

Berberine enhances the anti-HCC effect of NK cells through inhibiting interferon-gamma-mediated PD-L1 expression

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Research Article

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Abstract

Berberine (BBR) is an isoquinoline alkaloid with various functions in anti-inflammation, blocking tumor immune escape and enhancing the anti-tumor activity of immune cells. However, its immunomodulatory effect on natural killer (NK) cell activity in Hepatocellular carcinoma (HCC) is still unclear. Here, we determined whether BBR enhances the anti-HCC effect of NK cells and its mechanism by focusing on the regulation of the Programmed death-ligand 1 (PD-L1) pathway. It was found that BBR enhanced the cytotoxicity of NK92-M1 cells to HCC cells *in vitro* and *in vivo*. The combination of BBR and PBMCs inhibited the proliferation and induced the apoptosis of HCC cells. BBR increased the frequency of CD3⁻CD56⁺ and CD3⁻CD16⁺ NK cells in peripheral blood isolated from healthy volunteers. Furthermore, the expression of PD-L1 in HCC cells was up-regulated after co-culture with NK-92MI cells or PBMCs. PD-L1 knockdown increased the sensitivity of HCC cells to NK-92MI cells and PBMCs. Mechanisms for BBR blocked the secretion of Interferon Gamma (IFN- γ), thereby inhibiting PD-L1 expression caused by the interaction of NK92-M1 cells/PBMCs and HCC cells. Collectively, we are the first to demonstrate that BBR plays an immunomodulatory role by enhancing the cytotoxic effect of NK cells and inhibiting tumor immune escape by reducing the expression of PD-L1. Our study provides a theoretical basis for the clinical application of BBR combined with NK cells in the treatment of HCC.

Introduction

Hepatocellular carcinoma (HCC) is the fourth leading cause of cancer-related death and the sixth most common malignancy[1]. Although the treatment of HCC has improved, the prognosis of most patients is still poor[2]. Natural killer (NK) cells are immune cells of the lymphoid lineage with natural cytotoxicity and the capacity to secrete cytokines[3]. Compelling evidence has suggested that patients with HCC show a significant reduction in the number of peripheral and intrahepatic NK cells, which are often functionally exhausted with impaired Interferon Gamma (IFN- γ) production and cytotoxicity in the tumor[4–6]. It has been reported that sorafenib elicits macrophages pyroptosis that enhances NK-cell effector function and ultimately effective tumor cell killing[7]. Cisplatin enhances the efficacy of NK cells immunotherapy via altering the androgen receptor (AR)-ULBP2 signals[8]. Therefore, searching for therapeutics that activate NK cells is urgently needed for HCC.

BBR is an isoquinoline alkaloid with anti-inflammatory and immunomodulatory effects[9–10]. BBR acts as a potential immunotherapeutic drug via enhancing the anti-tumor activity of immune cells and blocking tumor immune escape[11–12]. BBR derivatives have been reported to enhance the killing effect of NK cells on lung cancer cell A549 by activating the AMPK signaling axis and inhibiting STAT1 phosphorylation[11]. However, the immunomodulatory effect of BBR on NK cell activity in HCC is still unclear.

The programmed cell death-ligand 1(PD-L1) / programmed cell death protein-1 (PD-1) pathway has been identified as the most critical immune checkpoint in immunotherapy[13]. PD-L1 is normally up-regulated in various tumors and tumor-infiltrating immune cells, which promotes a host of immunosuppressive

effects upon binding to its receptor PD-1[14]. Tumor cells in the tumor microenvironment can up-regulate PD-L1 after encountering activated immune cells via their secretion of IFN- γ [15]. In this study, we investigated the contributions of BBR to the anti-HCC effect of NK cells and its mechanism by focusing on the regulation of the PD-L1 pathway.

Material And Methods

Cells and reagents

Human HCC cell line SMMC-7721 and NK cell line NK92-MI were obtained from the Cell Bank of Type Culture Collection (Chinese Academy of Sciences, Shanghai, China). Human HCC cell line Hep3B was obtained from the American Type Culture Collection(Manassas, VA, USA). Cells were cultured in Dulbecco's modified Eagle's medium(GIBCO; Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10%(V/V) fetal bovine serum(GIBCO) and maintained at 37°C in a 5% CO₂ incubator. BBR was obtained from Selleck Chemicals (Houston, TX, USA; cat. no. S2271).

PBMCs isolation

PBMCs were obtained from the peripheral blood provided by healthy volunteers using Ficoll standard density gradient centrifugation(Tianjin,China;cat.no.LDS1075). PBMCs were incubated in 1640 medium(GIBCO) and supplemented with 10%FBS (GIBCO) and 50IU/ml Recombinant Human Interleukin-2(IL-2, Jiangsu Jinsili Pharmaceutical Co. LTD, China), which were maintained at 37°C in a 5% CO₂incubator. Ethical approval for the study was given by the Research Ethics Committee of the Second Affiliated Hospital of Guangzhou Medical University. All healthy volunteers obtained informed consent before collecting the peripheral blood.

LDH cytotoxicity analysis, MTS assay, EdU incorporation assay and Apoptosis assay

HCC cells were co-incubated with NK92-MI, PBMCs, BBR(60 or 80 μ M), or their combination for 36h, respectively. The CytoTox 96® Non-Radioactive Cytotoxicity Assay (Promega; USA) was used to measure the release level of lactate dehydrogenase (LDH) enzyme according to the manufacturer's instruction. The viability of HCC cells was detected by MTS assay Cell Titer 96® AQueous One solution (Promega, USA; cat, no.G3588). The Cell-Light™ EdU Apollo567 In Vitro Imaging Kit (RiboBio, Guangzhou, China) was used to evaluate cell proliferation levels. The DeadEnd™ Fluorometric TUNEL System assay(Promega, cat. no. G3250; USA) and Annexin V-V450/PI Apoptosis Detection Kit (Thermo Fisher, USA) were performed to detect cellular apoptotic level.

In vivo tumor assay

SMMC-7721 cells(1×10^7) were injected into the dorsal area to establish a subcutaneous tumor model in male BALB/c nude mice (Guangdong Animal Center, China). When the tumor reached a size of 5 \times 5 mm², mice were treated either with DMSO (as control), BBR(10mg/kg/d; intraperitoneal injection twice a week), NK92-MI (1×10^6 per mouse; peritumor injection twice a week) or their combination, respectively. Tumor volumes were determined by a caliper and calculated according to the formula (width² \times length)/2. These

experiments were performed in accordance with the institutions of guidelines for the use of laboratory animals and approved by the second affiliated hospital of Guangzhou Medical University.

Flow cytometry analysis of PBMCs

After incubating PBMCs with SMMC-7721 cells, 80 μ M BBR, or their combination for 36h, the populations of T cell and NK cells were analyzed by flow cytometer. The following antibodies were used for phenotypic analysis of PBMCs: live/dead Mouse IgG1 kappa Isotype Control(eFluor 660; eBioscience,USA,cat.no.50-4714-82), PerCP-Cy™ 5.5 Mouse Anti-Human CD3 Clone UCHT1(BD,USA,cat.no.560835), FITC Mouse Anti-Human CD4 Clone RPA-T4(BD,USA,cat.no.555346), PE Mouse Anti-Human CD8 Clone RPA-T8(BD,USA,cat.no.555367), PE-Cy™ 7 Mouse Anti-Human CD56 Clone B159(BD,USA,cat.no.557747), BV421 Mouse Anti-Human CD16 Clone 3G8(BD,USA,cat.no.562847), PE Mouse Anti-Ki-67 (BD, USA, cat.no.556027).

siRNA transfection

Human PD-L1 siRNA and scramble siRNA were obtained from RiboBio company (Guangzhou, China), as following: scramble siRNA (sense: 5'-UUCUCCGAACGUGUCACGUTT-3', antisense:5'-ACGUGACACGUUCGGAGAATT-3');siPD-L1(sense:5'-GGAUCCAGUCACCUCUGAATT-3',antisense:5'-UUCAGAGGUGACUGGAUCCTT-3'). HCC cells were transfected with siRNA usingLipofectamine® RNAiMAX reagent (Invitrogen, Carlsbad, CA, USA).

Western Blot analysis

The extraction of total protein lysate and SDS-PAGE were performed according to previously described[16]. The primary antibodies included anti-poly (ADP-ribose) polymerase (PARP; 1:1,000; cat. no. 9532; Cell Signaling Technology, Inc.), anti-PD-L1 (1:1,000; cat. no.ab213524; Abcam, Cambridge, UK) and anti-GAPDH (1:1,000; cat. no. 2118; Cell Signaling Technology, Inc.).

Statistical Analysis

Statistical analyses were conducted using SPSS version 19.0 software (SPSS Inc., Chicago, IL, USA). The quantitative data were shown as means \pm SD from three independent experiments. Statistical analysis was performed using Student's t-test or one-way ANOVA. A value of $P < 0.05$ indicated a significant difference.

Results

BBR enhanced the cytotoxicity of NK92-MI cells to HCC cells

To detect the cytotoxicity of NK92-MI cells against HCC cells under BBR treatment, lactate dehydrogenase (LDH) release assay was performed, and the release of LDH from HCC cells in BBR and NK92-MI co-treated group was higher than that in other treatment groups (Fig. 1a). The expression of cleaved-PARP protein was up-regulated in the BBR, NK92-MI and HCC cells co-treated group (Fig. 1b). To verify the anti-tumor effect of the combination of BBR andNK92-MI in vivo, a subcutaneous xenograft tumor model was established by transplanting SMMC-7721 cells into nude mice. The combined treatment group exhibited

a reduced gain of tumor sizes compared with the control group, BBR, or NK92-MI single treated group(Fig. 1c). The tumor volume and weight of the combined treatment group were also reduced (Fig. 1d-e). These results demonstrate that BBR synergistically enhances the anti-HCC effect of NK92-MI.

PD-L1 knockdown increased the sensitivity of HCC cells to NK92-MI

To investigate whether the killing effect of NK92-MI was related to PD-L1, the expression of PD-L1 in HCC cells was knocked down by PD-L1 siRNA(Fig. 2c). We found that comparing with NK92-MI and HCC cells co-culture group, the viability and proliferation level of HCC cells decreased in NK92-MI and PD-L1 knocked-down HCC cells co-culture group(Fig. 2a-b), and the apoptosis level of HCC cells increased (Fig. 2c). As shown in Fig. 2d, PD-L1 expression was detected only in the presence of NK92-MI cells and up-regulated in the co-culture of NK92-MI cells and HCC cells. However, BBR could down-regulate PD-L1 expression caused by the interaction of NK92-MI and HCC cells. These results indicate that BBR enhances the anti-HCC effect of NK92-MI by regulating PD-L1 expression.

The combination of BBR and PBMCs inhibited the proliferation and induced the apoptosis of HCC cells

Since BBR can enhance the cytotoxicity of NK92-MI cells against HCC cells, we wonder whether BBR has a similar effect on PBMCs of healthy volunteers. HCC cells were treated with different concentrations of BBR, PBMCs, and their combination. Results of the EdU assay showed that the proliferation level of HCC cells decreased after BBR and PBMCs treatment(Fig. 3a). We observed an increase in apoptotic cells in the BBR and PBMCs co-culture group(Fig. 3b and Fig. S1), and the protein expression of cleaved-PARP was up-regulated (Fig. 3c). Collectively, these data demonstrate that BBR can also enhance the anti-HCC effect of PBMCs.

BBR increased the frequency of NK cells in PBMCs

Then, we analyzed the frequencies of T cells (CD3 + CD4+ / CD3 + CD8+) and NK cells (CD3-CD56+ / CD3-CD16+) in PBMCs by flow cytometry. After co-cultivation of PBMCs and BBR,the frequency of CD3 + CD4 + T cells decreased, while the percentage of T cells (CD3 + CD8+) and NK cells (CD3-CD56+ / CD3-CD16+) increased significantly compared with the control group (Fig. 4a-b). In addition, the frequency of NK cells in BBR, PBMCs, and SMMC-7721 co-culture group was also significantly higher than that in PBMCs and SMMC-7721 group (Fig. 4c-d). Therefore, NK cells maybe are the main immune cells that enhance the anti-tumor effect of PBMCs after treatment with BBR or co-culture with BBR and SMMC-7721 cells.

PD-L1 was involved in BBR enhancing the anti-HCC effect of PBMCs

To explore whether PD-L1 participated in the anti-HCC effect of PBMCs, PD-L1 expression was knocked down in HCC cells. When HCC cells were co-cultured with PBMCs, the viability and proliferation level of PD-L1 knockdown HCC cells decreased Fig. 5a-b), while the protein expression of cleaved-PARP was up-regulated(Fig. 5c). We also found that BBR alone could not induce PD-L1 expression. PD-L1 was up-regulated only when HCC cells were co-cultured with PBMCs, and BBR down-regulated PD-L1 expression was caused by the interaction of PBMCs and HCC cells (Fig. 5d).

BBR inhibited PD-L1 expression through blocking the secretion of IFN- γ

To search for cytokines that may regulate PD-L1 expression in PBMCs and HCC cells interaction, cellular supernatants were further collected from different groups, The results indicated that supernatants in the co-culture group of PBMCs and SMMC-7721 could induce PD-L1 expression, while the supernatants containing BBR inhibited its expression(Fig. S2a).

Subsequently, RayBio Human Cytokine Antibody Array detection was conducted, and we observed that these creation levels of IFN- γ , GM-CSF, IL-8, and IL-10 were increased in the conditioned medium of SMMC-7721 and PBMCs co-culture group, while decreased in the presence of BBR(Fig. 6a and Fig. S2b). However, after treatment with IL-8 and IL-10, the expression of PD-L1 could not be detected (data not shown). Only IFN- γ (Fig. 6b), but not GM-CSF (Fig. S2c), could induce PD-L1 expression. Furthermore, ELISA confirmed the change of IFN- γ secretion in the conditioned medium of SMMC-7721 and BBR, PBMCs, and their co-culture groups(Fig. 6c).

To further investigate whether BBR enhanced the anti-HCC effect of PBMCs / NK92-MI by inhibiting IFN- γ secretion and PD-L1 expression, IFN- γ was used in a rescue test. The results displayed that IFN- γ could rescue the viability of HCC cells in PBMCs and BBR co-culture groups (Fig. 6d).PD-L1 expression level increased, but cleaved PARP expression level decreased after IFN- γ was added into the PBMCs/NK92-MI and BBR co-culture groups (Fig. 6e-f). Compared with the PBMCs/NK92-MI and BBR co-cultured groups, the number of apoptotic HCC cells in the IFN- γ added group was reduced(Fig. 6g-h). Collectively, BBR inhibited PD-L1 expression by blocking the secretion of IFN- γ , thereby enhancing the anti-tumor effect of PBMCs/NK92-MI.

Discussion

This is the first study to elucidate the effect of BBR on the anti-HCC effect of NK cells. The results indicated that BBR could exert its immunomodulatory role by enhancing the anti-HCC effect of PBMCs and NK-92MI. When treated with BBR regardless of the presence or absence of HCC cells, the frequency of NK cells in PBMCs increases. Additionally, BBR inhibits tumor immune escape through down-regulating IFN- γ -mediated expression of PD-L1 in HCC cells after co-culture with immune cells(Fig. 7a-b).

BBR has been documented to possess many beneficial biological effects such as anti-inflammatory, antioxidant, and anti-tumor properties[17–19]. BBR exerts its anti-inflammatory activity through regulating the immune system, which has an affects on adaptive immunity by inhibiting the differentiation of Th1 and Th17 cells[20]and promotes the differentiation of Treg cells to ameliorate intestinal inflammation lesions[17]. Our study firstly revealed that BBR could enhance the cytotoxicity of NK92-MI cells to HCC cells. The combination of BBR and NK92-MI cells inhibited HCC cell proliferation and induced cellular apoptosis. A previous study has shown that Staphylococcal enterotoxin A enhances the proliferation and activation of human PBMCs, thereby inhibiting the proliferation and inducing the death of human lung carcinoma A549 cells when co-cultured with PBMCs[21]. In the present study, we found that BBR could increase the frequency of peripheral blood NK cells and enhance the anti-HCC effect of PBMCs. These findings indicate that BBR is a potential immunotherapy drug for liver cancer.

The immunosuppressive molecule PD-1 and its ligand PD-L1 have attracted much attention in tumor immunity. PD-L1 is typically expressed on tumor cells, enabling them to inhibit PD-1 + T cell function thereby enhancing the ability of tumors to escape the immune system[22–23]. Up-regulation of PD-L1 in HCC cells is associated with poor prognosis, which is a potential marker for poor prognosis and recurrence of liver cancer[24–25]. Furthermore, PD-L1 is expressed not only on tumor cells but also on immune cells such as NK cells, dendritic cells, and T cells within the tumor microenvironment[15, 26–27]. PD-L1 is up-regulated on T cells in cancer in response to antigen-presentation and PD-L1 + T cells have diverse tolerogenic effects on tumor immunity[27]. Besides, the expression of PD-L1 can be induced in NK cells when encountering tumor cells, and PD-L1 + NK cells had obviously enhanced cytotoxicity and IFN- γ production compared to PD-L1- NK cells[15]. Consistent with previous researches, we demonstrated that the expression of PD-L1 in HCC cells was up-regulated after co-culture with PBMCs or NK-92MI. However, this phenomenon was reversed by BBR. PD-L1 knockdown on HCC cells increased their sensitivity to PBMCs and NK92-MI cytotoxicity. These results indicate that BBR enhances the killing capacity of PBMCs and NK92-MI cells against HCC cells by down-regulating the expression of PD-L1.

IFN- γ is usually considered to be an anti-tumor cytokine[28]. NK cell-mediated production of IFN- γ has antiviral, immunoregulatory, and anti-tumor properties[28]. NK-secreted IFN- γ is responsible for shaping the adaptive immune response, and correlated with tumor stage and cancer prognosis[29]. Nevertheless, in certain circumstances, IFN- γ significantly induces tumor progression[30]. It acts as an inducer of the immune escape phenomenon through up-regulating PD-L1 expression[31–32]. In this study, RayBio Human Cytokine Antibody Array was performed to search for cytokines that regulate the expression of PD-L1 in the co-culture group of BBR, PBMCs, and SMMC-7721. In the PBMCs and SMMC-7721 cells co-culture group, we found that the secretion level of IFN- γ was increased, which led to the up-regulation of PD-L1 expression and induced the occurrence of immune escape. However, BBR could block the secretion of IFN- γ and inhibit the expression of PD-L1, thereby enhancing the anti-HCC effect of PBMCs and NK92-MI cells. Although IFN- γ is commonly thought to be an anti-tumor cytokine, our study further confirms that, in some cases, IFN- γ impairs tumor immunity via up-regulating PD-L1 expression. BBR can down-regulate IFN- γ -induced PD-L1 expression in HCC cells and ultimately restore the immune activity of PBMCs and NK92-MI cells.

The present study demonstrates that IFN- γ secreted by NK cells leads to the induction of PD-L1 molecule on the surface of HCC cells. HCC cells can escape the attack of immune cells. When BBR is co-culture with NK cells and HCC cells, BBR exerts an anti-HCC effect via increasing the frequency of NK cells and inhibiting the secretion of IFN- γ , which suppressed the expression of PD-L1. As a consequence, BBR promotes apoptosis of HCC cells and inhibits tumor immune escape. In summary, our study illustrates that BBR has an immunomodulatory role and can enhance the anti-HCC capacity of PBMCs/NK92-MI cells by strongly inhibiting IFN- γ -induced PD-L1 expression, thereby making HCC cells more sensitive to immune cells. The data from this research will provide a theoretical basis for the treatment of liver cancer with BBR and NK cells.

Declarations

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Competing interests

All authors declare that they have no competing interests.

Availability of data and materials

All data generated or analyzed in this study are available from the corresponding author upon reasonable request.

Authors' contributions

KW, CG, and GY designed and conducted the study, analyzed the data, and prepared the manuscript. JL, ZW, QL, YX, and DZ conducted the study and statistical analysis. XJ, JZ, and SL analyzed the data and reviewed the manuscript. HY generated the idea, designed the study, analyzed the data, and edited the manuscript. All authors have approved the final draft submitted.

Ethical approval

Ethical approval for the study was given by the Research Ethics Committee of the Second Affiliated Hospital of Guangzhou Medical University(No.2019-KY-0115C).

Consent to participate

All healthy volunteers obtained informed consent and agreed to publication before collecting the peripheral blood.

Consent for publication

All authors agree with publication.

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Figures

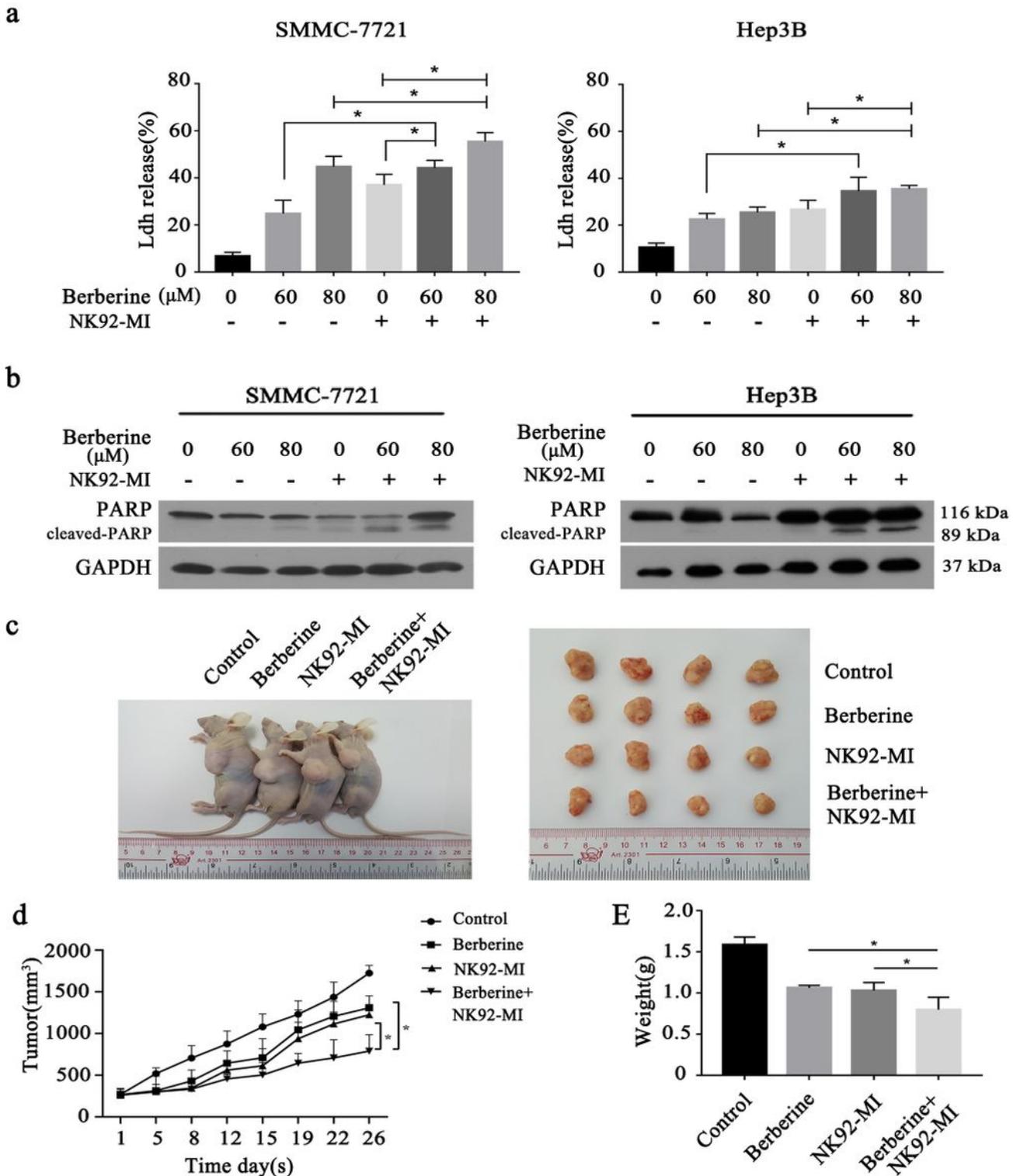


Figure 1

The cytotoxicity of NK92-MI was enhanced by BBR in vitro and in vivo. The cytotoxicity of NK92-MI cells against HCC cells was determined by LDH assay. Western blot analysis showed the expression level of cleaved-PARP protein in HCC cells that were co-cultured with BBR and NK92-MI cells. GAPDH served as a loading control. Treatments with DMSO, BBR, NK92-MI, or their combination were performed on nude mice. Representative images of xenograft tumors and tumors isolated from each group. Continuous

quantification of tumor volumes of each group during the experiment. e The weight of xenograft tumors was shown as the mean \pm SD of the four tumors resected in each group.*P<0.05.

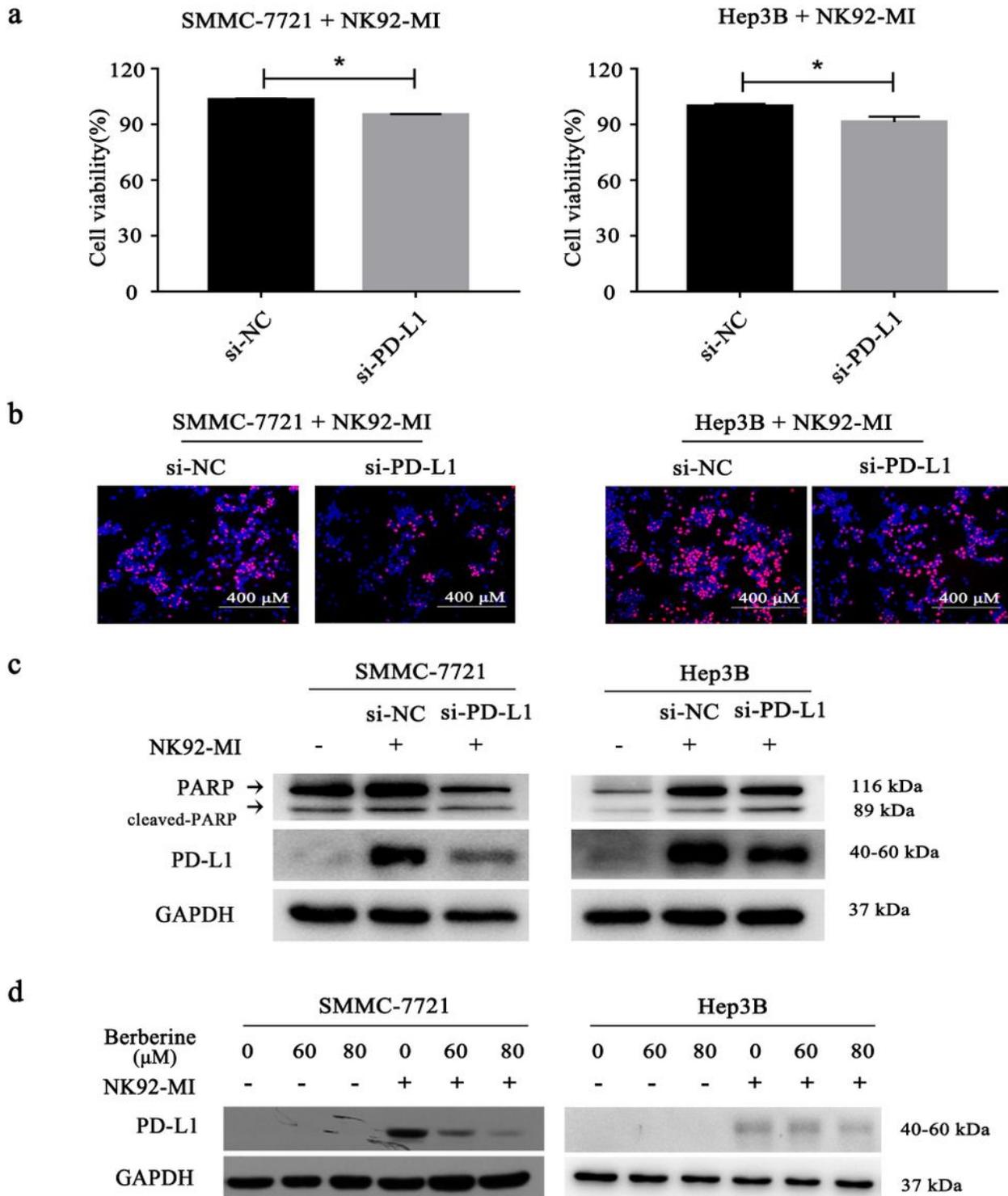


Figure 2

After down-regulating PD-L1, the anti-HCC effect of NK92-MI was enhanced a-b The viability and proliferative capacity of PD-L1 knockdown HCC cells were measured by MTS and EdU assays after co-cultured with NK92-MI. Scale bars represent 400 μ m. c The protein expression level of cleaved-PARP and

PD-L1 was detected by Western blot assay. d PD-L1 protein expression was detected in HCC cells after treatment with BBR, NK92-M1 cells, and their combination. *P<0.05.

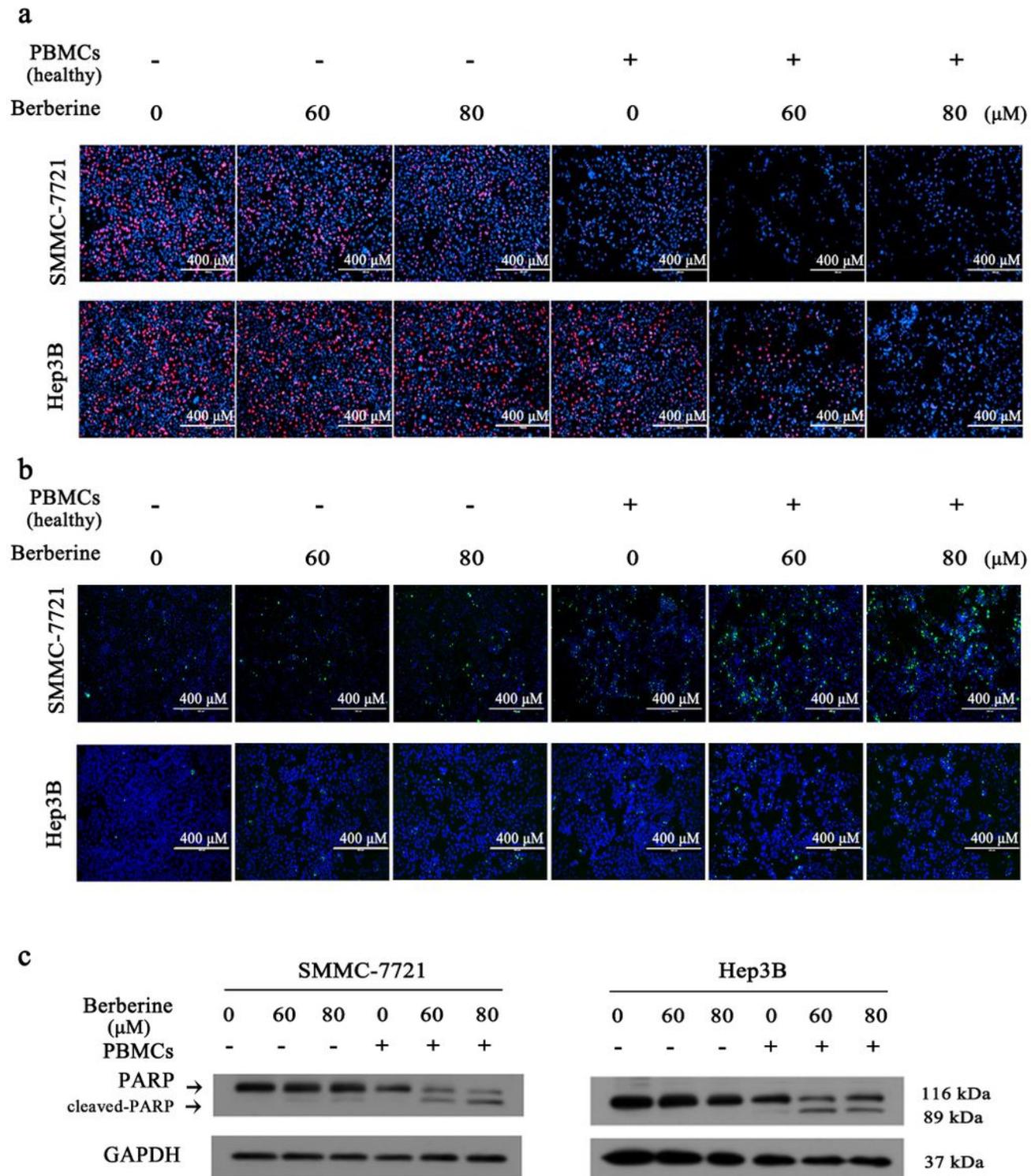


Figure 3

Effects of BBR and PBMCs alone or in combination on proliferation and apoptosis of HCC cells a The proliferative level of HCC cells was detected by EdU assay after treated with BBR, PBMCs, and their combination for 36h. Scale bars represent 400μm. b TUNel assay was used to measure the apoptotic level

of HCC cells. Scale bars represent 400 μ m. c The protein expression level of cleaved-PARP was examined by Western blot assay.

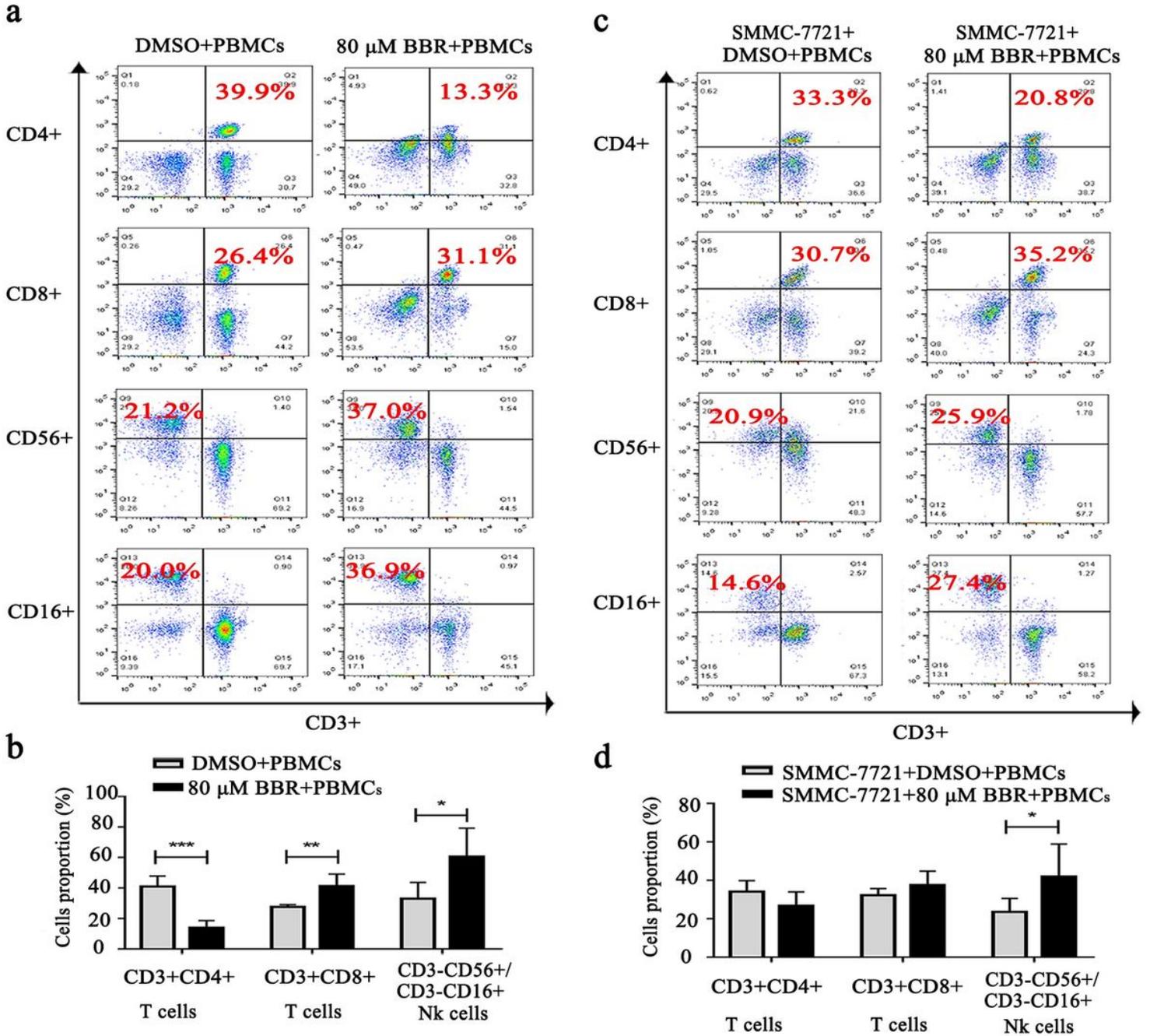


Figure 4

The frequencies of peripheral blood NK cells and T cells were analyzed by flow cytometry after treated with BBR or co-cultivation of SMMC-7721 and BBR a Representative FACS plots of CD3+CD4+ T cells, CD3+CD8+ T cells, CD3-CD56+ NK cells and CD3-CD16+ NK cell populations in PBMCs after BBR treatment. b Histograms of the percentage of peripheral blood T cells and NK cells. *P<0.05, **P<0.01. c Representative FACS plots of CD3+CD4+ T cells, CD3+CD8+ T cells, CD3-CD56+ NK cells and CD3-CD16+ NK cell populations in PBMCs co-cultured with SMMC-7721 alone or BBR and SMMC-7721 cells. d Histograms of the percentage of peripheral blood T cells and NK cells. *P<0.05, **P<0.01.

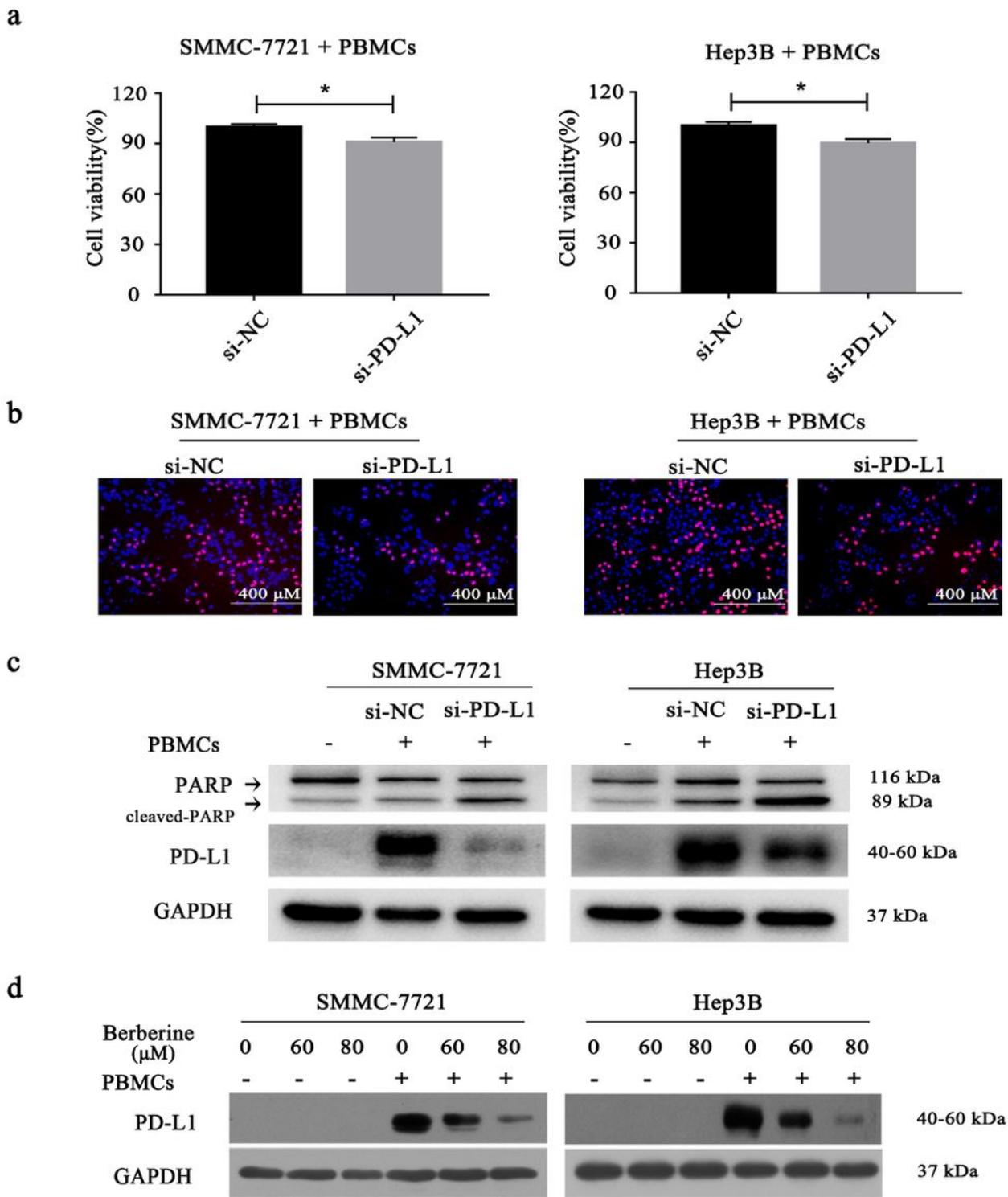


Figure 5

PD-L1 knockdown enhanced the anti-HCC effect of PBMCs a-b The viability and proliferative capacity of PD-L1 knockdown HCC cells after co-cultured with PBMCs, as determined by MTS and EdU assays. Scale bars represent 400μm. c The protein expression level of cleaved-PARP and PD-L1 was detected by Western blot assay. d PD-L1 protein expression was detected in HCC cells after treatment with BBR, PBMCs cells, and their combination. *P<0.05.

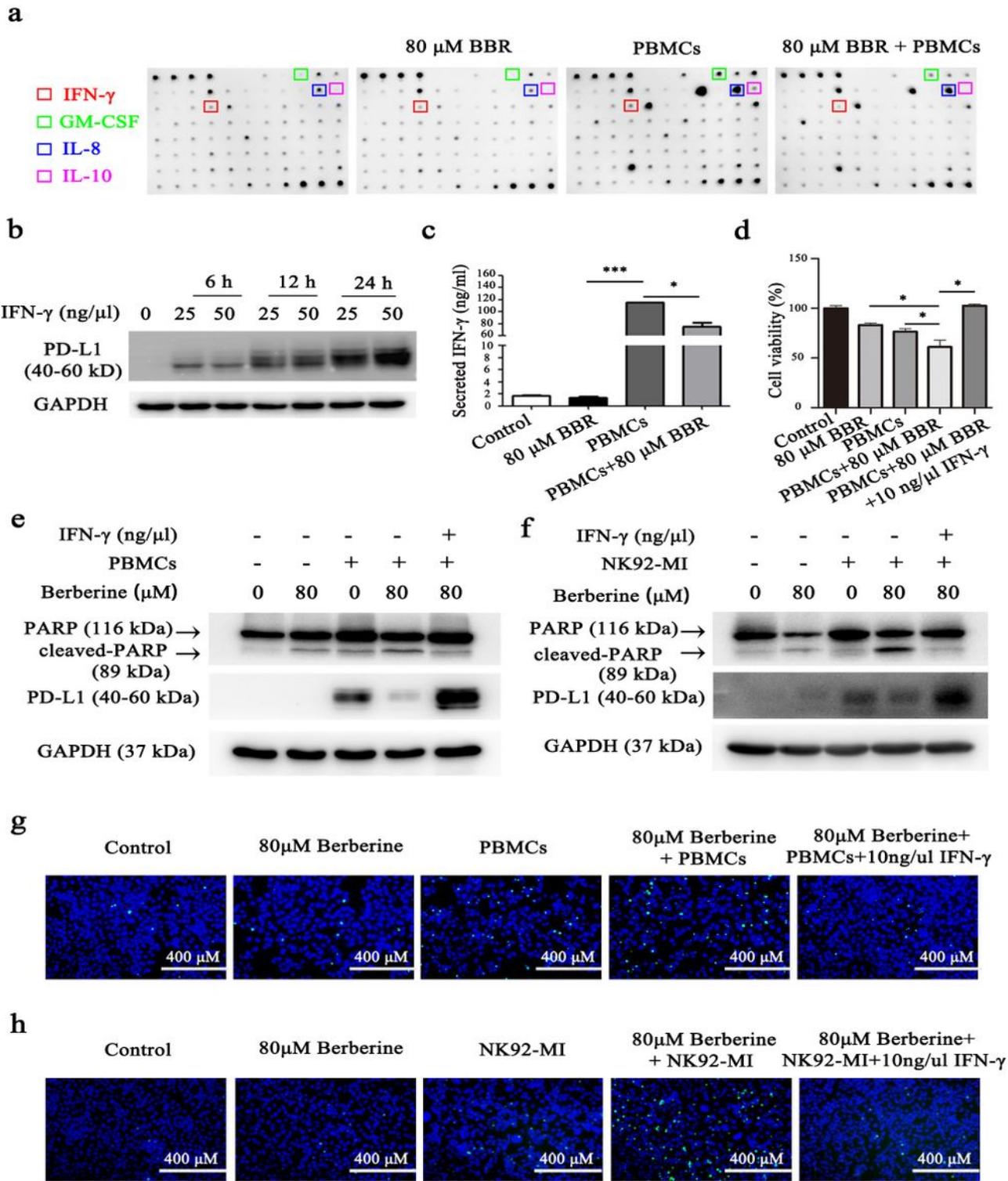


Figure 6

IFN- γ mediated the anti-HCC effects of PBMCs / NK92-MI induced by BBR a Cytokine arrays of the conditioned medium of HCC cells by treatment with BBR, PBMCs and combination thereof for 36h. b The protein expression of PD-L1 in HCC cells was detected by Western blot assay after IFN- γ treatment. c The secretion level of IFN- γ in PBMCs was detected by ELISA assay. d A rescue test of cell viability was performed by adding 10ng/ul IFN- γ to the combined treatment of 80 μ M BBR and PBMCs. e-f The protein

expression of cleaved-PARP and PD-L1 in SMMC-7721 cells were detected by Western blot assay after adding 10ng/ul IFN- γ to the combined treatment of 80 μ M BBR and PBMCs/NK92-MI. g-h Tunel assay was used to measure the apoptosis level of SMMC-7721 cells. Scale bars represent 400 μ m. *P<0.05, ***P<0.001.

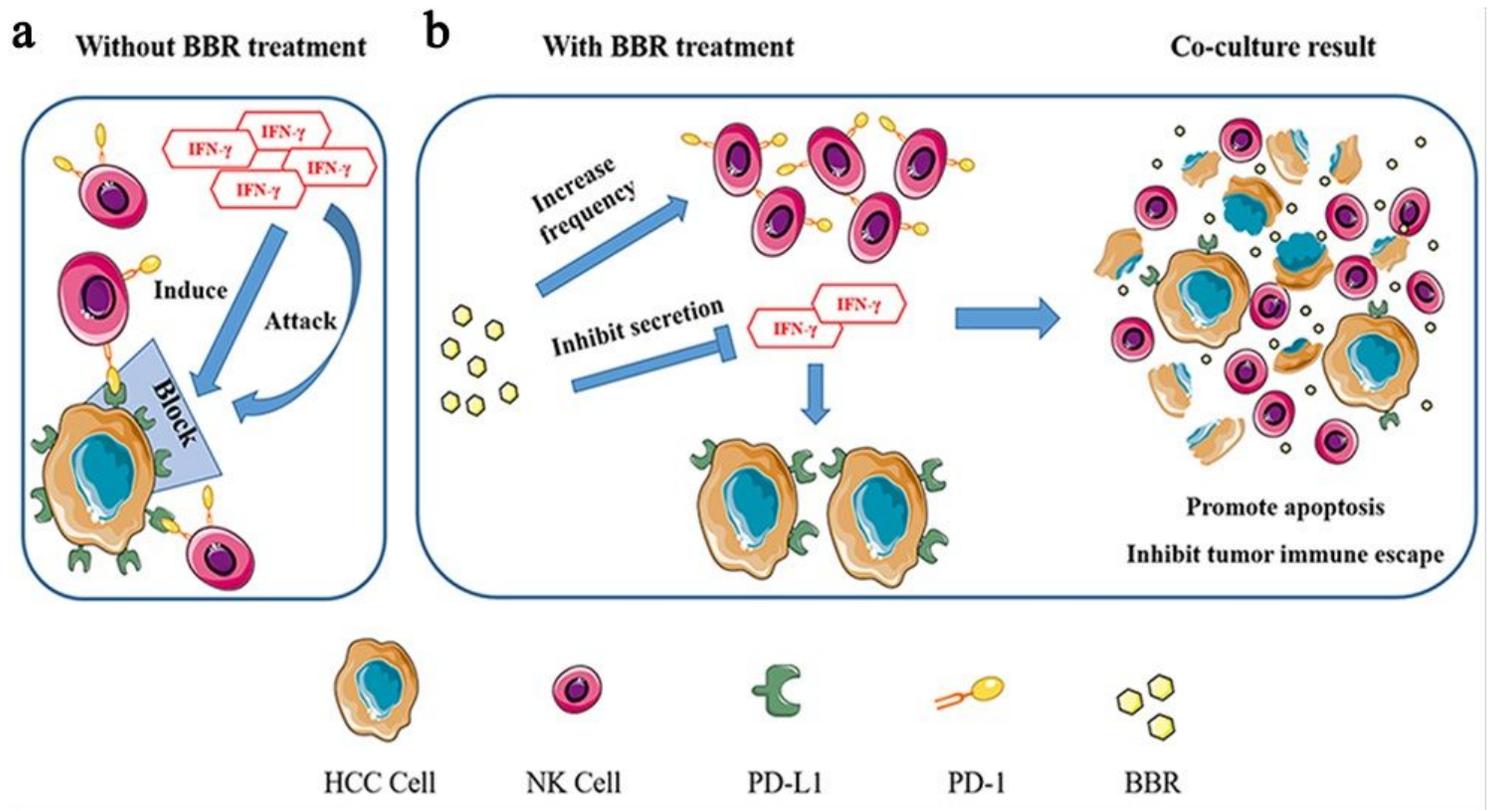


Figure 7

A schematic diagram of the immunomodulatory effects of BBR in HCC a IFN- γ acts as an anti-tumor cytokine without BBR treatment. IFN- γ secreted by immune cells in PBMCs leads to the induction of PD-L1 molecule on the surface of HCC cells, which can escape the attack of immune cells. b When BBR is co-culture with PBMCs / NK92-MI and HCC cells, BBR exerts its anti-HCC effect via increasing the frequency of NK cells and inhibiting the secretion of IFN- γ , thereby inhibiting PD-L1 expression. Therefore, BBR can promote apoptosis of HCC cells and inhibit tumor immune escape.

Supplementary Files

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