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# Pro-inflammation NF- $\kappa$ B signaling triggers a positive feedback via enhancing cholesterol accumulation in liver cancer cells

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## Abstract

**Background:** Hepatocellular carcinoma (HCC) develops in a complex microenvironment characterized by chronic inflammation. In recent years, cholesterol metabolic abnormalities have been implicated the importance in cancer cell physiology. This study was designed to investigate the relationship between inflammation and cholesterol accumulation in HCC cells.

**Methods:** Human HCC cells HepG2 and Huh7 were cultured and stimulated with lipopolysaccharide (LPS) for 24 h. The changes of HCC cells related to cholesterol metabolism including intracellular cholesterol concentrations, cholesterol uptake, and the expression of cholesterol-related genes 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR), LDL receptor (LDLR), sterol regulatory element-binding transcription factor 2 (SREBF2), and proprotein convertase subtilisin/kexin 9 (PCSK9) were comparatively analyzed. Simultaneously, the effects of nuclear factor-kappa B (NF- $\kappa$ B) signaling pathway on cholesterol metabolism were clarified by knocking-down of nuclear factor kappa-B kinase subunit alpha (IKK $\alpha$ ) and TGF-beta-activated kinase 1 and MAP3K7-binding protein 3 (TAB3) via RNAi and microRNA (miR)-195. Subsequently, the roles of cholesterol accumulation in LPS induced pro-inflammatory effects were further investigated.

**Results:** Pro-inflammatory factor LPS significantly increased intracellular cholesterol accumulation by upregulating the expression of HMGCR, LDLR, and SREBF2, while downregulating the expression of PCSK9. These effects were revealed to depend on NF- $\kappa$ B signaling pathway by knocking-down and overexpression of IKK $\alpha$  and TAB3. Additionally, miR-195, a regulator directly targeting IKK $\alpha$  and TAB3, blocked the effects of cholesterol accumulation, further supporting the critical role of pro-inflammation NF- $\kappa$ B signaling in regulating cholesterol accumulation. Intriguingly, the accumulation of cholesterol conversely exerted an augmented pro-inflammation effects by further activating NF- $\kappa$ B signaling pathway.

**Conclusions:** These results indicated that pro-inflammation effects of NF- $\kappa$ B signaling could be augmented by a positive feedback via enhancing the cholesterol accumulation in liver cancer cells.

**Keywords:** Lipopolysaccharide, Nuclear factor-kappa B, Cholesterol accumulation, microRNA-195, Hepatocellular carcinoma

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## Background

Metabolic reprogramming in the uncontrolled proliferation of cancer cells has been thought to play important roles in cancer biology [1]. Recently, many studies have demonstrated that cholesterol accumulate in a series of human cancers, including breast [2], colon [3], prostate [4], HCC [5], and others [6]. In addition, enzymes involved in cholesterol metabolism have been reported abnormal expression in cancer tissues [7, 8]. For instance, cholesterol acyltransferase (ACAT)2, is found to be induced and promotes esterification of excess oxysterols for secretion to avoid cytotoxicity in a subset of hepatocellular carcinomas (HCCs) for tumor growth [9], suggestive of a specific cholesterol metabolic pathway in HCCs. On the one hand, cholesterol is needed for the synthesis of membranes, signaling molecules, lipid raft formation, and other factors to support the rapid growth of tumor cells [8]. On the other hand, cholesterol oxidized products, namely oxysterols, exhibit inhibitory activities in cell growth and promote cell apoptosis and dampen antitumor responses [10–12]. However, mechanism and pathological significance underlying the aberrant cholesterol metabolism are still elusive.

Epidemiological studies have shown that 80% of HCCs develop in fibrotic or cirrhotic livers as a consequence of chronic liver injury [13, 14]. The NF- $\kappa$ B pathway, the key link of inflammatory responses, plays an important role in HCC promotion by increasing proliferation and preventing apoptosis [15]. In the inactive state, NF- $\kappa$ B transcription factors are complexed in the cytoplasm, either with members of the inhibitor of  $\kappa$ B (I $\kappa$ B) family (in the canonical pathway) or with the NF- $\kappa$ B precursor p100 (in the non-canonical pathway). Lipopolysaccharide (LPS), a component of the gram-negative bacterial wall, could activate NF- $\kappa$ B pathway and stimulate inflammatory responses. Previous studies have demonstrated that LPS/NF- $\kappa$ B signaling pathway promotes atherosclerotic progression and macrophage foam cell formation by increasing intracellular cholesterol accumulation [16, 17]. These reports inspired us to hypothesize whether there is a crosstalk between NF- $\kappa$ B signaling and cholesterol metabolism in cancer cells.

In the present study, we investigated the relationship between inflammation and cholesterol metabolism, and found that pro-inflammatory factor Lipopolysaccharide (LPS) increased intracellular cholesterol accumulation through NF- $\kappa$ B pathway in HCC cells. Cholesterol accumulation conversely promoted LPS/NF- $\kappa$ B pathway and inflammatory responses.

## Methods

### Cell culture

Human HCC cells HepG2 and Huh7 were purchased from Cell Bank of the institute of Biochemistry and Cell

Biology, China Academy of Sciences, Shanghai, China. Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% (v/v) fetal bovine serum (Gibco, BRL, USA), 10ug/mL streptomycin sulfate and 100ug/mL penicillin G. All cells were incubated at 37 °C in a humidified atmosphere containing 5% CO<sub>2</sub>.

### LDL preparation

LDL was purchased from AngYu Biotechnologies (Shanghai, China). It is isolated from blood bank produced human plasma. It is purified via ultracentrifugation to homogeneity determined by agarose gel electrophoresis. Each lot is analyzed on agarose gel electrophoresis for migration versus LDL.

### Measurement of intracellular cholesterol

Total cellular cholesterol measurement was determined with a detection kit from Applygen (Peking, China) according to the manufacturer's protocol. Intracellular lipids were extracted using a chloroform-methanol (2:1) mix and dried under vacuum. Total cholesterol contents were measured by an enzymatic assay. The value was normalized against total protein concentration from each sample, as determined with Bicinchoninic Acid assay (Beyotime biotechnology, China).

### DIL-LDL uptake

The low density lipoprotein labeled with 1,1'-Dioctadecyl-3,3,3',3'-tetramethyl-indocarbocyanideperchlorate (DIL-LDL) (Biomedical Technologies Inc., MA, USA) uptake represented the ability of cell cholesterol uptake. HCC cells were treated with different addressment, then changed to serum-free medium and incubated with 10ug/ml DIL-LDL at 37 °C for 5 h. The cells were detached by trypsin, washed and suspended by PBS and analyzed by flow cytometry using FL2 emission filter (FACScan, BD Biosciences, San Jose, CA, USA). Data were analyzed with FlowJo software.

### Total RNA isolation and quantitative real-time quantitative PCR

Total RNA, containing miRNA, was isolated from HCC cells with TRIzol reagent (Invitrogen, CA, USA) according to the provided protocol. For mRNA analysis, complementary DNA was synthesized using reverse transcription reagent kit (TaKaRa, Japan) from RNA of 500 ng. Primers for PCR were shown in Table 1. Quantitative PCR amplification was performed by ABI7500 real-time fluorescent measurement system (Applied Biosystems, USA) using PCR amplification Kit (TaKaRa, Japan). Values are derived from at least 3 separate experiments performed in duplicate and normalized to  $\beta$ -actin expression. The  $2^{-\Delta\Delta Ct}$  method was used for the data analysis [18]. For miRNA

**Table 1** Primers for PCR used in this study

Gene Name	Forward primer (5' → 3')	Reverse primer (5' → 3')
PCSK9	AGACCCACCTCTCGAGTC	GGAGTCCTCCTCGATGTAGTC
LDLR	GCTACCCCTCGAGACAGATG	CACTGTCCGAAGCCTGTTCT
SREBF2	GGTTGTCCGGTGCATGGG	TTGCAGCATCTCGTCGATGT
HMGCR	GAGCGTGCCTAAGGTGAGG	ACAGAATCCTTGGATCCTCCAG
IKK $\alpha$	GGCTTCGGGAACGTCTGTC	TTTGGTACTTAGCTCTAGGCGA
TAB3	AGCAGCCACAGCTTGATATT	ACTAGGAGAATGGATACCCAGGT
MiR-195	GTCATTCCCTATTCTTCTGCC	ATTGGACCATCTCCCTCTGT
VCAM1	GGGAAGATGGTCGTGATCCTT	TCTGGGGTGGTCTCGATTTTA
MMP9	GGAGGTTTCGACGTGAAGG	GGAACATCCGGTCCACCT
IL8	AGACAGCAGACACACAAGC	ATGGTTCCTCCGGTGGT
TNF $\alpha$	CTCGAACCCCGAGTGACAA	GCTGCCCTCAGCTTGAG
I $\kappa$ B $\alpha$	CATTGACATCAGCACCCAAG	CCACTCCATCCTGAAGGCTA
P100	CTCCTTAGGCCCATGTCAG	AGCCTGGTAGACACGTACCG
$\beta$ -actin	CGTGGACATCCGTAAGACC	ACATCTGCTGGAAGGTGGAC

analysis, the first-strand cDNA was synthesized using the reverse transcriptase with miRNAs-specific stem-loop primer (RiboBio, Guangzhou, China). PCR amplification was conducted in ABI7500 real-time fluorescent measurement system (Applied Biosystems, USA) using the Bulge-Loop miRNA qRT-PCR Starter Kit (RiboBio, Guangzhou, China) according to the protocol provided. The relative expression level of miRNA was normalized to that of endogenous control U6 by using  $2^{-\Delta\Delta Ct}$  method.

#### Western blot

HCC cells were lysed in cell lysis buffer containing 1nM PMSF for 30 min at 4 °C. Lysates were collected by centrifugation at 12,000 rpm for 30 min at 4 °C. Proteins from cell lysates were separated on the SDS-PAGE and transferred onto PVDF membrane (Immobion-P Transfer Membrane, Millipore Corp., Billerica, MA, USA). The membrane was blocked with TBST containing 5% non-fat dry milk for 1 h and further incubated overnight at 4 °C with primary antibodies against PCSK9, LDLR, HMGCR, SREBF2 (Abcam, Cambridge, MA, USA), NF- $\kappa$ B p65, phospho-NF- $\kappa$ B p65, IKK $\alpha$ , TAB3 (Cell Signaling Technology, USA) and  $\beta$ -actin (Santa Cruz, CA, USA). After that, the membrane was incubated with horseradish peroxidase (HRP)-linked secondary antibodies (Santa Cruz Biotechnology, USA) for 2 h at room temperature. All protein bands were visualized using an electrochemiluminescence kit (Thermo, USA). Intensity of each protein band was quantified by Quantity One 4.6.2 software (Bio Rad).

#### MiRNA inhibitor or mimic transfection

For transfections in 12-well plates, the HCC cells were seeded at a density of  $1 \times 10^5$  cells/well and incubated overnight and then transfected with 50 nM miR-195

mimic or 100 nM miR-195 inhibitor (GenePharma, Shanghai, China) using lipo2000 Transfection Agent (Invitrogen, USA) according to the manufacturer's instructions. The corresponding negative sequence of mimic or inhibitors (GenePharma, Shanghai, China) were transfected with the same concentration as controls. At 24 h after the transfection, cells were harvested or further incubated with LPS (1000 ng/mL) for the following experiments.

#### RNA interference

Gene silencing was performed by infecting HCC cells with siRNA oligonucleotides (GenePharma, Shanghai, China). The siRNA sequences were shown in Table 2. For transfections in 12-well plates,  $1.0 \times 10^5$  cells were seeded per well and incubated overnight, then transfected with 80nM TAB3-siRNA, IKK $\alpha$ -siRNA or negative sequence using lipo2000 Transfection Agent (Invitrogen, USA) according to the manufacturer's protocol. At 24 h after the transfection, cells were harvested or further incubated with LPS (1000 ng/mL) for the following experiments.

#### ELISA

HCC cells were stimulated with LPS (1000 ng/ml) for 24 h in serum-free medium in the presence or absence of LDL (200ug/ml). The concentrations of IL-8 and TNF- $\alpha$  in culture supernatants were measured with ELISA kits (R&D Systems, USA) according to the manufacturer's instruction. Absorbance was recorded at 450 nm using a microplate reader.

#### Statistical analysis

Data analysis was performed using SPSS16.0 statistical software (SPSS Inc, Chicago, IL). Student's *t*-tests were

**Table 2** The sequences of siRNA used in this study

Identifier	Sense Primer Sequence (5' → 3')	Anti-sense Primer Sequence (3' → 5')
NC siRNA	CCACCUCUGAUCGAUUUAUdTdT	dTdTAAUAAAUCGAUCAGAGGUGG
IKKa siRNA	GCAGGCUCUUUCAGGGACAdTdT	dTdTTCGUCCGAGAAAGUCCUCGU
TAB3 siRNA	CCAAGGUUCCAUGAAGAAdTdT	dTdTGGUUUCCAAGGUACUUCUU

used to assess significant differences among study groups.  $P < 0.05$  was considered statistically significant. All experiments have been performed at least three times.

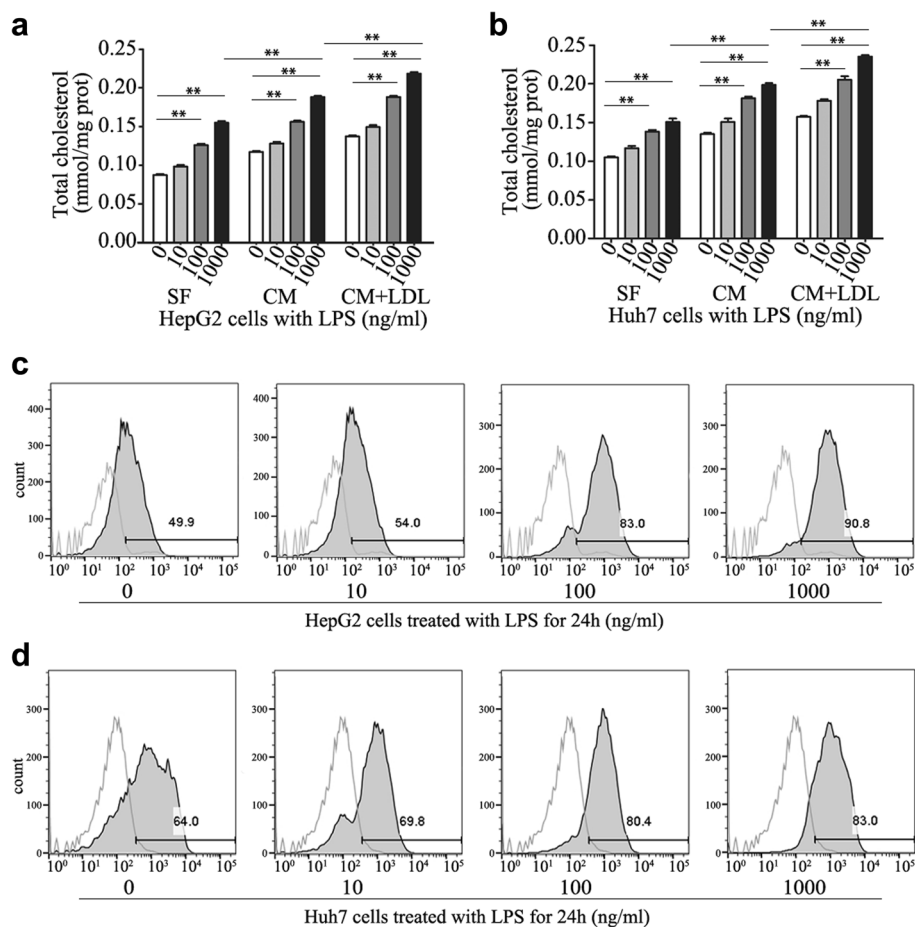
**Results**

**Role of NF-κB signaling in cholesterol accumulation**

To assess whether cholesterol metabolism could be affected by NF-κB pathway in HCC cells, we monitored intracellular cholesterol levels in response to LPS at dosages ranging from 0 to 1000 ng/ml in serum free (SF) medium or complete medium (CM)

or CM with LDL loading (200 μg/ml). As shown in Fig. 1a and b, LPS significantly increased the intracellular cholesterol concentration in a dose-dependent manner in HepG2 and Huh7 HCC cells. And the effects were not affected by constitutive cholesterol levels. Furthermore, we found that uptake of 3,3'-diiodocholesterol (DIL-LDL) in HCC cells was stimulated by LPS, suggesting that it may increase native LDL cholesterol uptake via LDLR (Fig. 1c and d).

Next, we examined the effect of LPS on the expression of proprotein convertase subtilisin/kexin 9 (PCSK9),



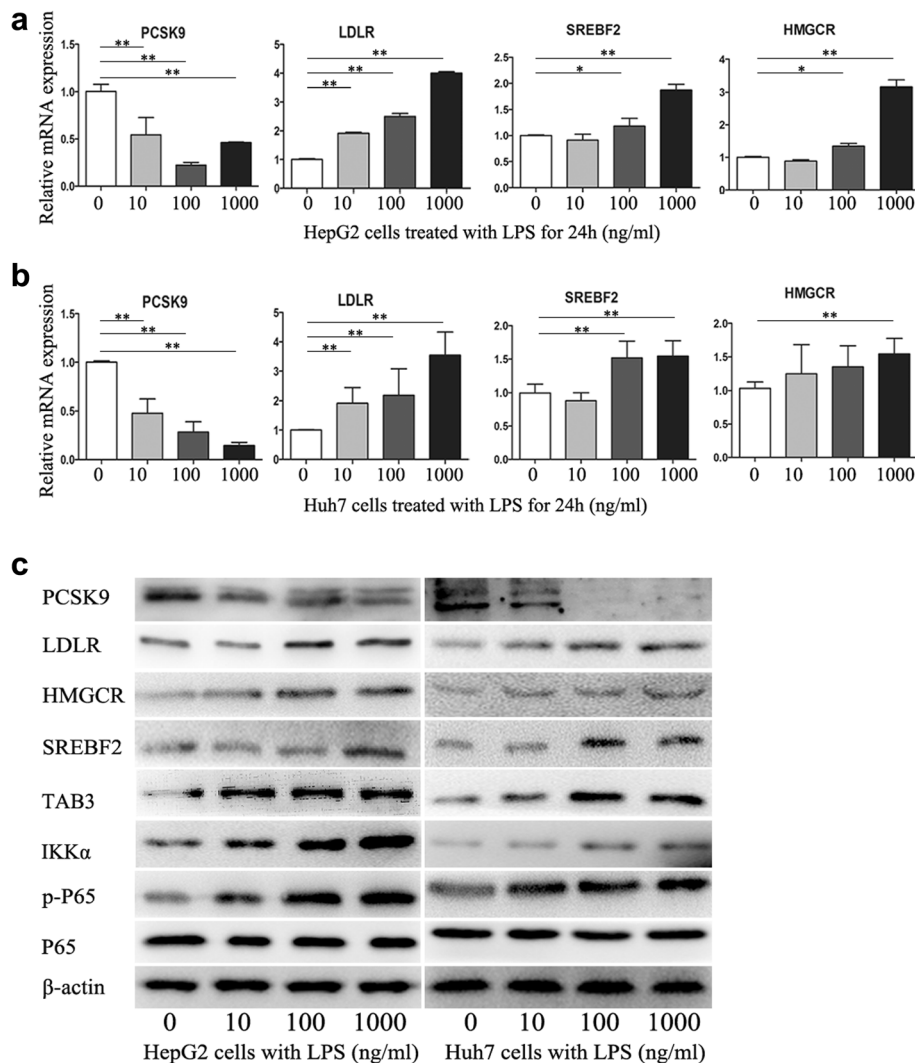
**Fig. 1** Effects of LPS on cholesterol metabolism in HCC cells. **a, b** Cells treated with 0, 10, 100, 1000 ng/ml LPS in serum free (SF) medium or complete medium (CM) in the absence or presence of LDL (200 μg/ml) for 24 h, the total cholesterol ester concentration was measured; **c, d** Cells treated with 0, 10, 100, 1000 ng/ml LPS for 24 h. DIL-LDL uptake was detected. Data represent the mean ± SD of triplicate samples; \*\* $P < 0.01$

LDLR, HMGCR and sterol regulatory element-binding transcription factor 2 (SREBF2) in HCC cells (Fig. 2a-c). After treatment with various concentrations of LPS for 24 h, PCSK9 mRNA and protein levels in HCC cells were significantly downregulated, and the expression of LDLR, HMGCR and SREBF2 were upregulated in a dose-dependent manner. In addition, LPS promotes the expression of IKK $\alpha$ , TAB3 and phosphorylated P65 (Fig. 2c), demonstrating the role of NF- $\kappa$ B pathway in HCC intracellular cholesterol accumulation.

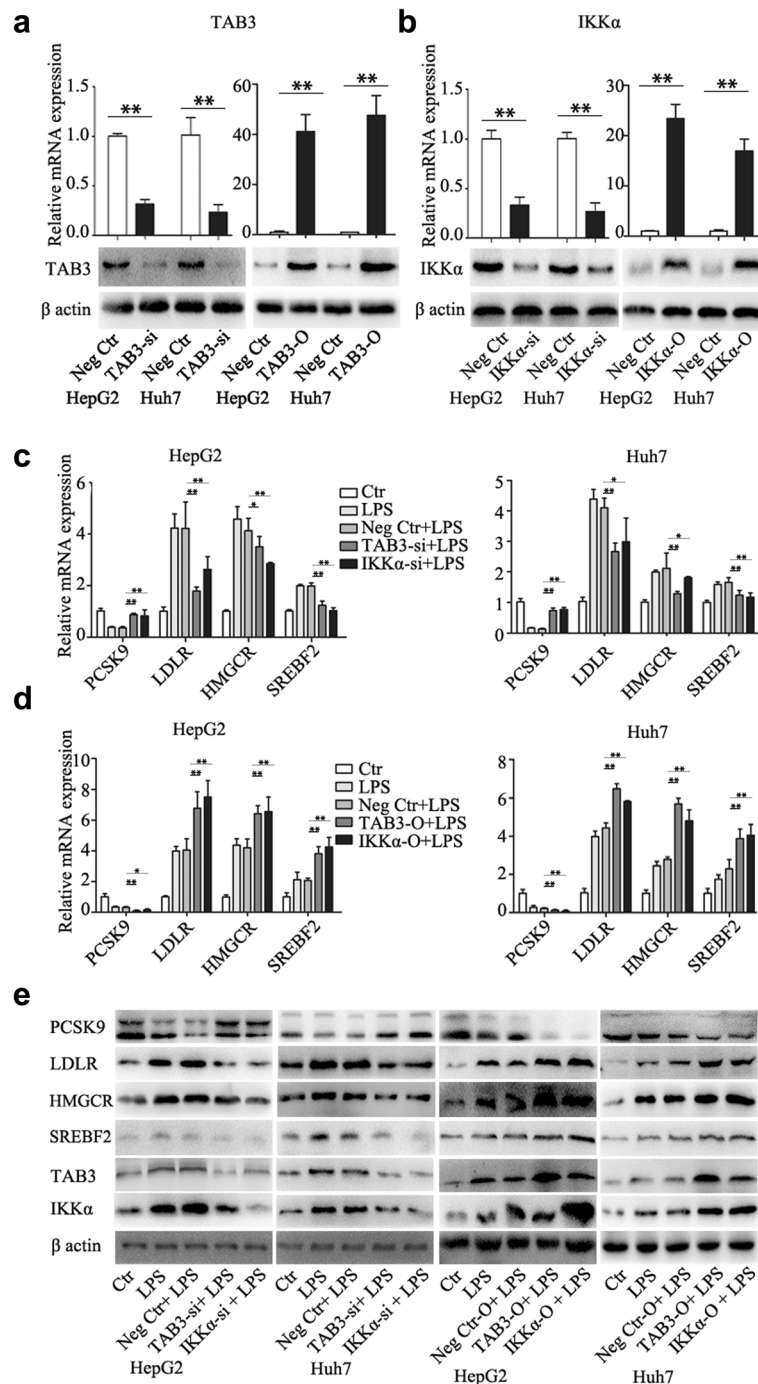
**IKK $\alpha$  and TAB3 regulates LPS-induced cholesterol accumulation**

To further examine whether LPS mediated cholesterol accumulation by NF- $\kappa$ B pathway, we synthesized siRNAs specifically against nuclear factor kappa-B kinase

subunit alpha (IKK $\alpha$ ) and TGF-beta-activated kinase 1 and MAP3K7-binding protein 3 (TAB3) and overexpressed IKK $\alpha$  and TAB3 in HepG2 and Huh7 cells. The down- and up-regulated efficacy of TAB3 and IKK $\alpha$  was confirmed by quantitative real-time PCR and western blotting (Fig. 3a and b). Twenty-four hours after transfection, HCC cells were treated with LPS (1000 ng/ml) for 24 h, and PCSK9, LDLR, HMGCR, and SREBF2 expression, total cellular cholesterol levels, and uptake of DIL-LDL were measured at the endpoints. As expected, down-regulation of TAB3 and IKK $\alpha$  increased PCSK9 expression and decreased LDLR, HMGCR, and SREBF2 expression, while up-regulation of TAB3 and IKK $\alpha$  decreased PCSK9 expression and increased LDLR, HMGCR, and SREBF2 expression (Fig. 3c-e). Additionally, down-



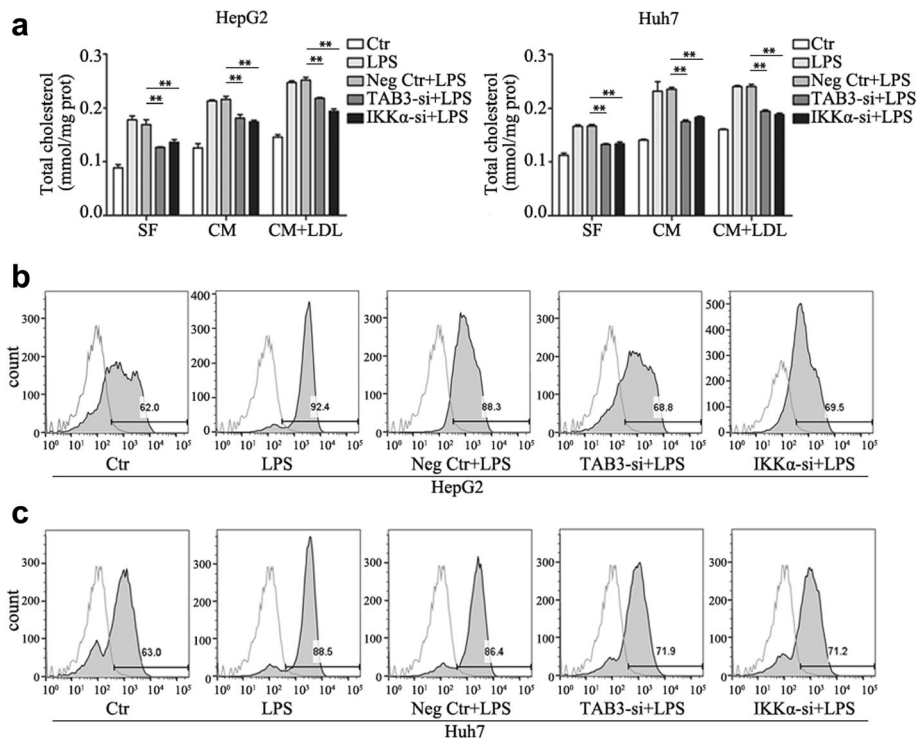
**Fig. 2** Expression of PCSK9, LDLR, HMGCR and SREBF2 in HCC cells stimulated with LPS. Cells treated with 0, 10, 100, 1000 ng/ml LPS for 24 h. The expression of PCSK9, LDLR, HMGCR, SREBF2 and the phosphorylation status of P65 were detected by qRT-PCR (a, b) and western blot (c). Data represent the mean  $\pm$  SD of triplicate samples; \* $P$  < 0.05, \*\* $P$  < 0.01



**Fig. 3** IKK $\alpha$  and TAB3 regulates cholesterol-related gene expression induced by LPS. **a, b** qRT-PCR and western blotting confirm the down- and up-regulation of IKK $\alpha$  or TAB3 expression in HCC cells. **c, d, e** Twenty-four hours after transfection, HCC cells were treated with LPS (1000 ng/ml) for 24 h, and then PCSK9, LDLR, HMGCR and SREBF2 expression were detected. Data represent the mean  $\pm$  SD of triplicate samples; \* $P < 0.05$ , \*\* $P < 0.01$

regulation of TAB3 and IKK $\alpha$  abrogated the effects of LPS on cholesterol accumulation (Fig. 4a–c). These findings indicated that TAB3 and IKK $\alpha$  were indeed functional in LPS-induced cholesterol accumulation.

**MiR-195 regulates cholesterol accumulation and cholesterol-related gene expression induced by LPS**  
 Previous findings have shown that miR-195 decreases the activity of NF- $\kappa$ B signaling and the expression of



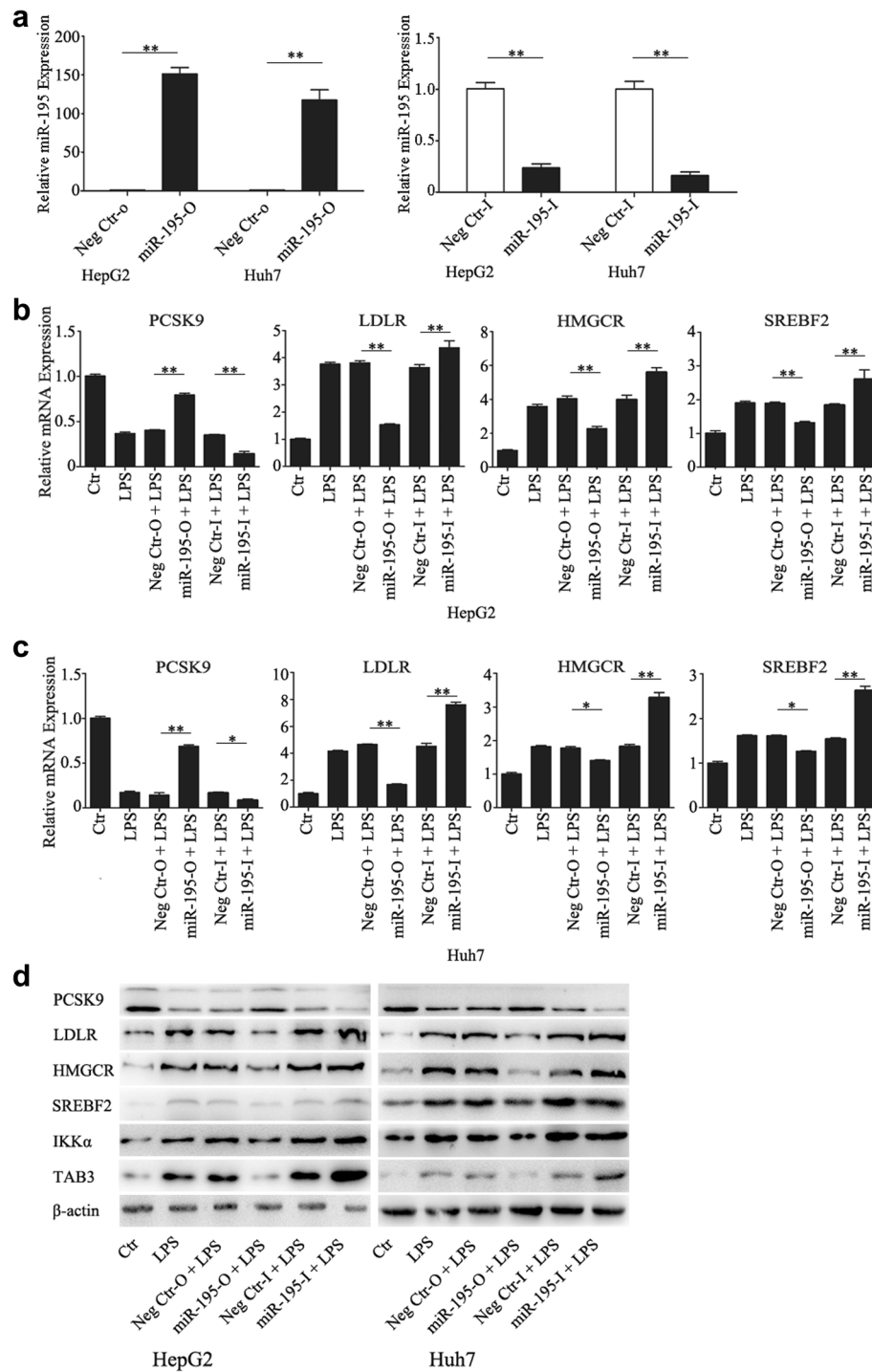
**Fig. 4** Down-regulation of IKKα and TAB3 expression suppressed LPS-induced cholesterol accumulation. **a** After transfection with IKKα, TAB3 siRNA or negative control for 24 h, cells treated with 1000 ng/ml LPS in serum free medium or complete medium in the absence or presence of LDL (200 ug/ml) for 24 h, then the total cholesterol ester concentration was measured. **b, c** After transfection with IKKα, TAB3 siRNA or negative control for 24 h, cells were exposed to LPS (1000 ng/mL) for 24 h, then DIL-LDL uptake was detected. Data represent the mean ± SD of triplicate samples; \*\**P* < 0.01

NF-κB downstream effectors by directly targeting TAB3 and IKKα [19]. To further investigate the involvement of NF-κB signaling in cholesterol metabolism, the cells overexpressing or knocking down miR-195 were established by transfecting with miR-195 mimic, miR-195 inhibitor. As shown in Fig. 5a, these cells achieved more than a 100-fold upregulation and 80% downregulation of mRNA expression, respectively (Fig. 5a). Twenty-four hours after transfection with miR-195 mimic, miR-195 inhibitor, or negative control, HCC cells were treated with 1000 ng/mL LPS for another 24 h and then analyzed for intracellular cholesterol levels and related gene expression. Relative to the controls, mRNA and protein expression levels of PCSK9 were downregulated by LPS, and LDLR, HMGCR, and SREBF2 expression was upregulated. Overexpression of miR-195 increased PCSK9 expression and decreased LDLR, HMGCR, and SREBF2 expression, while knockdown of miR-195 decreased PCSK9 expression and increased LDLR, HMGCR, and SREBF2 expression (Figs. 5b–d). In addition, overexpression of miR-195 inhibited the total intracellular cholesterol level (Fig. 6a and b) and DIL-LDL uptake (Fig. 6c). These data indicate that miR-195 negatively regulates LPS-induced cholesterol accumulation and expression of cholesterol-related genes.

Next, we investigated the phosphorylation status of p65 and the expression levels of TAB3 and IKKα, which were the direct gene targets of miR-195. We observed that overexpression of miR-195 significantly inhibited the phosphorylation status of p65 and the expression levels of TAB3 and IKKα, while knockdown of miR-195 increased the phosphorylation status of p65 and the expression levels of TAB3 and IKKα (Fig. 7), supporting our findings that NF-κB pathway played an important role in HCC intracellular cholesterol accumulation.

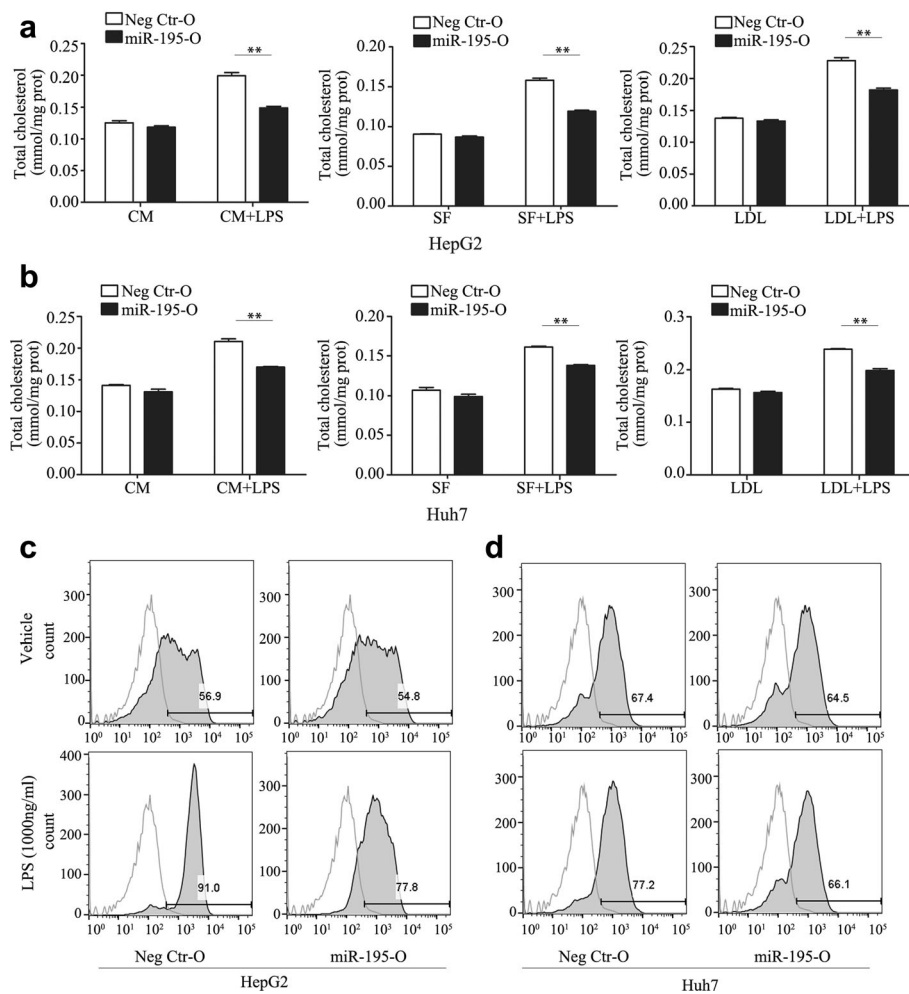
**Cholesterol increases LPS induced pro-inflammatory effects**

LDL, containing 49% cholesterol, could increase intracellular cholesterol contents in HCC cells (Fig. 8a). To elucidate the influence of cholesterol accumulation in inflammatory responses, we investigated the phosphorylation status of NF-κB signaling pathway and the expression levels of 20 NF-κB downstream effectors in the absence or presence of LDL loading. LPS at 1000 ng/ml activated NF-κB signaling pathway and stimulated the expression of NF-κB downstream effectors, while the phosphorylation level of p65 and IKKα (Fig. 8b) and the mRNA abundance of six effectors (Fig. 8c and d) and cytokines production (Fig. 8e and f) was further increased



**Fig. 5** MiR-195 regulated cholesterol-related gene expression induced by LPS. **a** validation of miR-195 mimic and inhibitor by qRT-PCR at 24 h after transfection (**b-d**) After transfection with miR-195 mimic or inhibitor for 24 h, cells were exposed to LPS (1000 ng/mL) for 24 h. The expression levels of PCSK9, LDLR, HMGR and SREBF2 in HCC cells were detected by qRT-PCR (**b, c**) and western blot (**d**). Data represent the mean  $\pm$  SD of triplicate samples; \* $P < 0.05$ , \*\* $P < 0.01$





**Fig. 6** MiR-195 inhibited LPS-mediated cholesterol accumulation. **a, b** After transfection with miR-195 mimic for 24 h, cells treated with 1000 ng/ml LPS in serum free medium or complete medium in the absence or presence of LDL (200 ug/ml) for 24 h, then the total cholesterol ester concentration was measured. **c, d** After transfection with miR-195 mimic for 24 h, cells were exposed to LPS (1000 ng/mL) for 24 h. DIL-LDL uptake was detected. Data represent the mean  $\pm$  SD of triplicate samples; \*\* $P < 0.01$

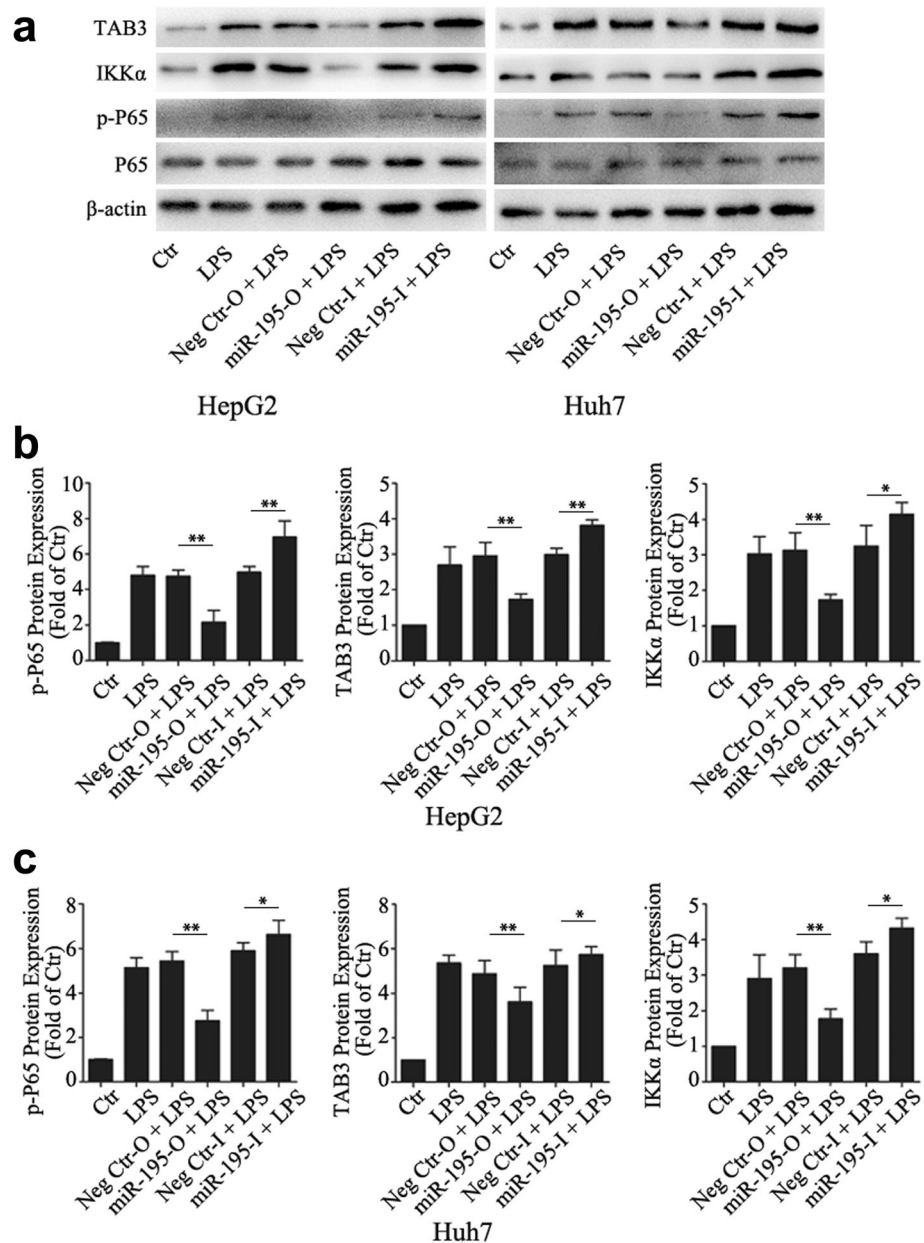
by LDL at 200ug/ml in both HepG2 and Huh7 cells. The data suggest that accumulated cholesterol increases LPS/NF- $\kappa$ B-induced inflammatory effects by increasing p-IKK $\alpha$  in HCC cells.

**Discussion**

HCC, among the most malignant of human cancers [20], develops in a complex microenvironment characterized by chronic inflammation [13, 14]. Emerging evidence has demonstrated that perpetuating hepatic inflammation promotes HCC initiation and progression [13, 21, 22]. Meanwhile, cholesterol metabolic abnormalities have been implicated the importance in cancer cell physiology in recent years [2–4, 23]. However, the relationship between the aberrant cholesterol metabolism and inflammation in HCCs are not completely understood. In the present study, we demonstrated that LPS/

NF- $\kappa$ B pathway induced a significant increase in the intracellular cholesterol concentration and in DIL-LDL uptake in HCC cells, suggesting a key role of NF- $\kappa$ B pathway in promoting cholesterol accumulation. Additionally, the cholesterol accumulation conversely promoted LPS/NF- $\kappa$ B-induced inflammatory effects, indicating a positive feedback of inflammation and cholesterol metabolism (Fig. 9).

MicroRNAs (miRNAs) are a class of small non-coding RNAs, which repress translation or induce cleavage of target mRNAs by base pairing with their 3' untranslated regions. MiR-195 is a member of the miR-15/16/195/424/497 family [24]. It has been reported that miR-195 suppresses cancer development and is downregulated in multiple types of cancer, such as prostate [25, 26], lung [27], osteosarcoma [28], HCCs [29], and so forth. Genome-wide screening suggests that miR-195 mediates

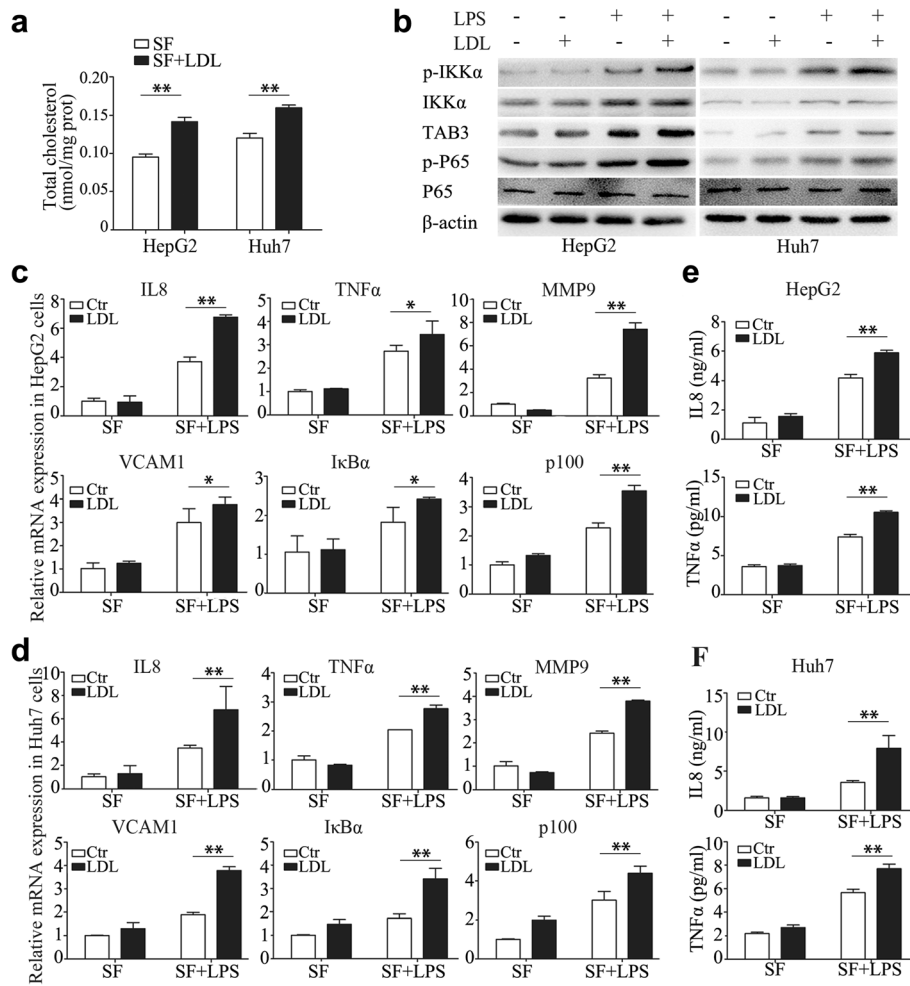


**Fig. 7** MiR-195 inhibited the activation of NF-κB pathway. **a, b, c** After transfection with miR-195 mimic for 24 h, cells treated with 1000 ng/ml LPS for 24 h, then the phosphorylation status of P65 and the expression levels of TAB3 and IKKα were detected by western blot. Data represent the mean ± SD of triplicate samples; \* $P < 0.05$ , \*\* $P < 0.01$

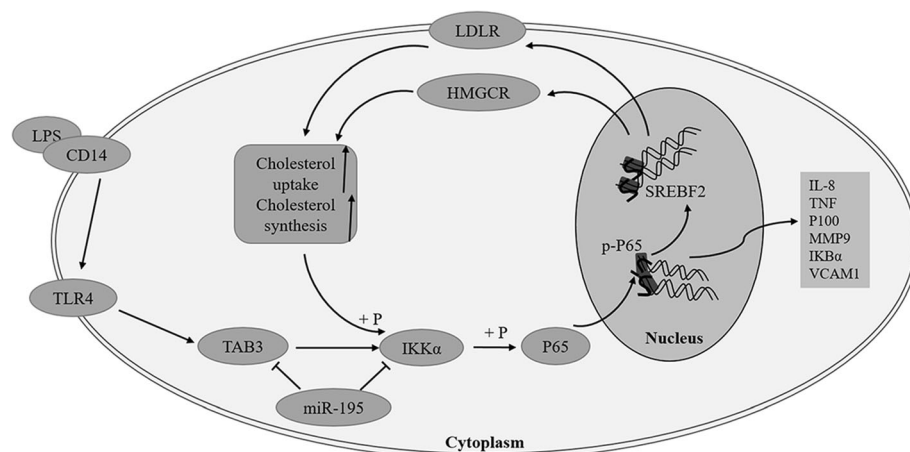
NF-κB activity by directly targeting the expression of IKKα TAB3 in HCCs [19]. Here, we demonstrated that not only miR-195 but also its targeted genes TAB3 and IKKα mediate LPS/NF-κB-induced cholesterol accumulation and cholesterol-related gene expression, providing further evidence that NF-κB pathway play an important role in HCC intracellular cholesterol accumulation.

PCSK9, the ninth member of the proteinase K subfamily of subtilases, plays an important role in post-transcriptional degradation of LDLR, which decreases intracellular

cholesterol uptake [30–32]. Decreased PCSK9 and increased LDLR expression have been demonstrated in HCC tissues, supporting a constant cholesterol supply in the HCC microenvironment [33]. HMGCR is the rate-limiting enzyme in *de novo* synthesis of cholesterol in vivo. Recent studies have reported that HMGCR is upregulated in several types of cancer including gastric [34], ovarian [7] and breast cancers [35], suggesting that HMGCR plays an oncogenic role. SREBF2 is a membrane-bound transcription factor that regulates cholesterol homeostasis in cells. It



**Fig. 8** LDL increases LPS induced pro-inflammatory effects. LDL increases intracellular cholesterol contents (a), LDL significantly activated the phosphorylation of p65 and IKKα (b), increased the expression of NF-κB target genes (c, d), promoted the production of IL-8 and TNF-α in HCC cells with administration of LPS for 24 h (e, f). Data represent the mean ± SD of triplicate samples; \*P < 0.05, \*\*P < 0.01



**Fig. 9** Illustration of the schematic representation of the working

has been demonstrated that PCSK9, LDLR, and HMGCR expression are co-regulated by SREBF2 [36–38]. When cholesterol levels fall, SREBF2 is activated to up-regulate the expression of genes responsible for cholesterol synthesis, such as HMGCR, and for cholesterol uptake, such as LDLR. In this study, the expression of PCSK9, LDLR, HMGCR, and SREBF2 were investigated in HCC cells after stimulation with LPS. We found that LPS significantly inhibited the expression of PCSK9 and increased LDLR, HMGCR, and SREBF2 expression, suggesting that LPS may increase native LDL cholesterol uptake via LDLR and promote *de novo* cholesterol synthesis via HMGCR.

There are growing evidences that cholesterol, as an important molecule, impacts upon cancer cell physiology, however, the concrete role of cholesterol in cancer progression remains elusive and controversial. Analyses of the cancer Genome Atlas (TCGA) database revealed a correlation between increased activity of the cholesterol synthesis pathway and decreased survival in patients with sarcoma, acute myeloid leukemia and melanoma [39, 40], supporting the concept that cholesterol promotes carcinogenesis. However, some epidemiological studies have reported objective observation that poor prognosis in HCC patients were linked to decreased serum cholesterol [41, 42]. In this study, we have suggested that cholesterol further activated the NF- $\kappa$ B signaling pathway and promotes the expression of NF- $\kappa$ B target genes, indicating the pro-inflammatory effects of cholesterol in HCC cells.

## Conclusions

In summary, we have experimentally demonstrated that LPS/NF- $\kappa$ B signaling pathway triggers an increase in intracellular cholesterol levels by promoting the expression of HMGCR and LDLR in HCC cells. Cholesterol accumulation conversely promotes LPS/NF- $\kappa$ B induced pro-inflammatory effects. MiR-195, as a regulator of NF- $\kappa$ B pathway, inhibited cholesterol accumulation by decreasing the expression of TAB3 and IKK $\alpha$ . These data provide us with a better understanding of the relationship between LPS/NF- $\kappa$ B pathway and cholesterol abnormalities in cancer cells.

## Abbreviations

DIL-LDL: Low density lipoprotein labeled with 1,1'-Diiodo-3,3',3'-tetramethyl-indocarbocyanideperchlorate; HCC: Hepatocellular carcinoma; HMGCR: 3-hydroxy-3-methylglutaryl-CoA reductase; IKK $\alpha$ : Nuclear factor kappa-B kinase subunit alpha; LDLR: LDL receptor; LPS: Lipopolysaccharide; miR-195: microRNA-195; NF- $\kappa$ B: Nuclear factor-kappa B; PCSK9: Proprotein convertase subtilisin/kexin 9; SREBF2: Sterol regulatory element-binding transcription factor 2; TAB3: TGF-beta-activated kinase 1 and MAP3K7-binding protein 3

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## Availability of data and materials

Not applicable.

## Authors' contributions

JLX and LSW conceived and designed the study. MYH, WHZ, YYD performed the experiments. TTF, WQT, BL, GLC, BWY, PXH analyzed the data. All authors read and approved the final manuscript.

## Competing interests

The authors declare that they have no competing interests.

## Consent for publication

Not applicable.

## Ethics approval and consent to participate

Not applicable.

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