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MicroRNA-145 inhibits proliferation and promotes apoptosis of HepG2 cells by targeting ROCK1 through the ROCK1/NF-κB signaling pathway

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Abstract. – OBJECTIVE: Hepatocellular carcinoma (HCC) is a malignant cancer with a high fatality rate, and the expression of microRNA-145 (miR-145) is significantly low in HCC tissue. Therefore, the effect of miR-145 on HCC was explored.

PATIENTS AND METHODS: Primary hepatocellular carcinoma samples and corresponding normal samples, and HepG2 cells were analyzed using flow cytometry, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, Real-time quantitative reverse transcription- polymerase chain reaction, Western blotting, ad dual-luciferase reporter assay.

RESULTS: miR-145 expression was significantly downregulated in HCC tissue and HepG2 cells as compared to normal liver tissue. After HepG2 cells were transfected with miR-145 mimics, miR-145 expression was recovered, accompanied by a significantly lower cell number, inhibition of the G1/S phase transition, and promotion of the apoptosis of HepG2 cells, as well as changes in levels of G1/S-specific cyclin-E1 (CCNE1) and activated caspase-3. Furthermore, the rho-associated protein kinase 1 (ROCK1) levels were opposite the levels of miR-145 expression *in vivo* and *in vitro*, and additional experiments with co-transfection of miR-145 mimics and pEGFP-N3-3'UTR provided the direct evidence that the ROCK1 gene is a target of miR-145. Moreover, a significant decrease or increase in the expression of ROCK1 was associated with nuclear factor-kB (NF-κB)(p65) activity, and lipopolysaccharide (LPS) significantly increased NF-κB(p65) activity, accompanied by recovery of the reduction in the number of HepG2 cells for miR-145 mimics. The NF-κB activity and cell number were significantly ($p < 0.05$, $p < 0.01$) increased in response to the overexpression of the ROCK1 gene in HepG2 cells.

CONCLUSIONS: We showed that miR-145 can target and downregulate ROCK1 expression, and it controls HCC by inhibiting the cell cycle and activating apoptosis via the ROCK1/NF-κB signaling pathway. Our findings will provide a new perspective for the therapy of HCC.

Key Words:
miR-145, ROCK1/NF-κB, Cell cycle, Cell apoptosis, Hepatocellular carcinoma.

Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignant tumors, with a high fatality rate due to its capacity for proliferation and invasion. MicroRNAs (miRNAs) extensively regulate gene expression by targeting gene-3'UTR to participate in individual development, cell proliferation, cell apoptosis, cell differentiation, etc.[1,2]. It was reported that some miRNAs (such as miR-99b, -101, -155, -181b, and -370) play an important role in the occurrence and development of tumors[3-7]. Therefore, it is feasible to choose miRNA as a new target for cancer therapy. miR-145 (5q32-33) is located near the important fragile site 5q31 on chromosome 5. Fragile sites are usually stable in somatic cells, but are often absent or rearranged in many tumor cells. The low expression of miR-145 in various tumor tissues regulates tumor proliferation, apoptosis, and invasion ability by controlling the target protein, and inhibits tumor growth[8-11]. In addition, miR-145 has a significant inhibitory effect on tumor cell invasion and metastasis[12-14]. miR-145 targets multiple genes, especially N-cadherin and vimentin, which can affect the epithelial-mesenchymal transition so that invasion and metastasis are inhibited[10,15]. It was reported that the expression level of miR-145 is significantly lower in HCC tissue than in normal liver tissue[16], suggesting that miR-145 may play a negative role in the occurrence and development of HCC. Using HCC tissue and HepG2

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human liver carcinoma cells, the effects of miR-145 on cell proliferation and apoptosis in the occurrence and development of HCC were investigated in this study, which will enrich the theory and also provide a new perspective on HCC.

Materials and Methods

Ethics

The present study was approved by the Ethics Committee of JiNing No. 1 People's Hospital (NO. JNPH-HEC2018-01-015; JiNing, Shandong, China). Written informed consent was obtained from each patient.

Tissue Specimen Collection

A total of 9 primary hepatocellular carcinoma samples and corresponding normal samples were collected from patients at the JiNing No. 1 People's Hospital. None of the patients had received blood transfusions, radiotherapy, or chemotherapy prior to surgery. All samples were immediately snap-frozen in liquid nitrogen following surgical removal and stored at -80°C until use.

HepG2 Cell Culture and Treatment

HepG2 cells (purchased from the National Infrastructure of Cell Line Resource of China) were cultured with Dulbecco's modified Eagle's medium (DMEM) (Gibco, Grand Island, NY, USA) containing 10% fetal bovine serum (FBS) (Gibco, Grand Island, NY, USA) in a 5% CO_2 incubator at 37°C. The medium was changed every 3 days. After reaching 80% confluence, cells were passaged, and cells at passage 2-4 were used for further experiments.

HepG2 cells with 20% confluence were plated in 96-well or 100-mm dishes to use for transfection of miR-145 mimics (RiboBio Co., Ltd, Guangzhou, China). After transfection for 48 or 60 h, cells were collected for further examination. In addition, two groups of HepG2 cells were treated for 2 h with 5 μg/ml lipopolysaccharides (LPS) (Solarbio, Beijing, China) or miR-145 mimics. According to the sequences of the rho-associated protein kinase 1 (ROCK1) gene in the Ensembl database (ENSG00000067900), a pEGFP-N3 eukaryotic expression vector carrying the coding sequence (CDS) of ROCK1 gene was constructed, and would be subsequently transfected into HepG2 cells.

3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) Assay

After HepG2 cells were cultured in 96-well plates with different treatments for 60 h, the cell number was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. First, 20 μl of 5 mg/ml MTT (Solarbio, Beijing, China) was added to each well, and the plates were then incubated for 4 h at 37°C. After removing the supernatant, formazan crystals were dissolved in 200 μl of DMSO (Solarbio, Beijing, China), and the absorbance was measured at 490 nm. There were eight replicate wells for each group to ensure experimental accuracy.

Flow Cytometry (FCM)

HepG2 cells that underwent different treatments were collected to use for the analysis of cell cycle or cell apoptosis using a FACSCanto II flow cytometer (Becton, Dickinson and Company, Brea, CA, USA). For cell cycle, cells were mixed with 0.25 % Triton X-100 and 5 μl propidium iodide (PI) (Solarbio, Beijing, China), and incubated for 30 min at room temperature in the dark. Cells were resuspended in 0.5 ml of phosphate-buffered saline (PBS) and immediately analyzed. For cell apoptosis, cells were digested with trypsin, centrifuged at 300 × g for 5 min, and washed with ice-cold phosphate-buffered saline (PBS) (Gibco, Grand Island, NY, USA). The cell pellets were re-suspended in 100 μl Annexin V-binding buffer, transferred to a 5-ml culture tube containing 5 μl Annexin V-FITC (BioVision) (San Francisco, CA, USA), and mixed with 10 μl PI. The tube was gently vortexed and incubated for 15 min at room temperature in the dark. Subsequently, 300 μl of binding buffer was added, and the cells were immediately analyzed.

Real-Time Quantitative Reverse Transcription-Polymerase Chain Reaction (qPCR)

Total RNA was extracted from the cells using TRIzol reagent (Invitrogen; Carlsbad, CA, USA) according to the manufacturer's instructions. First strand cDNA was synthesized using PrimeScript RT Master kit (TaKaRa, Dalian, China). Q-PCR was performed using SYBR Green reagents with a 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). The specific primers of the ROCK1 gene (F:5'-GGTGGTCGGTTGGGGTATTT-3'; R:5'-AACTGGTGCTACAGTGTCTCG-3') and miR-145 (F:5'-GTCCAGTTTTCCCAGGAATC CCT-3';

R:5'-GCT GTCAACATACGCTACGTAACG-3') were used, and actin beta (β-actin) (F:5'-GCTC-GTCGTCGACAACGGCTC-3'; R:5'-CAAA-CATGATCTGGGTCATCTTCTC-3') and U6 (F:5'-CTC GCTTCGGCAGCACA-3'; R:5'-GC-GAGCACAGAATTAATACGAC-3') expression was used as the normalization control by the $2^{-\Delta\Delta Ct}$ method. A melting curve was constructed to verify the single amplified PCR product. Samples were assayed in triplicate, with standard deviations of cycle threshold (CT) values that did not exceed 0.5 on a within-run basis.

Dual-Luciferase Reporter Assay

According to the sequences of the human ROCK1 gene in the GALGAL5.0 database (ENSG00000067900), the pEGFP-N3 eukaryotic expression vector carrying the 3'-untranslated region (UTR), and the mutant of the 3'-UTR region of human ROCK1 gene were constructed. Then, the pEGFP-N3, pEGFP-N3-ROCK1-3'UTR, or the pEGFP-N3-ROCK1-mutant-3'UTR plasmid was co-transfected with miR-145 mimics into 293T cells in a dual-luciferase reporter system. After 36 h incubation, firefly and Renilla luciferase activities were measured using the dual-luciferase reporter assay kit (Promega, Madison, WI, USA) according to the manufacturer's instructions.

Western Blotting

The nuclear factor-kB (NF-κB) (p65) activity, and the levels of ROCK1, (NF-κB)(p65), and G1/S-specific cyclin-E1 (CCNE1) were detected in HepG2 cells that had been given different treatments. The total protein levels of all samples were assayed with a Bicinchoninic acid (BCA) Protein Assay Kit (Pierce) (Thermo Fisher, Waltham, MA, USA). The following monoclonal antibodies were purchased and used in this research: anti-β-tubulin, anti-NF-κB(p65), anti-pNF-κB(p65), anti-CCNE1, anti-ROCK1 (Abcam, Cambridge, MA, USA), anti-Caspase-3 (CASP3), and anti-Cleaved-CASP3 (Cell Signaling Technology, Danvers, MA, USA).

Statistical Analysis

All statistical analyses were performed using one-way ANOVA with post-hoc pairwise comparisons by the least significant difference (LSD) test using Statistical Analysis Systems software (Version 8.2, SAS Institute, Cary, NC, USA, 2001) to determine significance (accepted at $p < 0.05$). The data are expressed as the mean ± SEM.

Results

miR-145 Induces a Decrease in the Hepatocellular Carcinoma Cell Number

As demonstrated in Figure 1A, the qPCR results showed that the level of miR-145 expression was significantly downregulated ($p < 0.01$) in the hepatocellular carcinoma tissues as compared to normal liver tissues. Furthermore, the miR-145 expression was found to be notably decreased ($p < 0.01$) in HepG2 cell lines as compared to normal liver tissue. Compared with the control HepG2 cells without treatment, the level of miR-145 expression in HepG2 cells transfected with miR-145 mimics for 60 h had been significantly upregulated ($p < 0.01$) (Figure 1A), but the cell number was significantly lower ($p < 0.05$) (Figure 1B). Accordingly, these results indicate that miR-145 may inhibit the development of hepatocellular carcinoma.

miR-145 Inhibits the Proliferation and Promotes the Apoptosis of HepG2 Cells

To investigate the effect of miR-145 on the observed inhibition of HepG2 cell numbers, the cell cycle and apoptosis of HepG2 cells transfected with miR-145 mimics were detected by flow cytometric (FCM) assay. As shown in Figure 1C, after transfection with the miR-145 mimics compared with no transfection, the percentage of HepG2 cells in the S and G1 phases was significantly lower and higher, respectively. Additionally, miR-145 induced an obvious increase in PI-negative and Annexin V-positive cells (Figure 1D), with the cytotoxic agent etoposide being used as a positive control for apoptosis detection. Genetically, the CCNE1 (G1/S-specific cyclin E1) protein level was significantly downregulated, and CASP3 (a key factor in apoptosis) and activated C-CASP3 levels were markedly increased in HepG2 cells with transfection by miR-145 mimics (Figure 1E).

ROCK1 is the Target of miR-145 in HepG2 Cells

The expression of ROCK1 was detected according to a previously published procedure[17]. The qPCR and Western blotting results showed that the mRNA ($p < 0.01$, $p < 0.01$) and protein levels of ROCK1 were significantly upregulated in the hepatocellular carcinoma tissues and HepG2 cells as compared to normal liver tissues (Figure 2A), which was the opposite change as compared to miR-145 expression. However, after the HepG2 cells were transfected with the miR-145 mimics, then the mRNA ($p < 0.01$) and protein levels of ROCK1 were obviously downregulated (Figure

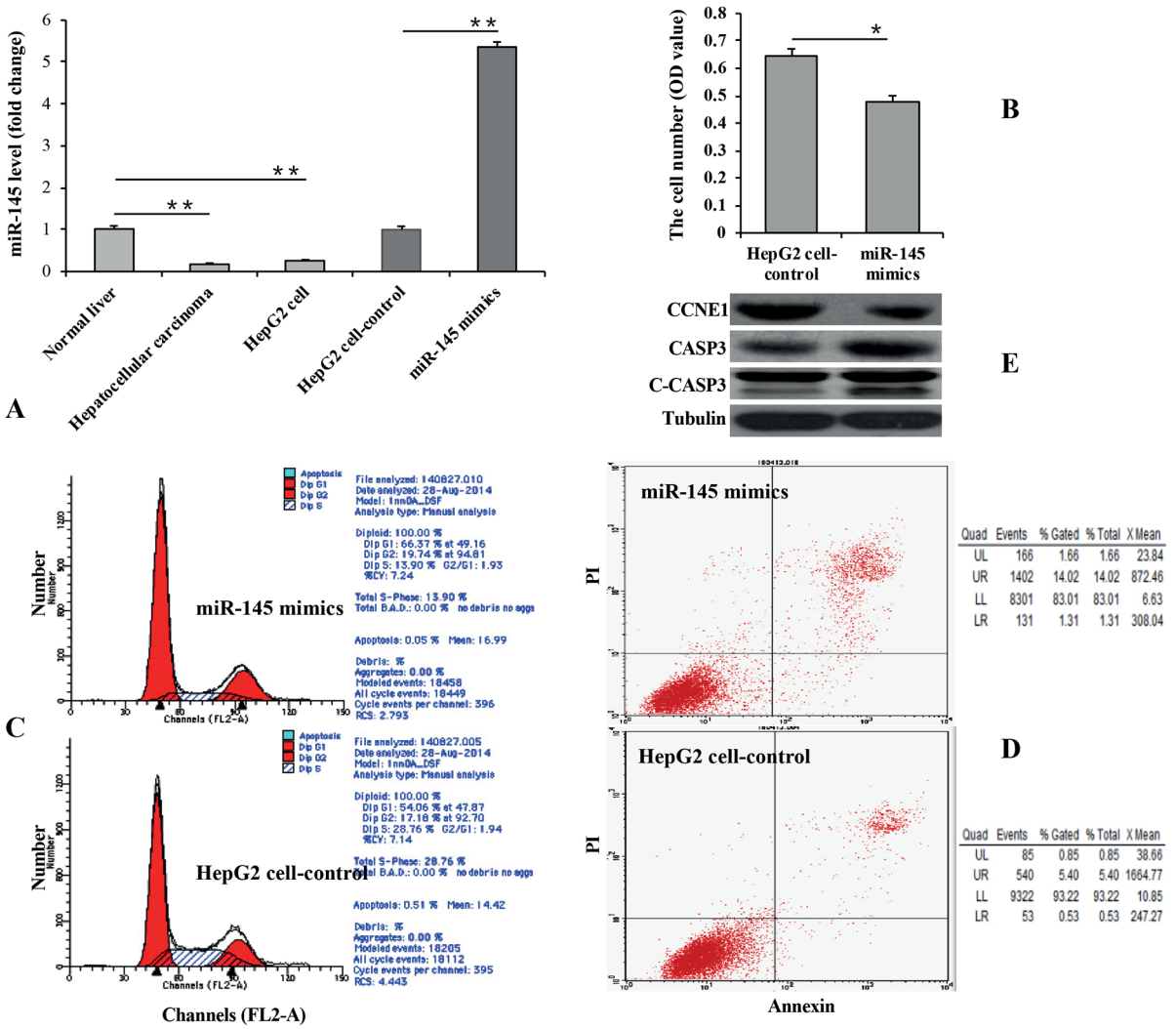

Figure 1. The effect of miR-145 on hepatocellular carcinoma. ***A*,** The comparison of miR-145 expression in hepatocellular carcinoma tissue, normal liver tissue, HepG2 cells, and HepG2 cells transfected with miR-145 mimics or not for 48 h by Q-PCR. ***B*,** The change in cell number of HepG2 cells transfected with miR-145 mimics or not for 60 h by MTT; ***C*** and ***D*,** The comparison by FCM of cell cycle or cell apoptosis between HepG2 cells after treatment by miR-145 mimics or not; ***E*,** The levels of CCNE1, CASP3, and C-CASP3 in HepG2 cells after treatment by miR-145 mimics or not. The data are expressed as the mean ± SEM; n=3 or 8. **p* < 0.05, ***p* < 0.01.

2B). Additional experiments by dual-luciferase reporter assay also were performed to explore the regulatory relationship between miR-145 and ROCK1, and the results revealed that the positive signal significantly ($p < 0.01$) increased in 293T cells after co-transfection with miR-145 mimics and the pEGFP-N3-3'UTR vector of ROCK1 gene, providing the direct evidence that ROCK1 is the target gene of miR-145 (Figure 2C).

NF-κB Signaling Pathway Mediated the Effect of miR-145 in HepG2 Cells

The NF-κB(p65) activity, which is considered to be an important regulator of cell cycle and apoptosis, was analyzed. The results showed that the NF-κB(p65) levels and activities were obviously higher in hepatocellular carcinoma tissues and HepG2 cells as compared to normal liver tissues (Figure 3A). After a significant increase in the miR-145 level in HepG2 cells after transfection with miR-145 mimics, the NF-κB(p65) activity also exhibited the same significant decrease as that of the expression of ROCK1 (Figure 3B). However, after the HepG2 cells were treated with LPS plus transfected with the miR-145 mimics, then the NF-κB (p65) activity significantly increased, accompanied by significant ($p < 0.05$) recovery of HepG2 cell numbers (Figure 3C). In addition,

after transfecting the pEGFP-N3-CDS vector containing the ROCK1 gene into HepG2 cells, the NF-κB(p65) activity in HepG2 cells was significantly increased, accompanied by a significant increase in the ROCK1 protein level (Figure 3D).

Discussion

The changes in microRNA expression in various human tumors are closely related to the occurrence, development, diagnosis, treatment, and prognosis of the cancer[18,19]. HCC is a common malignant cancer with a high fatality rate, and microRNA-145 (miR-145) expression was significantly downregulated in HCC tissue as compared to normal liver tissue[16], which may play an important negative role in the occurrence and development of HCC. Therefore, the effects of miR-145 on the cell proliferation and apoptosis of liver cancer cells were detected in this study using HCC tissue and

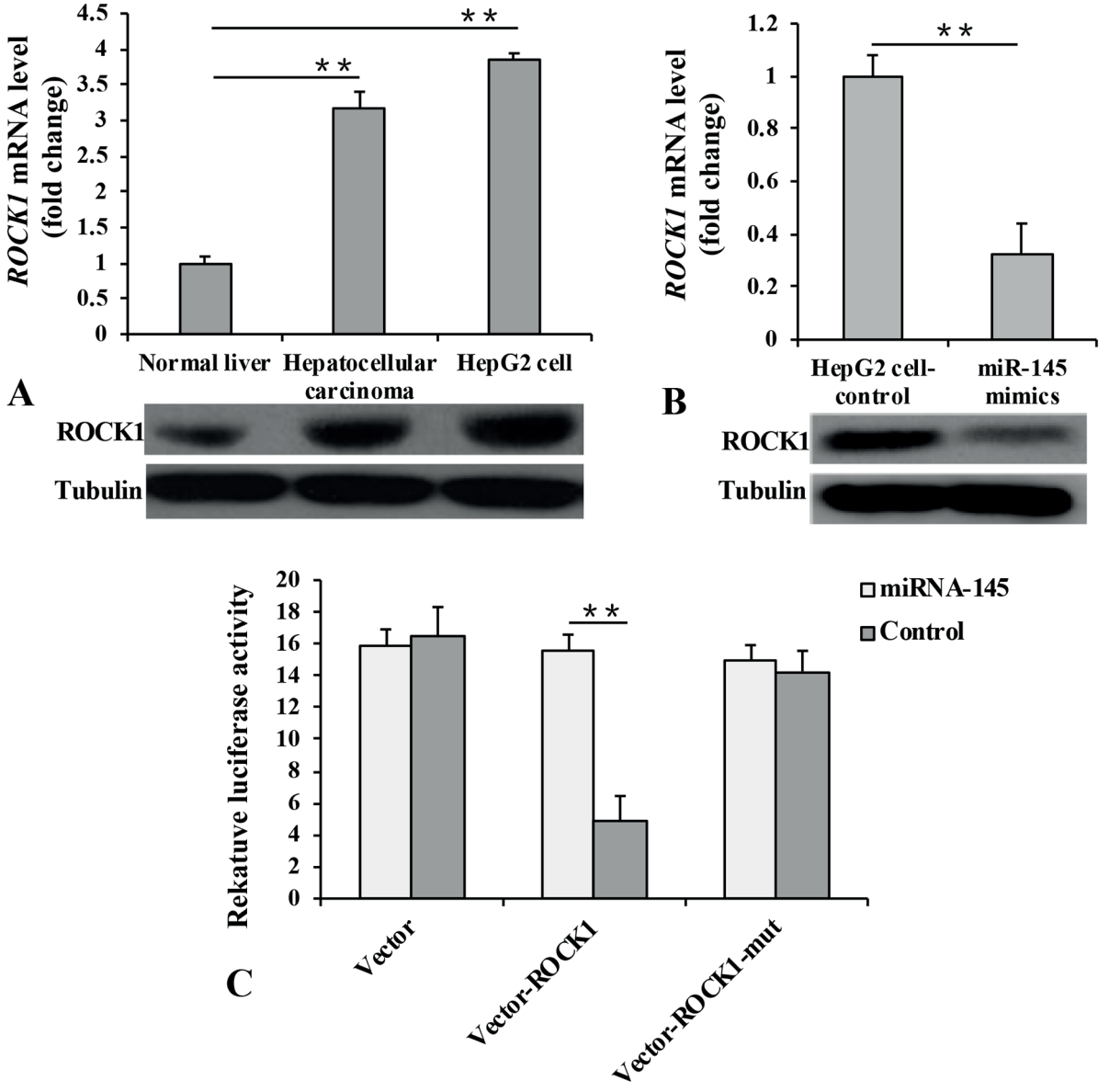

Figure 2. miR-145 directly downregulates the expression of target gene ROCK1 in HepG2 cells. ***A,*** The expression level of ROCK1 by Q-PCR and western blotting in hepatocellular carcinoma tissue, normal liver tissues, and HepG2 cells; ***B,*** The expression changes of ROCK1 by Q-PCR and western blotting in HepG2 cells transfected with miR-145 mimics or not for 48 h; ***C,*** The relative luciferase activity as measured by the dual luciferase system between miR-145 and *ROCK1*. The data are expressed as the mean ± SEM; n=3. $^{**}p < 0.01$.

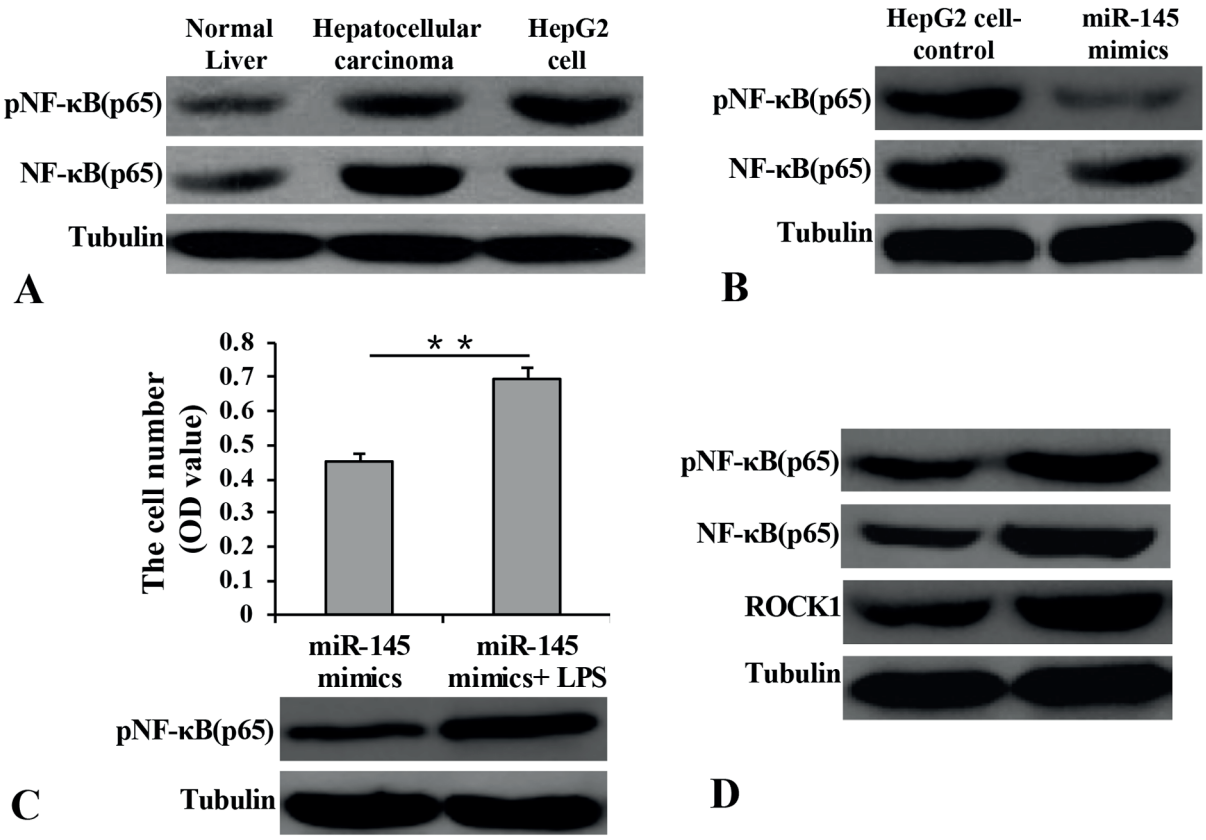

Figure 3. The ROCK1/NF-κB(p65) signaling pathway involved in the regulation of miR-145 and subsequent control of hepatocellular carcinoma. ***A*,** The changes in the NF-κB(p65) level and activity by Western blotting in hepatocellular carcinoma tissue, normal liver tissues, and HepG2 cells; ***B*,** A comparison of the NF-κB(p65) level and activity by Q-PCR and Western blotting in HepG2 cells transfected with miR-145 mimics or not for 48 h; **C:** The changes in the NF-κB(p65) level and activity, and cell number of HepG2 cells with additional LPS on the basis of miR-145 mimics for 2 h or 60 h. **D:** The changes in the NF-κB(p65) level and activity in HepG2 cells after effective overexpression treatment of ROCK1; the data are expressed as the mean ± SEM; n=3 or 8. $*p < 0.05$.

HepG2 cells. The qPCR results revealed that miR-145 expression downregulated HCC tissue and HepG2 cells as compared to normal liver tissue. Further *in vitro* experiments showed that miR-145 expression upregulated HepG2 cells inducted with miR-145 mimics, accompanied by a decrease in the number of HepG2 cells. All of these results showed that miR-145 inhibited the occurrence and development of HCC. It has been reported that microRNAs extensively regulate gene expression by targeting gene-3'UTR to participate in the individual development, cell proliferation, cell apoptosis, cell differentiation, etc.[1,2]. Moreover, the inhibition in the number of HepG2 cells after the upregulation of the miR-145 level was analyzed by FCM to investigate the status of cell proliferation or apoptosis in HepG2 cells after treatment with miR-145 mimics. The percentages of HepG2 cells in S and G1 phases revealed that miR-145 inhibited the G1/S phase transition of HepG2 cells. Additionally, the significant increase in PI-negative and Annexin V-positive cells revealed that miR-145 promoted the apoptosis of HepG2 cells. Genetically, the CCNE1 protein level was significantly downregulated, and the activated C-CASP3 level was markedly increased in HepG2 cells treated with miR-145 mimics compared with control cells without treatment. Considering the important role of CCNE (G1/S-specific cyclin E1) in the cell cycle[20], and the activated C-CASP3 in cell apoptosis[21], it was detected that miR-145 inhibits proliferation and promotes apoptosis of HepG2 cells. The microRNA combines with the 3'-UTR sequence of the target mRNA and regulates the expression level of the target mRNA[22]. As previously reported[8-11], miR-145 plays a broad role in biological action by controlling the different levels of the target protein. The target gene of miR-145

was determined in order to explore how miR-145 implements the regulation of cell proliferation and apoptosis of HepG2 cells. According to the known information on the relationship between miR-145 and ROCK1[23,24], ROCK1 was detected and verified. The results revealed that the expression levels of ROCK1 exhibited the opposite change as those of miR-145 with different status (hepatocellular carcinoma tissue *vs.* normal liver tissue, HepG2 cells *vs.* normal liver tissue, and HepG2 cells *vs.* HepG2 cells transfected with miR-145 mimics). Moreover, further results by dual-luciferase reporter assay provided the direct evidence that ROCK1 is the target gene of miR-145, as previously reported[25]. It is well known that the NF-κB signaling pathway is an important regulator of cell proliferation and apoptosis[26-29] in carcinogenesis, and therefore, the NF-κB activity in the liver cells also was analyzed. Here, the NF-κB(p65) activities were significantly higher in the hepatocellular carcinoma tissues and HepG2 cells as compared to the normal liver tissues, but significantly lower in HepG2 cells transfected with the miR-145 mimics for a significant increase in the miR-145 level, accompanied by the same expression of ROCK1. Based on the transfection with the miR-145 mimics, the NF-κB(p65) activity significantly increased after induction by LPS (as a specific activator of NF-κB[30]), accompanied by an increase in the HepG2 cell number. These results effectively indicated that the NF-κB signaling pathway mediated the effect of miR-145 on cell proliferation and apoptosis of liver cancer cells. In addition, the pEGFP-N3-CDS vector of the ROCK1 gene was used to investigate the relationship between ROCK1 and NF-κB(p65), which were regulated together by miR-145 in this study. After transfection, the ROCK1 expression and NF-κB activity were significantly increased, confirming that ROCK1 and NF-κB(p65) mediated the effect of miR-145 with the joint ROCK1/NF-κB signaling pathway, which was in accordance with previous studies[31,32]. Given all the results together, we propose a molecular regulatory network that inhibits hepatocellular carcinoma cells by miR-145, as presented in Figure 4.

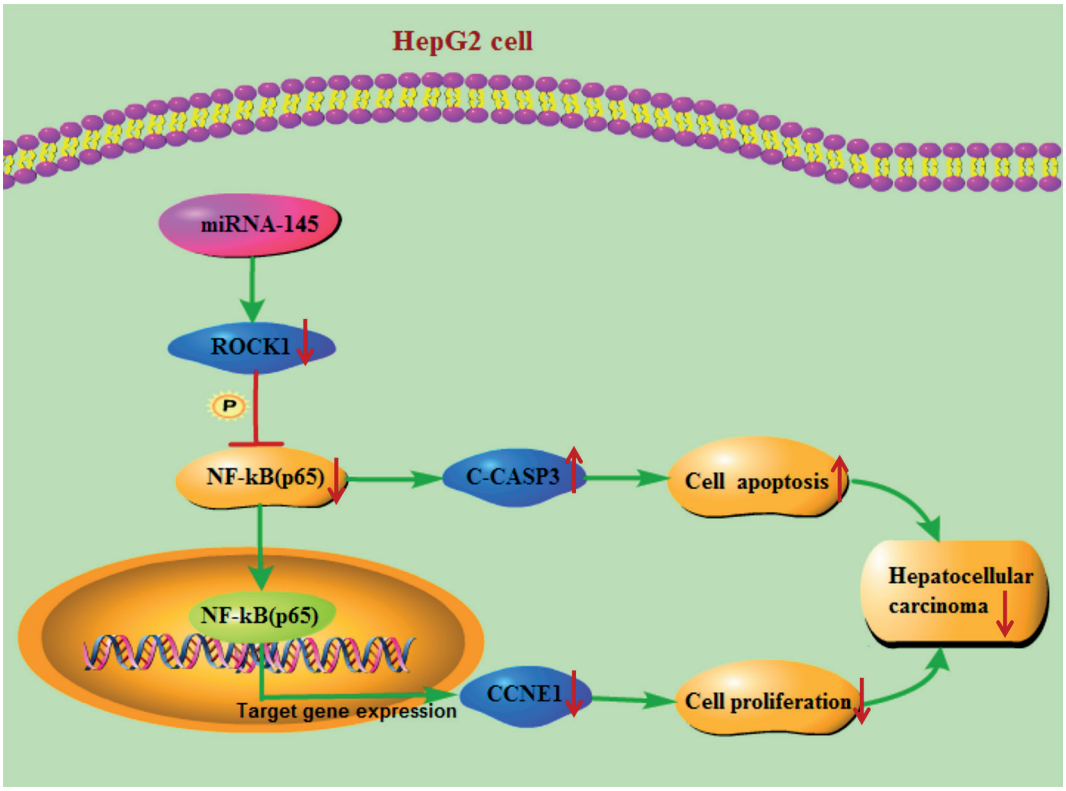

Figure 4. The underlying regulatory mechanism that miR-145 uses to control hepatocellular carcinoma. miR-145 can directly downregulate the expression of the target ROCK1 gene in hepatocellular carcinoma cells. The increase in miR-145 controls hepatocellular carcinoma by inducing cell cycle inhibition and activation of cell apoptosis through the ROCK1/NF-κB signaling pathway.

Conclusions

We showed for the first time that miR-145 directly downregulates the expression of target gene ROCK1 to control hepatocellular carcinoma by inhibiting cell proliferation and activating cell apoptosis through the ROCK1/NF-κB signaling pathway. Our findings suggest a new regulatory mechanism underlying hepatocellular carcinoma, which will provide a perspective for the therapy of hepatocellular carcinoma.

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Authors' contributions

Ruike Wang and Xiaomei Shao contributed to the design and performing of the study, the interpretation of data, and writing of the manuscript. Jiping Yang and Huiling Yan contributed to interpretation of data and writing of the manuscript. Ying Shao contributed to the design of the study. All authors submitted comments on drafts, and read and approved the final manuscript.

Conflict of Interest

The Authors declare that they have no conflict of interest.

References

1) Volk N, Shomron N. Versatility of MicroRNA biogenesis. PLoS One 2011; 6:e19391.
2) Esquela-Kerscher A, Slack FJ. Oncomirs - microRNAs with a role in cancer. Nat Rev Cancer 2006; 6: 259-269
3) Xu L, Beckebaum S, Iacob S, Wu G, Kaiser GM, Radtke A, Liu C, Kabar I, Schmudt HH, Zhang X, Lu M, Cicinnati VR. MicroRNA-101 inhibits human hepatocellular carcinoma progression through EZH2 down regulation and increased cytostatic drug sensitivity. J Hepatol 2014; 60: 590-598.
4) Ezzat WM, Amr KS, Raouf HA, Elhosary YA, Hegazy AE, Fahim HH, Kamel RR. Relationship between serum microRNA155 and telomerase expression in hepatocellular carcinoma. Arch Med Res 2016; 47: 349-355.
5) Zhou Q, Zheng X, Chen L, Xu B, Yang X, Jiang J, Wu C. Smad2/3/4 pathway contributes to TGF-β-induced MiRNA-181b expression to promote gastric cancer metastasis by targeting Timp3. Cell Physiol Biochem 2016; 39: 453-466.
6) Pan XP, Huang LH, Wang X. MiR-370 functions as prognostic marker in patients with hepatocellular carcinoma. Eur Rev Med Pharmacol Sci 2017; 21: 3581-3585.
7) Liu CJ, Yang JH, Huang FZ, Yang JH, Liu CP, Mao XH, Yi WM, Shen XB, Peng C, Chen MF, Jiang B, Wu JS. The role of miR-99b in mediating hepatocellular carcinmoma in vasion and migration. Eur Rev Med Pharmacol Sci 2018; 22: 2273-2281.
8) Chen X, Gong J, Zeng H, Chen N, Huang R, Huang Y, Nie L, Xu , Xia J, Zhao F, Meng W, Zhou Q. MicroRNA145 targets BNIP3 and suppresses prostate cancer progression. Cancer Res 2010; 70: 2728-2738.
9) Shi B, Sepp-Lorenzino L, Prisco M, Linsley P, Deangelis T, Baserga R. Micro RNA 145 targets the insulin receptor substrate-1 and inhibits the growth of colon cancer cells. J Biol Chem 2007; 282: 32582-32590.
10) Singh N, Liu G, Chakrabarty S. Isolation and characterization of calcium sensing receptor null cells: a highly malignant and drug resistant phenotype of colon cancer. Int J Cancer 2013; 132: 1996-2005.
11) Michael MZ, O' Connor SM, van Holst Pellekaan NG, Young GP, James RJ. Reduced accumulation of specific microRNAs in colorectal neoplasia. Mol Cancer Res 2003; 1: 882-891.
12) Sachdeva M, Mo YY. MicroRNA-145 suppresses cell invasion and metastasis by directly targeting mucin 1. Cancer Res 2010; 70: 378-387.
13) Zhang Y, Wen X, Hu XL, Cheng LZ, Yu JY, Wei ZB. Downregulation of miR-145-5p correlates with poor prognosis in gastric cancer. Eur Rev Med Pharmacol Sci 2016; 20: 3026- 3030.
14) Li Y, Liu J, Liu ZZ, Wei WB. MicroRNA-145 inhibits tumour growth and metastasis in osteosarcoma by targeting cyclin-dependent kinase, CDK6. Eur Rev Med Pharmacol Sci 2016; 20: 5117-5125.
15) Ren D, Wang M, Guo W, Zhao X, Tu X, Huang S, Zou X, Peng X. Wild-type p53 suppresses the epithelial-mesenchymal transition and stemness in PC-3 prostatecancer cells by modulating miR-145. Int J Oncol 2013; 42: 1473-1481.
16) Varnholt H, Drebber U, Schulze F, Wedemeyer I, Schirmacher P, Dienes HP, Odenthal M. MicroRNA gene expression profile of hepatitis C virus-associated hepatocellular carcinoma. Hepatology 2008; 47: 1223-1232.
17) Lu J, Getz G, Miska EA, Alvarez-Saavedra E, Lamb J, Peck D, Sweet-Cordero A, Ebert BL, Mak RH, Ferrando AA, Downing JR, Jacks T, Horvitz HR, Golub TR. MicroRNA expression profiles classify human cancers. Nature 2005; 435: 834-838.
18) Voortman J, Goto A, Mendiboure J, Sohn JJ, Schetter AJ, Saito M, Dunant A, Pham TC, Petrini I, Lee A, Khan MA, Hainaut P, Pignon JP, Brambilla E, Popper HH, Filipits M, Harris CC, Giaccone G. MicroRNA expression and clinical outcomes in patients treated with adjuvant chemotherapy after complete resection of non-small cell lung carcinoma. Cancer Res 2010; 70: 8288-8298.
19) Liu J, Zheng M, Tang YL, Liang XH, Yang Q. MicroRNAs, an active and versatile group in cancers. Int J Oral Sci 2011.; 3: 165-175.

20) Li M, Ouyang L, Zheng Z, Xiang D, Ti A, Li L, Dan Y, Yu C, Li W. E3 ubiquitin ligase FBW7α inhibits cholangiocarcinoma cell proliferation by down-regulating c-Myc and cyclin E. Oncol Rep 2017; 37: 1627-1636.

21) Pu X, Storr SJ, Zhang Y, Rakha EA, Green AR, Ellis IO, Martin SG. Caspase-3 and caspase-8 expression in breast cancer:caspase-3 is associated with survival. Apoptosis 2017; 22: 357-368.

22) Ambros V. microRNAs: tiny regulators with great potential. Cell 2001; 107: 823-826.

23) Ding W, Tan H, Zhao C, Li X, Li Z, Jiang C, Zhang Y, Wang L. MiR-145 suppresses cell proliferation and motility by inhibiting ROCK1 in hepatocellular carcinoma. Tumour Biol 2016; 37: 6255-6260.

24) Zheng M, Sun X, Li Y, Zuo W. MicroRNA-145 inhibits growth and migration of breast cancer cells through targeting oncoprotein ROCK1. Tumour Biol 2016; 37: 8189-8196.

25) Chen M, Zhang Y, Li W, Yang J. MicroRNA-145 alleviates high glucose-induced proliferation and migration of vascular smooth muscle cells through targeting ROCK1. Biomed Pharmacother 2018; 99: 81-86.

26) Xia Y, Shen S, Verma IM. NF-κB, an active player in human cancers. Cancer Immunol Res 2014; 2: 823-830.

27) Jiao L, Jiang M, Liu J, Wei L, Wu M. Nuclear factor-kappa B activation inhibits proliferation and promotes apoptosis of vascular smooth muscle cells. Vascular 2018; 1: 1708538118787125.

28) Xia ZB, Meng FR, Fang YX, Wu X, Zhang CW, Liu Y, Liu D, Li GQ, Feng FB, Qiu HY. Inhibition of NF-κB signaling pathway induces apoptosis and suppresses proliferation and angio -genesis of human fibroblast-like synovial cells in rheumatoid arthritis. Medicine (Baltimore) 2018; 97: e10920.

29) Lee J, Park Jr, Lee H, Jang S, Ryu SM, Kim H, Kim D, Jang A, Yang SR. L-carnosine induces apoptosis/cell cycle arrest via suppression of NF-κB/STAT1 pathway in HCT116 colorectal cancer cells. In Vitro Cell Dev Biol Anim 2018; 54: 505-512.

30) Long Y, Wang G, Li K, Zhang Z, Zhang P, Zhang J, Zhang X, Bao Y, Yang X, Wang P. Oxidative stress and NF-κB signaling are involved in LPS induced pulmonary dysplasia in chick embryos. Cell Cycle 2018; 2: 1-15.

31) Miyata K, Satou R, Shao W, Prieto MC, Urushihara M, Kobori H, Navar LG. ROCK/NF-κB axis-dependent augmentation of angiotensinogen by angiotensin II in primary-cultured preglomerular vascular smooth muscle cells. Am J Physiol Renal Physiol 2014; 306: F608- 618.

32) Zhu L, Chen T, Chang X, Zhou R, Luo F, Liu J, Zhang K, Wang Y, Yang Y, Long H, Liu Y, Yan T, Ma C. Salidroside ameliorates arthritis-induced brain cognition deficits by regulating Rho/ROCK/NF-κB pathway. Neuropharmacology 2016; 103: 134-142.