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MicroRNA-127-5p targets the biliverdin reductase B/nuclear factor-κB pathway to suppress cell growth in hepatocellular carcinoma cells

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Key words

BLVRB, growth, hepatocellular carcinoma, miR-127-5p, NF-kB activation

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Nuclear factor-κB (NF-κB) activation is one of the major mediators of inflammation-induced cancer cell growth and progression. In previous studies, we screened a series of microRNAs (miRNAs) that targeted the NF-κB signaling pathway. In this study, we showed that miR-127-5p suppressed NF-κB activity through inhibition of p65 nuclear translocation. In addition, miR-127-5p also inhibited the transcription of downstream targets of the NF-κB signaling pathway. While exploring the mechanism of the inhibition of NF-κB activity by miR-127-5p, we found that miR-127-5p decreased the phosphorylation of p65. MicroRNA-127-5p inhibited the growth and colony formation of hepatocellular carcinoma (HCC) cells and decreased biliverdin reductase B (BLVRB) expression by directly binding to its 3′-UTR. RNA interference of BLVRB suppressed HCC cell growth, whereas the overexpression of BLVRB promoted HCC cell growth. Furthermore, BLVRB blockade inhibited the phosphorylation of p65 protein and the expression of downstream targets of the NF-κB signaling pathway, mimicking the function of miR-127-5p. The restoration of BLVRB in HCC cells overexpressing miR-127-5p impaired the suppression of HCC growth by miR-127-5p. Moreover, miR-127-5p was downregulated in 58% of HCC samples. In summary, we found that miR-127-5p suppressed NF-κB activity by directly targeting BLVRB in HCC cells, and this finding improves our understanding of the molecular mechanism of inflammation-induced HCC growth and proliferation and the successful inhibition of NF-κB activity by cancer treatment.

It has been well established that chronic inflammation promotes the initiation and progression of cancers. The first evidence linking inflammation to cancer was presented in 1863 by Virchow, who observed leukocytes in tumor tissues.[1] Later, that observation was confirmed in several clinical cases.[2] Chronic inflammation occurs when inflammatory signaling cannot be stopped, resulting in recruitment of inflammatory cells, increased production of reactive oxygen species and reduction of DNA repair.[3] Moreover, chronic inflammation is regarded as an important cause of tumorigenesis in humans, accounting for ~20% of human cancers worldwide.[3] The development of hepatocellular carcinoma (HCC) is one of the most extensively illustrated inflammation-induced carcinogenic processes because more than 90% of HCCs are attributed to chronic liver damage and inflammation.[4] Hepatocellular carcinoma represents the fifth most common cancer in men and the seventh most common cancer in women and is the third leading cause of cancer-related death worldwide.[5]

In recent years, research has been dedicated to exploring the underlying mechanisms of inflammation-induced cancers. Two major regulating signaling pathways have been shown to participate in the crosstalk between inflammation and cancers. One is the signal transducer and activator of transcription (STAT) pathway, in particular, the STAT pathway member STAT3, which can be activated by a series of inflammation factors.[6] Another is the nuclear factor-kappa B (NF-κB) signaling pathway, which is recognized as a critical player in incipient neoplasia and progression of inflammation-induced cancers.[7] Under normal circumstances, NF-κB is a heterodimer of p50 and p65, and it binds to inhibitory molecules of the I-κB family. Nuclear factor-κB is activated by a number of signals from the cell surface. The I-κB kinase (IKK) complex, composed of the IKKa and IKKb catalytic subunits and the regulatory subunit, is activated, result-

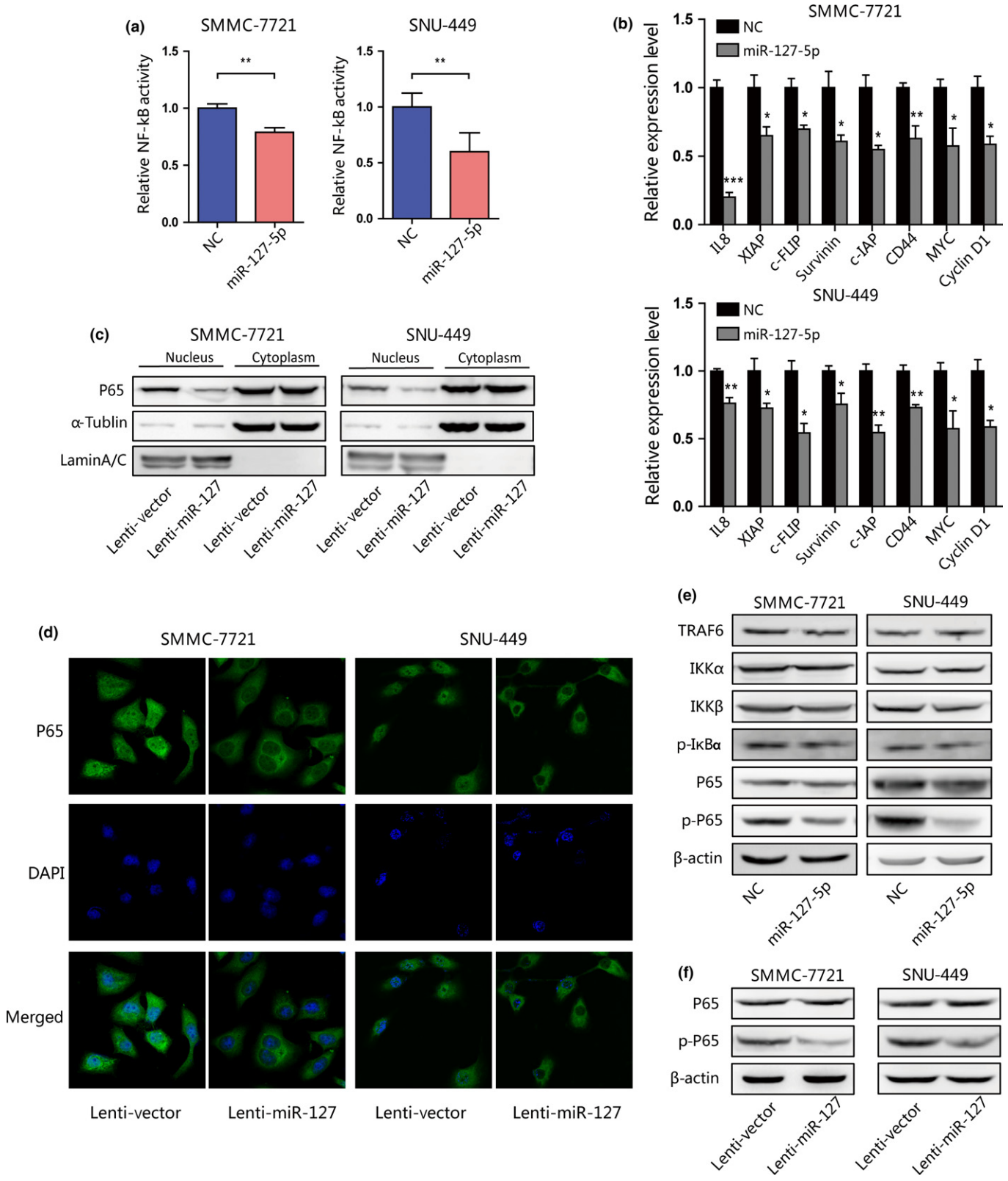


Fig. 1. MicroRNA (miR)-127-5p inhibits nuclear factor (NF)-κB transcriptional activity in hepatocellular carcinoma cells. (a) SMMC-7721 and SNU-449 cells were cotransfected with NF-κB luciferase reporter and miRNA mimic. A luciferase assay was carried out after 48 h. (b) The mRNA levels of NF-κB downstream effectors were determined by quantitative real-time PCR analysis after transfection with miR-127-5p mimic or negative control (NC) in SMMC-7721 and SNU-449 cells. β-actin served as an internal control. (c) Separation and preparation of cytoplasmic and nuclear extracts were carried out in SMMC-7721 and SNU-449 stable cells. The protein level of p65 was determined by Western blot analysis. (d) Confocal microscopic images show that ectopic expression of miR-127 inhibited the nuclear translocation of p65, which suggests that NF-κB signaling is suppressed. (e) The protein levels of NF-κB signaling were analyzed by Western blot after transfection with miR-127-5p. (f) The protein levels of NF-κB signaling were determined by Western blot in lenti-miR-127 stable cell lines. $^{**}P < 0.01$.

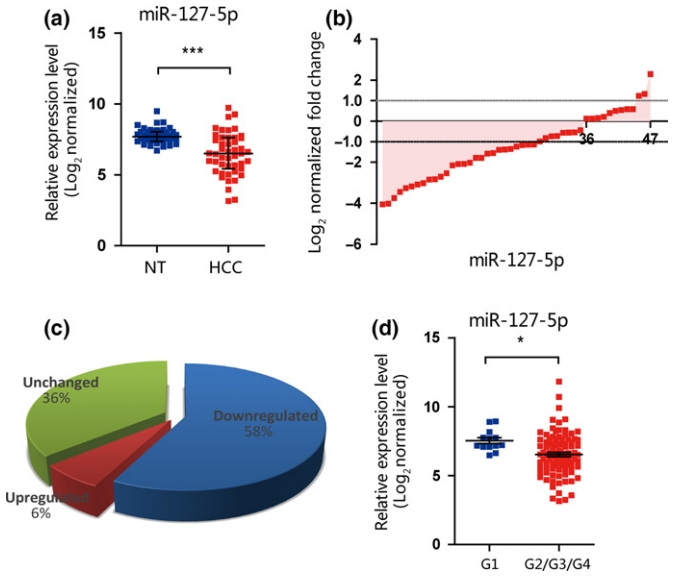


Fig. 2. MicroRNA (miR)-127-5p is frequently downregulated in hepatocellular carcinoma (HCC). (a) The expression level of miR-127-5p was determined using data from The Cancer Genome Atlas (http://cancergenome.nih.gov/). The expression level of miR-127-5p in 47 pairs of HCC tissues and matching adjacent normal tissues. Data are presented as $\log_2$ fold change. (b) Significant downregulation of miR-127-5p expression in HCC samples compared to paired adjacent normal tissue samples was observed. (c) Pie chart of the proportions of HCC samples in which miR-127-5p expression was upregulated (red), downregulated (blue), or unchanged (green). (d) HCC samples with poor histological grade have lower miR-127-5p expression than samples with higher histological grade. $^*P < 0.05$, $^{***}P < 0.001$.

ing in I-κB phosphorylation and degradation. Once released from I-κB family members, the phosphorylation of RelA results in NF-κB translocation into the nucleus and binds to the κB motif of its target genes.[8] Nuclear factor-κB signaling can be regulated by many positive and negative regulatory factors. Recently, regulation by non-coding RNAs, especially microRNAs (miRNAs), has been discovered as a new mechanism of NF-κB signaling regulation.

MicroRNAs constitute a class of short endogenous non-coding RNAs. Increasing evidence has shown that miRNAs play a conserved role in various biological processes by regulating the expression of sets of genes.[9] In previous work, we used high-throughput luciferase reporter assays to screen for miRNAs that regulate the NF-κB signaling pathway. For example, miR-195 targets the NF-κB signaling pathway by directly downregulating I-κBα and TAB 3.[10] In addition to miRNAs that direct target molecules in NF-κB signaling pathway, we also identified miRNAs that regulate NF-κB activities without targeting molecules that directly participate in NF-κB signaling pathway. Among these miRNAs, miR-127-5p significantly decreased NF-κB activities and inhibited cell growth by targeting biliverdin reductase B (BLVRB) in HCC.

Materials and Methods

Cell culture. HEK-293T, SMMC-7721, and SNU-449 cells were cultured in DMEM supplemented with 10% FBS and antibiotics at 37°C in an atmosphere of 5% CO_2.

Oligonucleotide transfection. MicroRNA-127-5p mimic and BLVRB siRNA were synthesized by GenePharma (Shanghai, China). The sequences are shown in Table S1. In each well of

Table 1. Correlation of the clinicopathological features with tumor miR-127-5p expression in HCC

Clinical variables	*P*	High miR-127-5p		Low miR-127-5p		Total no.	Unknown no.
		Case no.	%	Case no.	%		
Age							
>50	0.47455	42	76.36	46	83.64	110	1
≤50		13	23.64	9	16.36		
Gender							
Male	0.84441	33	60.00	35	63.64	110	1
Female		22	40.00	20	36.36		
Risk factor							
Hepatitis	0.18524	17	36.96	11	22.45	95	16
Other		29	63.04	38	77.55		
AFP							
Positive	0.11211	23	74.19	20	52.63	69	42
Negative		8	25.81	18	47.37		
Inflamation extent							
Mild/Severe	0.89303	13	46.43	18	41.86	71	40
None		15	53.57	25	58.14		
Liver fibrosis score							
≥1	0.67781	11	34.38	16	42.11	70	41
0		21	65.62	22	57.89		
Histologic grade							
G2/G3/G4	0.00395	43	79.63	53	98.15	108	3
G1		11	20.37	1	1.85		
Pathologic stage							
II/III/IV	0.56863	33	64.71	28	57.14	100	11
I		18	35.29	21	42.86		
Vascular invasion							
Micro/Macro	0.04113	10	21.28	21	42.86	96	15
None		37	78.72	28	57.14		

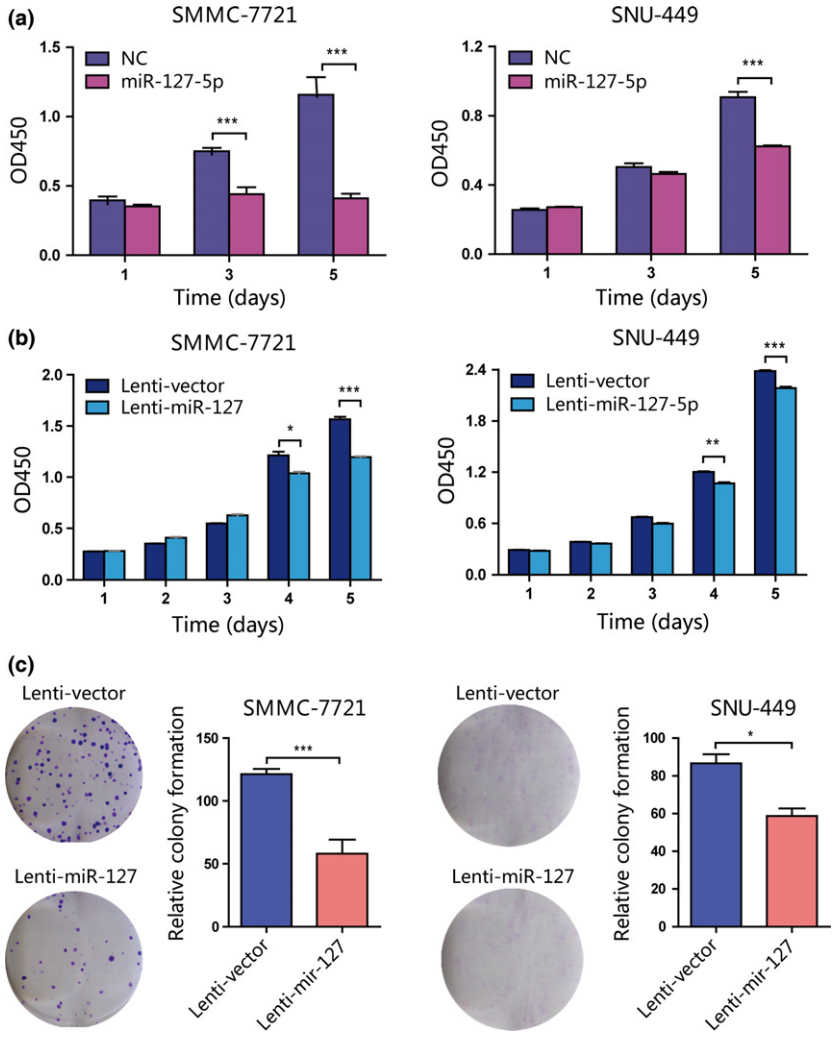


Fig. 3. MicroRNA (miR)-127-5p suppresses the proliferation of hepatocellular carcinoma cells. (a) Proliferation of SMMC-7721 cells and SNU-449 cells transfected with miR-217-5p mimic or negative control (NC) was measured by CCK8 assay. (b) Proliferation of SMMC-7721 and SNU-449 stable cells overexpressing miR-127 were measured by CCK8 assay. (c) Colony formation assay for SMMC-7721 and SNU-449 cells infected with lentivirus expressing miR-127 or mock control. A total of 1000 cells per well were seeded and cultivated for 2 weeks. The colonies were fixed and stained in a dye solution containing 0.1% crystal violet and 20% methanol. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

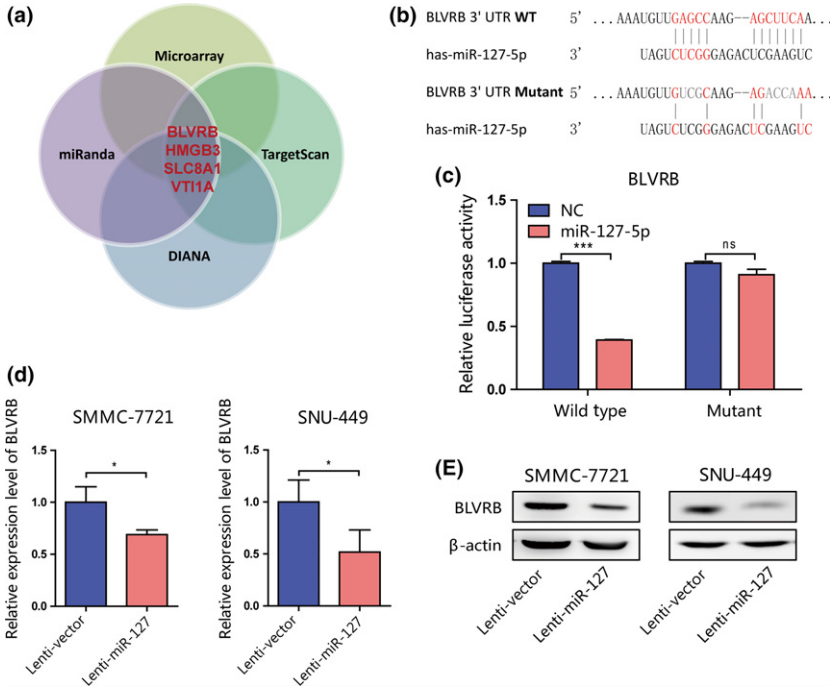


Fig. 4. Biliverdin reductase B (BLVRB) is a direct target of microRNA (miR)-127-5p. (a) Candidate genes identified by prediction algorithms based on gene expression profiles. (b) Putative miR-127-5p binding sites in the BLVRB 3′-UTR. Mutant binding sequences are highlighted in gray. (c) Luciferase activity assays of luciferase reporters with wild-type or mutant BLVRB 3′-UTRs were carried out after cotransfection with miR-127-5p mimic or negative control (NC) in HEK-293T cells. The mean ± SEM of a representative experiment in triplicate is shown. (d) mRNA levels of BLVRB in SMMC-7721 and SNU-449 stable cells were determined by quantitative real-time PCR analysis. (e) Protein levels of BLVRB in SMMC-7721 and SNU-449 stable cells were determined by Western blot analysis. $*P < 0.05$, $***P < 0.001$. ns, not significant.

a six-well plate, the cells were transfected with 5 μL miRNA mimic or siRNA (20 μM) using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). The cells were harvested 48 h after transfection.

Luciferase assay. The 3′UTR of BLVRB was amplified from the genomic DNA of HEK-293T and subcloned directly downstream of the stop codon of luciferase (p-LUC-UTR). HEK-293T cells were seeded into 96-well plates at a density of 5000 cells per well. After 24 h, the cells were transfected with 5 ng pRL-CMV renilla luciferase reporter, 50 ng of either pLUC or p-LUC-UTR, and 5 pmol miR-127-5p mimic. After 48 h, firefly and renilla luciferase activities were measured using a dual-luciferase reporter system (Promega, Madison, WI, USA). To measure NF-κB activity, SMMC-7721 and SNU-449 cells were seeded in 96-well plates and transfected with 5 pmol miRNA mimic or BLVRB siRNA, 50 ng pNF-κB-Luc, and 5 ng pRL-CMV renilla luciferase reporter.

RNA isolation and quantitative real-time PCR. Total RNA was extracted using TRIzol reagent (Invitrogen). Reverse-transcribed cDNA was synthesized using the PrimeScript RT Reagent Kit (TaKaRa, Dalian, China). Real-time PCR was carried out using SYBR Premix Ex Taq (TaKaRa). Reverse-transcribed cDNA for miRNA was synthesized using the MicroRNA Reverse Transcription Kit (Life Technologies, Carlsbad, CA, USA). Expression levels of NF-κB downstream effectors were measured using the Universal Probe Library (Roche, Shanghai, China). The primers used are presented in Table S2. Real-time PCR for miRNA was carried out by TaqMan MiRNA Assays (Applied Biosystems, Foster City, CA, USA).

Lentivirus production and infection. The pri-miR-127 fragment and ORF fragment of BLVRB were cloned into the pWPXL lentiviral vector. The primers used are presented in Table S3. Lentiviruses were generated by cotransfecting the lentiviral vector, the packaging plasmid PAX2, and the VSV-G envelope plasmid pMD2.G (pWPXL and pMD2.G were gifts from Dr. Didier Trono, Ecole Polytechnique Fédérale de Lausanne, 1015 Lausanne, Switzerland) into HEK-293T cells using Lipofectamine 2000 (Invitrogen). Supernatants were collected 48 h after transfection, filtered through a 0.45-mm membrane, and used to infect HCC cells.

Cell proliferation and colony formation assays. The cell proliferation assay was carried out using the Cell Counting Kit-8 (CCK-8) (Dojindo, Kumamoto, Japan). Ten microliters of CCK-8 solution was added to each well once a day. The absorbance at 450 nm was measured after 2 h of incubation at 37°C. For colony formation assays, 1000 cells were seeded into each well of a six-well plate. On the 14th day after seeding, the cells were stained with 0.1% crystal violet and 20% methanol. Images were taken and the colonies were counted.

Western blot analysis. Proteins were analyzed by SDS-PAGE and transferred to nitrocellulose membranes (Bio-Rad, Hercules, CA, USA). After blocking with 5% non-fat milk, the membranes were probed with primary antibodies against P65 or p-P65 (both from Cell Signaling Technology, Danvers, MA, USA) or BLVRB (Abgent, San Diego, CA, USA) overnight at 4°C. Immune complexes were detected using the SuperSignal West Femto Maximum Sensitivity Substrate (Thermo Fisher Scientific, Waltham, MA, USA).

Immunofluorescence. Cells on glass slides were incubated in 4% paraformaldehyde for 20 min at room temperature. After washing with PBS three times, the samples were solubilized with 0.1% Triton X-100 and blocked with Immunol Staining Fix Solution (Beyotime, Shanghai, China) for 30 min. The cells were then probed with anti-P65 antibody diluted in Immunol Staining Primary Antibody Dilution Buffer (Beyotime) for 1 h, followed by incubation with secondary antibody probed with Alexa 488 for 1 h; DAPI was used to stain the nuclei of the cells.

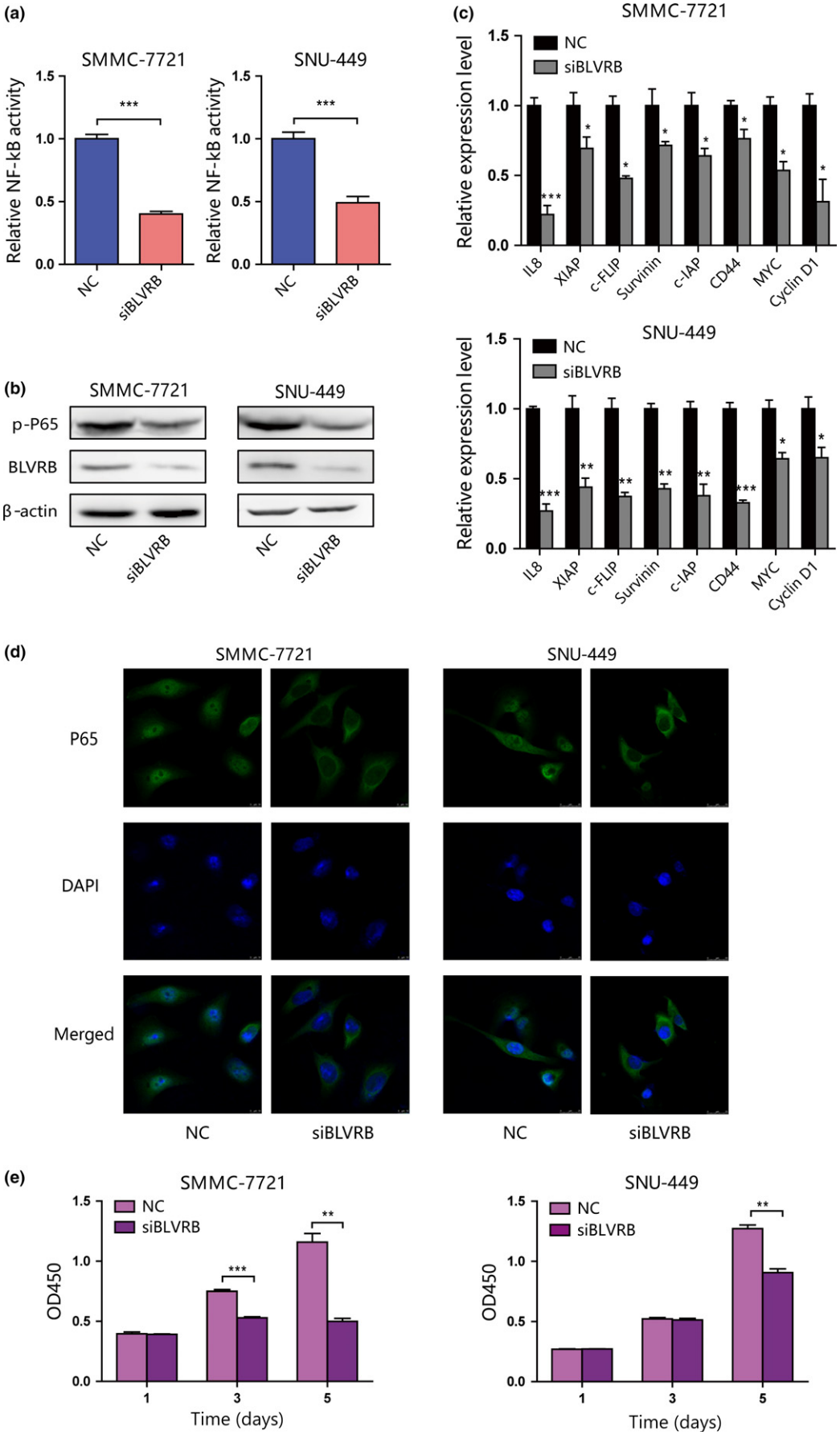
Statistical analysis. The data are presented as means and SD of at least three independent experiments. Paired and unpaired Student's *t*-tests were used to compare the collected results. A *P*-value of <0.05 was considered statistically significant ($*P < 0.05$, $**P < 0.01$, and $***P < 0.001$).

Results

MicroRNA-127-5p inhibits NF-κB activation in HCC cells. We first determined the effect of miR-127-5p on NF-κB activation. A luciferase reporter harboring four inducible NF-κB binding sites upstream of the luciferase gene was co-transfected with miR-127-5p mimic or negative control (NC) into two HCC cell lines, SMMC-7721 and SNU-449. The results showed that luciferase activity was notably decreased by miR-127-5p in both cell lines (Fig. 1a), which indicates that miR-127-5p influenced NF-κB activation. A set of NF-κB downstream effectors were detected after miR-127-5p overexpression, and the results showed miR-127-5p also decreased the expression of these targets (Fig. 1b). Furthermore, a negative correlation between miR-127-5p expression and NF-kB activity was noted in six HCC cell lines (Fig. S1). While exploring the mechanism through which miR-127-5p regulated NF-κB activation, we found that ectopic expression of miR-127-5p decreased the nuclear fraction of the RelA/p65 subunit of NF-κB in SMMC-7721 and SNU-449 cells (Fig. 1c). Moreover, immunofluorescence assays also indicated that the nuclear location of RelA/p65 was reduced after miR-127-5p overexpression (Figs. 1d, S2). We next examined the influence of miR-127-5p on the major components of the NF-κB signaling pathway and found that ectopic expression of miR-127-5p in HCC cells reduced the phosphorylated form of RelA/p65 (Fig. 1e,f). Taken together, overexpression of miR-127-5p inhibited NF-κB activation by reducing phosphorylation and nuclear translocation of RelA/p65 proteins in HCC cells.

MicroRNA-127-5p frequently downregulated and suppresses cell proliferation in HCC. As miR-127-5p inhibited the activation of NF-κB in HCC cells, we wondered whether miR-127-5p could act as a tumor suppressor in HCC. Therefore, we investigated the expression of miR-127-5p in HCC samples reported in the Cancer Genome Atlas (http://cancergenome.nih.gov/) database. Compared to normal liver, miR-127-5p expression is lower

Fig. 5. Biliverdin reductase B (BLVRB) blockade inhibited nuclear factor (NF)-κB signaling pathway and the proliferation of hepatocellular carcinoma cells. (a) SMMC-7721 and SNU-449 cells were cotransfected with BLVRB siRNA and NF-κB luciferase reporter. A luciferase assay was carried out after 48 h. (b) The protein levels of phosphorylated p65 were analyzed by Western blot after transfection with BLVRB siRNA. (c) mRNA levels of NF-κB downstream effectors were determined in SMMC-7721 and SNU-449 cells by quantitative real-time PCR analysis after transfection with BLVRB siRNA or negative control (NC). β-actin served as an internal control. (d) Confocal microscopic images show that knockdown of BLVRB inhibited the nuclear translocation of p65. (e) Proliferation of SMMC-7721 cells and SNU-449 cells transfected with BLVRB siRNA or NC were measured by CCK8 assay. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.



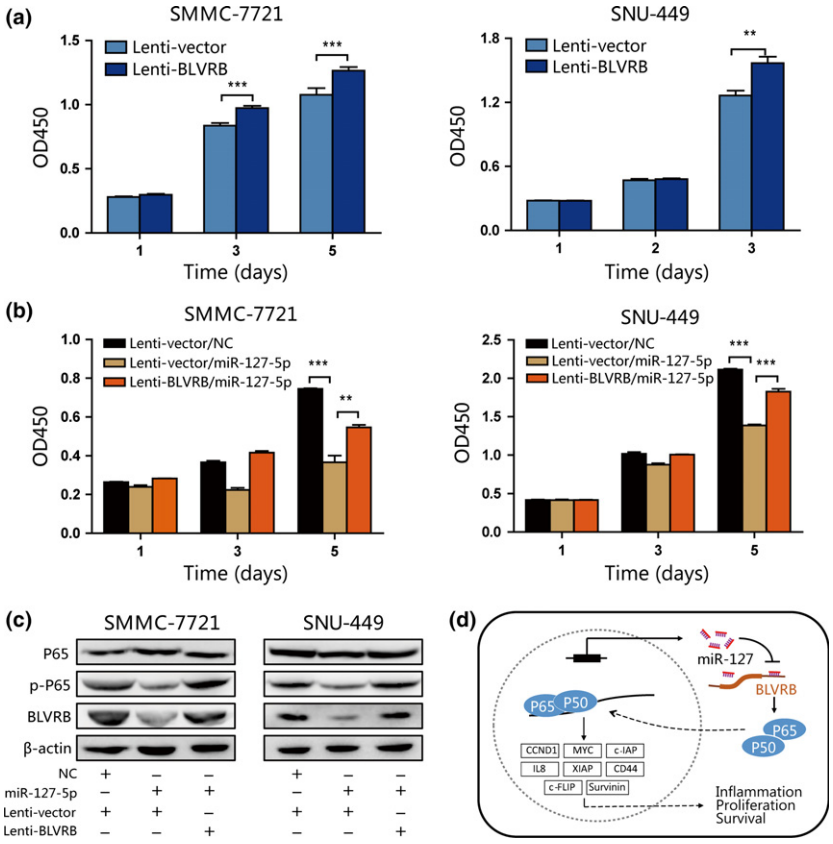


Fig. 6. MicroRNA (miR)-127-5p inhibits the nuclear factor (NF)-κB signaling pathway and the proliferation of hepatocellular carcinoma cells by inhibiting biliverdin reductase B (BLVRB). (a) Proliferation of SMMC-7721 and SNU-449 stable cells overexpressing BLVRB was measured by CCK8 assay. (b, c) CCK8 assay and Western blot analysis of SMMC-7721 and SNU-449 HCC cells transfected with miR-127-5p mimic following transduction with BLVRB lentivirus. (d) Proposed model of the tumor suppressor role of miR-127-5p in hepatocellular carcinoma.

in HCC tissues (Fig. 2a,b, Table 1). Downregulation of miR-127-5p (greater than twofold change) was observed in 58% (27/47) of HCC tissues compared to matched adjacent normal tissues (Fig. 2c). In addition, HCC tissues with poor histologic grade showed significantly lower expression of miR-127-5p than tissues with higher histologic grade (Fig. 2d, Table S1). These results, together with the known function of miR-127-5p, indicate that miR-127-5p is a potential tumor suppressor in HCC. Furthermore, we found that miR-127-5p mimic suppressed the growth of SMMC-7721 and SNU-449 cells (Fig. 3a). Moreover, stable expression of miR-127-5p through a lentiviral system also decreased the growth of both cell types (Fig. 3b). We also examined the effects of miR-127-5p on the colony formation ability of HCC cells and found that stable expression of miR-127-5p significantly reduced the colony formation ability of SMMC-7721 and SNU-449 cells (Fig. 3c).

Biliverdin reductase B is a direct downstream target of miR-127-5p in HCC cells. To determine the mechanism of the inhibition of proliferation and NF-κB signaling in HCC by miR-127-5p, we attempted to identify the target genes of miR-127-5p. We carried out a microarray analysis to determine differentially expressed genes after miR-127-5p transfection and combined the results with the targets predicted by three bioinformatics tools (TargetScan, miRanda, and DIANA) (Fig. 4a). Four candidate genes were identified, and BLVRB was confirmed by qPCR to be the most significantly downregulated by miR-127-5p (Fig. S3). Therefore, we selected BLVRB for further exploration. The 3′-UTR of BLVRB contains one binding site for miR-127-5p. We constructed luciferase reporter plasmids containing the 3′-UTR of BLVRB with a wild or mutant miR-127-5p binding site and cotransfected those plasmids with NC or miR-127-5p into HEK-293T cells (Fig. 4b). The luciferase assays showed that miR-127-5p significantly reduced luciferase activity in the wild-type group, but not in the mutant group (Fig. 4c). In addition, ectopic expression of miR-127 suppressed the expression of BLVRB at both the mRNA and protein levels (Fig. 4d,e). A negative correlation between miR-127-5p and BLVRB was noted among 346 patients from the Cancer Genome Atlas database (Fig. S4). These findings indicate that BLVRB is a direct target of miR-127-5p in HCC cells.

MicroRNA-127-5p inhibits NF-κB signaling pathway and HCC cell proliferation by targeting BLVRB. The biological function of BLVRB in HCC remains unclear. As a target of miR-127-5p in HCC, we first determined whether BLVRB influences NF-κB activation. Biliverdin reductase B siRNAs were cotransfected with a luciferase reporter plasmid containing NF-κB binding sites into SMMC-7721 and SNU-449 cells. The luciferase reporter assays indicated that BLVRB siRNAs decreased NF-κB activity (Fig. 5a,b). In addition, knockdown of BLVRB also inhibited the expression of NF-κB downstream targets (Fig. 5c), the nuclear translocation of p65 (Fig. 5d), and significantly suppressed the proliferation of HCC cells (Fig. 5e). Then, we obtained a lentiviral vector harboring the ORF of BLVRB and used it to establish SMMC-7721 and SNU-449 cell lines that stably expressed the ORF of BLVRB (Fig. S5). The CCK-8 assays indicated that ectopic expression of BLVRB facilitated the proliferation of HCC cells (Fig. 6a).

To determine whether BLVRB is a functional target of miR-127-5p, the expression of BLVRB was restored in cells overexpressing miR-127-5p. The CCK-8 assays showed that miR-127-5p significantly inhibited the proliferation of HCC cells and suppressed the phosphorylation of p65, and restoration of BLVRB expression abrogated the inhibition of

NF-κB activation and proliferation of HCC by miR-127-5p (Fig. 6b,c).

Discussion

This research has been dedicated to determining the mechanisms underlying NF-κB signaling dysregulation in the development of cancer. Nuclear factor-κB acts as the central coordinator of inflammatory signaling and has a critical role in cancer initiation and progression. Activation of NF-κB enhances the anti-apoptotic ability of cells.[11] Deficiency in the NF-κB component RelA in mouse embryos facilitates apoptosis in the liver.[12]

In the past decade, an increasing number of studies have shown that miRNAs have important roles in the initiation and progression of cancers. Our group and others have found that miRNAs also regulate the NF-κB signaling pathway by directly or indirectly targeting genes that participate in this signaling pathway.[10,13,14] In this study, we first observed that miR-127-5p, which is frequently downregulated in HCC, regulates NF-κB signaling activity in HCC cells. Previous studies have reported that miR-127 is downregulated in various cancers, including glioblastoma,[15] gastric cancer,[16] and HCC,[17] which indicated that downregulation of miR-127-5p is also a common event in cancers. In addition, expression of miR-127-5p is negatively associated with the histological grade of HCC. Li Wang's group reported that miR-433 and its twin miR-127 were regulated by DNA methylation in HCC cells.[18] Treatment with the DNA methylation inhibitor 5′-aza-2′ deoxycytidine resulted in a dose-dependent induction of miR-127-5p. Consistently, a reduction of BLVRB was noted in cells treated with DNA methylation inhibitor (Fig. S6). Further functional studies showed that miR-127-5p significantly suppresses HCC cell proliferation. Together, these results indicate that suppression of this regulatory miR-127-5p facilitated the proliferation of HCC cells.

In the current study, we found that BLVRB is a novel downstream target of miR-127-5p. Biliverdin reductase B has two activities in the cell: riboflavin reductase activity and biliverdin reductase activity.[19–21] It was previously identified as a component of antioxidant stress that shows a cell protective function.[22] Several studies have reported that BLVRB is upregulated at the protein level in esophageal squamous cell carcinoma,[23] acute lymphoblastic leukemia,[24] and HCC.[25] Here, we found that the knockdown of BLVRB inhibited the proliferation of HCC cells and inhibited NF-κB activity. Accordingly, the ectopic expression of BLVRB promoted the proliferation of HCC cells. It has been reported that an isoenzyme of BLVRB, biliverdin reductase A, enhanced the activation of NF-κB in MCF-7 cells. Biliverdin inhibited NF-κB activity in a concentration- and time-dependent manner.[26] In addition, analysis of the structure of BLVRB showed that it accommodates a wide range of tetrapyrrole substrates, including biliverdin Ix beta, meso-biliverdin Iv alpha, and biliverdin Ix alpha[27]. We infer that BLVRB may regulate NF-κB activity by the same mechanism. In summary, our findings show that miR-127-5p, which is frequently downregulated in HCC, indirectly affects the NF-κB signaling pathway by targeting BLVRB (Fig. 6d). Moreover, miR-127-5p was found to suppress the proliferation of HCC. This newly found miR-127-5p/BLVRB axis provides another link between miRNA, inflammation, and cancer.

Acknowledgments

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Disclosure Statement

The authors have no conflict of interest.

References

1 Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. *Nature* 2008; **454**: 436–44.
2 Bryant T. Remarks on some cases of inflammation of the breast simulating cancer. *Br Med J* 1868; **2**: 608–9.
3 Coussens LM, Werb Z. Inflammation and cancer. *Nature* 2002; **420**: 860–7.
4 Nakagawa H, Maeda S. Inflammation- and stress-related signaling pathways in hepatocarcinogenesis. *World J Gastroenterol* 2012; **18**: 4071–81.
5 El-Serag HB. Hepatocellular carcinoma. *N Engl J Med* 2011; **365**: 1118–27.
6 Yu H, Pardoll D, Jove R. STATs in cancer inflammation and immunity: a leading role for STAT3. *Nat Rev Cancer* 2009; **9**: 798–809.
7 Karin M. Nuclear factor-kappaB in cancer development and progression. *Nature* 2006; **441**: 431–6.
8 Lee JI, Burckart GJ. Nuclear factor kappa B: important transcription factor and therapeutic target. *J Clin Pharmacol* 1998; **38**: 981–93.
9 He L, Hannon GJ. MicroRNAs: small RNAs with a big role in gene regulation. *Nat Rev Genet* 2004; **5**: 522–31.
10 Ding J, Huang S, Wang Y *et al.* Genome-wide screening reveals that miR-195 targets the TNF-alpha/NF-kappaB pathway by down-regulating IkappaB kinase alpha and TAB 3 in hepatocellular carcinoma. *Hepatology* 2013; **58**: 654–66.
11 Liu Z-G, Hsu H, Goeddel DV, Karin M. Dissection of TNF receptor 1 effector functions: JNK activation is not linked to apoptosis while NF-κB activation prevents cell death. *Cell* 1996; **87**(3): 12.
12 Beg AA, Sha WC, Bronson RT, Ghosh S, Baltimore D. Embryonic lethality and liver degeneration in mice lacking the RelA component of NF-kappa B. *Nature* 1995; **376**(6536): 167–70.
13 Ma X, Becker Buscaglia LE, Barker JR, Li Y. MicroRNAs in NF-kappaB signaling. *J Mol Cell Biol* 2011; **3**(3): 159–66.
14 Bao C, Li Y, Huan L *et al.* NF-κB signaling relieves negative regulation by miR-194 in hepatocellular carcinoma by suppressing the transcription factor HNF-1α. *Sci Signal* 2015; **8**(387): ra75-ra.
15 Jiang H, Jin C, Liu J *et al.* Next generation sequencing analysis of miRNAs: MiR-127-3p inhibits glioblastoma proliferation and activates TGF-beta signaling by targeting SKI. *OMICS* 2014; **18**(3): 196–206.
16 Guo LH, Li H, Wang F, Yu J, He JS. The tumor suppressor roles of miR-433 and miR-127 in gastric cancer. *Int J Mol Sci* 2013; **14**: 14171–84.
17 Zhou J, Lu S, Yang S *et al.* MicroRNA-127 post-transcriptionally downregulates Sept7 and suppresses cell growth in hepatocellular carcinoma cells. *Cell Physiol Biochem* 2014; **33**: 1537–46.
18 Yang Z, Tsuchiya H, Zhang Y, Hartnett ME, Wang L. MicroRNA-433 inhibits liver cancer cell migration by repressing the protein expression and function of cAMP response element-binding protein. *J Biol Chem* 2013; **288**: 28893–9.
19 Xu F, Quandt KS, Hultquist DE. Characterization of NADPH-dependent methemoglobin reductase as a heme-binding protein present in erythrocytes and liver. *Proc Natl Acad Sci USA* 1992; **89**: 2130–4.
20 Maines MD, Polevoda BV, Huang TJ, McCoubrey WK Jr. Human biliverdin IXalpha reductase is a zinc-metalloprotein. Characterization of purified and *Escherichia coli* expressed enzymes. *Eur J Biochem* 1996; **235**(1–2): 372–81.
21 Shalloe F, Elliott G, Ennis O, Mantle TJ. Evidence that biliverdin-IX beta reductase and flavin reductase are identical. *Biochem J* 1996; **316**(Pt 2): 385–7.
22 Sedlak TW, Snyder SH. Bilirubin benefits: cellular protection by a biliverdin reductase antioxidant cycle. *Pediatrics* 2004; **113**: 1776–82.
23 Pallua JD, Schaefer G, Seifarth C *et al.* MALDI-MS tissue imaging identification of biliverdin reductase B overexpression in prostate cancer. *J Proteomics* 2013; **91**: 500–14.

24 Silveira VS, Scrideli CA, Moreno DA *et al.* Gene expression pattern contributing to prognostic factors in childhood acute lymphoblastic leukemia. *Leuk Lymphoma* 2013; **54**: 310–4.
25 Melle C, Ernst G, Scheibner O *et al.* Identification of specific protein markers in microdissected hepatocellular carcinoma. *J Proteome Res* 2007; **6**(1): 306–15.
26 Gibbs PE, Maines MD. Biliverdin inhibits activation of NF-kappaB: reversal of inhibition by human biliverdin reductase. *Int J Cancer* 2007; **121**: 2567–74.
27 Pereira PJ, Macedo-Ribeiro S, Parraga A *et al*. Structure of human biliverdin IXbeta reductase, an early fetal bilirubin IXbeta producing enzyme. *Nat Struct Biol.* 2001; **8**(3): 215–20.

Supporting Information

Additional supporting information may be found in the online version of this article:

Table S1. Sequences of oligonucleotide used in this study.

Table S2. Probes used in this study.

Table S3. Primer sequences used in this study.

Fig. S1. Negative correlation between microRNA-127-5p and nuclear factor-κB activity.

Fig. S2. Establishment of stable cell lines expressing microRNA-127.

Fig. S3. Screening of potential target of microRNA-127-5p in the cell.

Fig. S4. Correlation between microRNA-127-5p and BLVRB.

Fig. S5. Establishment of stable cell lines expressing BLVRB.

Fig. S6. MicroRNA-127-5p was silenced by DNA methylation.