were identified with a fold change  $\geq 1.5$  upon *Dicer* overexpression (Supplementary Figure S2, Supplementary Table S1). Among the aberrantly expressed miRNAs, two miRNAs that suppress VEGF-A expression (miR-622, miR-378a-5p) were upregulated and three miRNAs that promote VEGF-A expression (miR-210-3p, miR-132-5p, miR-874-3p) were downregulated,<sup>52-56</sup> whereas one miRNA



Figure 4 Bevacizumab enhances the inhibition induced by Dicer in vivo. (A) Fluorescence image of mice bearing HCC cells xenografts; (B) Tumor growth was monitored by measuring tumor volume for HCC xenografts; (C) Quantification of results of (B); (D) Western blot of VEGF-A in xenograft tumor tissue; (E) Quantification of results of (D). (F) Immunohistochemical analysis of CD31; (G) Quantification of results of (F). n=4. \*p≤0.05, \*\*p≤0.01.

that represses VEGF-A expression (miR-342-5p) was downregulated.<sup>57,58</sup> In summary, Dicer regulated five miRNAs that suppress VEGF-A expression and one miRNA that promotes VEGF-A expression. Obviously, the effect of suppression VEGF-A expression caused by Dicer was greater than that of promotion. In addition, four anti-angiogenic miRNAs related to other VEGF family members (miR-4485, miR-148a-5p, miR-338-3p, miRwere upregulated and one pro-angiogenic 374b-5p) downregulated.59-63 (miR-1247-5p) miRNA was Therefore, Dicer may mediate angiogenesis through regulating these miRNAs related to VEGF.

Because our sample size of HCC xenografts was small, bevacizumab seemed to have the tendency to decrease VEGF-A level in pCMV-*Dicer* xenografts and MVD in pCMV-NC xenografts, albeit the difference was not statistically significant. In addition, the volume of pCMV-*Dicer* xenografts treated with bevacizumab significant decreased when compared with that of pCMV-NC xenografts at an early stage after implantation. These data indicate the clinical potential of *Dicer* to synergize with bevacizumab on HCC treatment. Several preclinical studies did suggest that the use of bevacizumab in combination with other agents may be a choice for HCC treatment.<sup>64</sup> We found that calcitriol inhibited the growth of gastric cancer cells by inducing Dicer expression,<sup>65</sup> however a suitable concentration of calcitriol should be assessed and validated preclinically in HCC xenografts.

## Conclusion

Dicer enhanced bevacizumab-related inhibition of HCC cell and xenograft via the VEGF pathway, therefore factors that induce *Dicer* expression may be considered in combination with bevacizumab as an alternative option for HCC therapy.

## Abbreviations

VEGF, vascular endothelial growth factor; HCC, hepatocellular carcinoma; MVD, microvessel density; PLC, primary liver cancer; HBV, hepatitis B virus; VEGFR, VEGF receptor; TKIs, tyrosine kinase inhibitors; NCCN, National Comprehensive Cancer Network; mPFS, median progression-free survival; PD-L1, programmed cell death 1 ligand 1; miRNAs, microRNAs; 3'UTR, 3'-untranslated region; RCC, renal cell carcinoma, FBS, fetal bovine serum; GFP, green fluorescent protein; CCK-8, cell counting kit-8; SPSS, Statistical Package for the Social Sciences; ANOVA, one-way analysis of variance; PDGF, platelet-derived growth factor; bFGF, basic fibroblast growth factor; ECs, vascular endothelial cells; PI, phosphatidylinositol.

## **Data Sharing Statement**

The datasets used and/or analyzed during the current study are available from Zhanjun Guo upon reasonable request.

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## Disclosure

The authors report no competing interest in this work.

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