OncoTargets and Therapy

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ORIGINAL RESEARCH

Downregulation of IRAK1 Prevents the Malignant Behavior of Hepatocellular Carcinoma Cells by Blocking Activation of the MAPKs/NLRP3/IL-1β Pathway

This article was published in the following Dove Press journal:
OncoTargets and Therapy

Wei Chen[1]
Tao Wei[1]
Yinghua Chen[2]
Lan Yang[3]
Xiaomin Wu[1]

[1]Department of Oncology, People's Hospital of Huadu District, Guangzhou 510800, People's Republic of China; [2]Department of Histology and Embryology, School of Basic Medical Sciences, Southern Medical University, Guangzhou 510515, People's Republic of China; [3]Department of Laser Beauty, People's Hospital of Huadu District, Guangzhou 510800, People's Republic of China

Introduction: Interleukin-1 receptor-associated kinase 1 (IRAK1) was shown to contribute to a variety of cancer-related processes. However, the function of IRAK1 in hepatocellular carcinoma (HCC) pathogenesis has not been investigated in detail.

Methods: IRAK1 expression in HCC was examined by immunohistochemistry, qRT-PCR, and Western blot assays. In addition, Huh7 and Hep3B cells were transfected with IRAK1 siRNAs and/or a NOD-like receptor family pyrindomain containing 3 (NLRP3) plasmid. Western blot, EdU staining, and Transwell assays were performed to determine changes of apoptosis, proliferation, migration, and invasion in HCC cells. Moreover, changes in the expression of proteins involved in the MAPKs/NLRP3/IL-1β pathway were confirmed by Western blotting.

Results: IRAK1 was found to be highly upregulated in HCC tissues and cells. Knockdown of IRAK1 signaling prevented the proliferation, invasion, migration, epithelial–mesenchymal transition (EMT) of HCC cells. Mechanistically, we found that activation of the MAPKs/NLRP3/IL-1β pathway could be markedly suppressed by IRAK1 knockdown in HCC cells. Furthermore, our data showed that NLRP3 could partially reverse the reduced aggressive biological behaviors of HCC cells which were caused by RAK1 knockdown.

Conclusion: Knockdown of *IRAK1* prevented HCC progression by inhibiting the ability of NLRP3 to block the MAPKs/IL-1β pathway, suggesting that approach as a strategy for treating HCC.

Keywords: hepatocellular carcinoma, interleukin-1 receptor-associated kinase 1, NOD-like receptor family pyrindomain containing 3, epithelial–mesenchymal transition

Introduction

Hepatocellular carcinoma (HCC) is one of the most frequently diagnosed malignant tumors and has the fifth highest incidence and third highest mortality rate of all malignancies worldwide.[1] In China, HCC is also one of the most common malignancies of the digestive system, and accounts for 50% of all new and fatal HCC cases worldwide.[2] The five-year survival rate of HCC patients remains low, due to the disease's occult onset, rapid progression, and early metastasis.[3] While comprehensive treatments that include surgery have achieved better results, the high rates of HCC recurrence and metastasis after surgery remain major obstacles to further improving the prognosis of HCC patients.[4,5] Even a small HCC (<5 cm) has

Correspondence: Wei Chen
Department of Oncology, People's Hospital of Huadu District, Xinhua Road #48, Huadu District, Guangzhou 510800, People's Republic of China
Tel +86 20 86832256
Email 13926260691@163.com

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http://doi.org/10.2147/OTT.S260793

a recurrence rate >50% during a 3-year period after radical resection or liver transplantation.[6] Rapid growth, invasion, and metastasis are important biological characteristics of HCC, and also vital factors that account for the poor prognosis and low 5-year survival rate of patients with HCC.[7] Therefore, it is important for researchers to gain a better understanding of the biological mechanisms that lead to HCC cell proliferation and metastasis.

Interleukin-1 receptor-associated kinases comprise a class of serine-threonine kinases that include IRAK1, IRAK2, IRAK3, and IRAK4.[8] Several studies have confirmed that IRAKs are associated with the pathophysiological processes of metabolic, inflammatory, and malignant diseases.[9,10] IRAK1, as a cytoplasmic and nuclear kinase, has a specific kinase activity.[11] Research has shown that IRAK1 modulates the cascade of proteins involved in the interleukin-1 (IL-1) receptor family signaling pathway, and helps to regulate the secretion of inflammatory factors such as TNF-α.[12] Furthermore, IRAK1 can also mediate multiple cell receptor signals, including those generated by toll-like receptors (TLRs),[13] and also function as a downstream effector of the TLR signaling pathway.[14] Research studies have confirmed that abnormal activation of TLR signaling can result in a variety of human diseases, including cancers.[14,15] In recent years, studies have revealed that IRAK1 is closely associated with various malignant cancers,[11] such as hepatocellular carcinoma[16] and lung cancer,[17] etc. Cheng et al[16] suggested that IRAK1 promotes hepatocellular carcinoma stemness and drug resistance via the AP-1/AKR1B10 signaling pathway.[16] Interestingly, Fernandes-Alnemri T et al[18] showed that IRAK1 activation leads to the activation of NLRP3 inflammasomes.[18] However, the role played by IRAK1 in HCC has not been fully elucidated.

HCC progression is closely related to chronic inflammation, which usually results from hepatitis, cirrhosis or liver neoplasms.[19] The nucleotide-binding oligomerization domain (NOD)-like receptor protein (NLRP) inflammasome is an intracellular multiprotein complex that is closely associated with inflammation, and a crucial component of innate immunity.[20] Recently, more studies have focused on NLRP1, NLRP3, NLRC4, NLRC5, NLRP6, and AIM2 inflammasomes,[20,21] and among those, the NLRP3 inflammasome has been the most thoroughly studied.[22] NLRP3 can form an inflammasome complex by recruiting the precursors of apoptosis-associated speck-like protein containing a CARD (ASC) and caspase-1, eventually resulting in the mature secretion of pro-inflammatory cytokines (IL-1β and IL-18), which mediate the occurrence of inflammation.[23] At present, studies of NLRP3 inflammasomes have mainly focused on innate immune cells, rather than cancer cells.[24] Therefore, further exploration of whether IRAK1 can influence the malignant behavior of HCC via NLRP3 will contribute to a comprehensive understanding of HCC pathogenesis.

In this study, we investigated the biological function and regulatory mechanism of IRAK1 in HCC progression. We demonstrated for the first time that downregulation of IRAK1 could suppress activation of the NLRP3 inflammasome, and prevent the proliferation, migration, and invasion of HCC cells, and accelerate the apoptosis of HCC cells. Additionally, we provide an in-depth discussion of the possible molecular mechanism of IRAK1 in HCC. Our results indicate that the molecular mechanism of IRAK1 in HCC is related to NLRP3 signaling, and suggest IRAK1 as a potential target for treating HCC.

Materials and Methods

Clinical Samples

Samples of cancer tissue and corresponding adjacent tissue were collected from 20 HCC patients at the People's Hospital of the Huadu District. All tissue samples were immediately stored at −80°C after removal. None of the patients had received any preoperative intervention prior to their surgery, and all the specimens were confirmed by a pathological examination performed after the operation. The corresponding adjacent tissues were obtained from locations that were 5 cm away from the HCC tissues, and those tissues were also pathologically confirmed. All patients provided their written Informed Consent for study participation, and the study protocol was approved by the Ethics Committee of the People's Hospital of Huadu District.

Cell Culture

HL-7701 cells and HCC cell lines (Bel-7405, HCCLM3, Huh7, and Hep3B) were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). The cells were cultured in DMEM medium containing 10% fetal bovine serum (FBS; Gibco, cat. no. 10099–141) at 37°C in a 5% CO_2 atmosphere. Cells in logarithmic growth phase were used for all experiments.

Cell Transfection

An NLRP3-overexpression plasmid was purchased from Wuhan Hualian Biotechnology Co. Ltd (Wuhan, China). IRAK1 siRNA#1, IRAK1 siRNA#2, and a negative control

(NC) were purchased from GenePharma (Shanghai, China). Huh7 and Hep3B cells were cultured in 6-well plates (1×10^5 cells/well) and then transfected with IRAK1 siRNAs, the NLRP3 plasmid or the negative control by using Lipofectamine 3000 reagent (Invitrogen, Carlsbad, CA, USA) in accordance with the manufacturer's instructions.

qRT-PCR Assays

TRIzol® reagent (Takara, Japan) was used to isolate the total RNA from HCC tissues and cells. Next, samples of total RNA were reverse transcribed into cDNA by using a Double-Strand cDNA Synthesis kit (Takara) in accordance with the manufacturer's instructions. Gene expression was quantified by performing qRT-PCR assays with SYBR Green PCR Master (Takara) on an ABI StepOnePlus Real-Time PCR system. The relative levels of gene expression were calculated using the $2^{-\Delta\Delta CT}$ method.

Western Blot Assays

RIPA buffer containing 1% protease inhibitor was added to cells or tissues, which were subsequently lysed on ice for 30 mins. The lysed cells or tissues were then centrifuged for 20 mins, and the supernatants were collected. The total protein concentration in each supernatant was determined by the BCA method. Next, a 30 μg aliquot of protein from each sample was separated by 10% SDS-PAGE, and the protein bands were transferred onto PVDF membranes (Millipore, Burlington, MA, USA), which were subsequently blocked with 5% skim milk for 1.5 hrs. Next, the membranes were washed and then incubated overnight at 4°C with the following primary antibodies (1∶1000, Abcam, Cambridge, UK): IRAK1 (Abcam, ab238), E-cadherin (Abcam, ab15148), N-cadherin (Abcam, ab18203), vimentin (Abcam, ab137321), IL-1β (Abcam, ab9722), NLRP3 (Abcam, ab214185), ASC (Abcam, ab227502), ERK1/2 (Abcam, ab17942), JNK1/2 (Abcam, ab112501), p-ERK1/2 (Abcam, ab223500), p-JNK1/2 (Abcam, ab4821), Caspase 3 (Abcam, ab32042), and GAPDH (Abcam, ab9485). The next day, the membranes were washed and then incubated with a 1∶5000 diluted secondary antibody (Abcam) for 2 hrs. The immunostained proteins were detected using an ECL substrate kit (Thermo Scientific, Waltham MA, USA), and results were displayed on a Bio-Rad Gel Doc/Chemi Doc Imaging System.

Immunofluorescence (IF) Assays

Cells in logarithmic growth phase were collected and their total concentration was adjusted. Next, a 1 mL aliquot containing 2×10^5 cells was inoculated into each well of a 6-well plate that had small slides at the bottom, and the cells were incubated for 8 hrs. After washing, 200 μL of 4% paraformaldehyde was added to each slide and the attached cells were fixed for 15 mins. The slides were then washed 3 times with PBS, and the cells were permeated with 0.5% Triton X-100 (Fisher Scientific, Cat. no. BP15) for 20 mins. Next, the slides were sealed with 10% goat serum at 37°C for 30 mins; after which, 30 μL of IRAK1 primary antibody (10 g/mL; Abcam, ab238) was added to the cells on each slide, and the slides were incubated at 4°C overnight. After washing, the cells were incubated with a FITC-conjugated fluorescent secondary antibody (1∶200, Abcam) at 37°C for 60 mins. The cells were then washed again and subsequently stained with DAPI (1:10; Invitrogen, Cat no. D1306) for 5 mins. After sealing, the slides were photographed under an inverted fluorescence microscope.

Flow Cytometry

Transfected Hep3B and Huh7 cells were collected, washed, and the cell density was adjusted to 1×10^6 cells/mL. Next, the cells were labeled by using reagents in an Annexin V-FITC Apoptosis Detection Kit (Cat. no. ALX-850-020-KI02) in accordance with the manufacturer's instructions. The results were analyzed by flow cytometry (FACS Calibur, Becton Dickinson, Franklin Lakes, NJ, USA).

EdU Staining

Cell proliferation was monitored using an EdU kit (Solarbio, cat. no. CA1170). The collected Hep3B and Huh7 cells were incubated with 100 μL of complete medium containing 50 μmol/L EduEdU reagent for 2 hrs. The cells were then fixed and subsequently decolored with 50 μL of glycine solution (2 mg/mL). EdU-positive cells were detected using a fluorescence microscope.

Transwell Assays

Transfected Hep3B and Huh7 cells were suspended in serum-free medium and the total cell concentration was adjusted to 5×10^5 cells/mL. Next, 200 μL of cell suspension was added to the upper chamber of a Transwell plate, and 600 μL of complete medium (10% FBS) was added to the lower chamber. After incubation for 24 hrs, the migrated cells were fixed with 4% paraformaldehyde for 10 mins, and then stained with 0.1% crystal violet solution (Sigma-Aldrich, St. Louis, MO, USA) for 10 mins. Five

visual fields were randomly selected under a microscope, and the number of migrated cells in each field was counted. Assays for cell invasion were performed using the methods described above, with the exception that the Transwell chambers had been pre-coated with 40 μL of Matrigel.

Ethics Approval and Consent to Participate

The protocol for this study was approved by the Ethics Committee of the People's Hospital of Huadu District. Written informed consent was obtained from each participant.

Statistical Analysis

All data were analyzed using IBM SPSS Statistics for Windows, Version 19 (IBM Corp., Armonk, NY, USA). Results are shown as the mean value ± SD. A P-value <0.05 was considered to be statistically significant.

Results

IRAK1 Was Upregulated in HCC Tissues and Cells

A review of the Kaplan Meier Plotter database (http://kmplot.com/analysis/) revealed that HCC patients with a high level of IRAK1 expression had a shorter overall survival time than patients with a low level of IRAK1 expression, suggesting that high expression of IRAK1 was indicative of a poor prognosis for patients with HCC (Figure 1A). To further examine the change in IRAK1 expression in HCC, samples of HCC and adjacent tissues were collected from HCC patients. First, IRAK1 expression was examined by using the online database tool, The

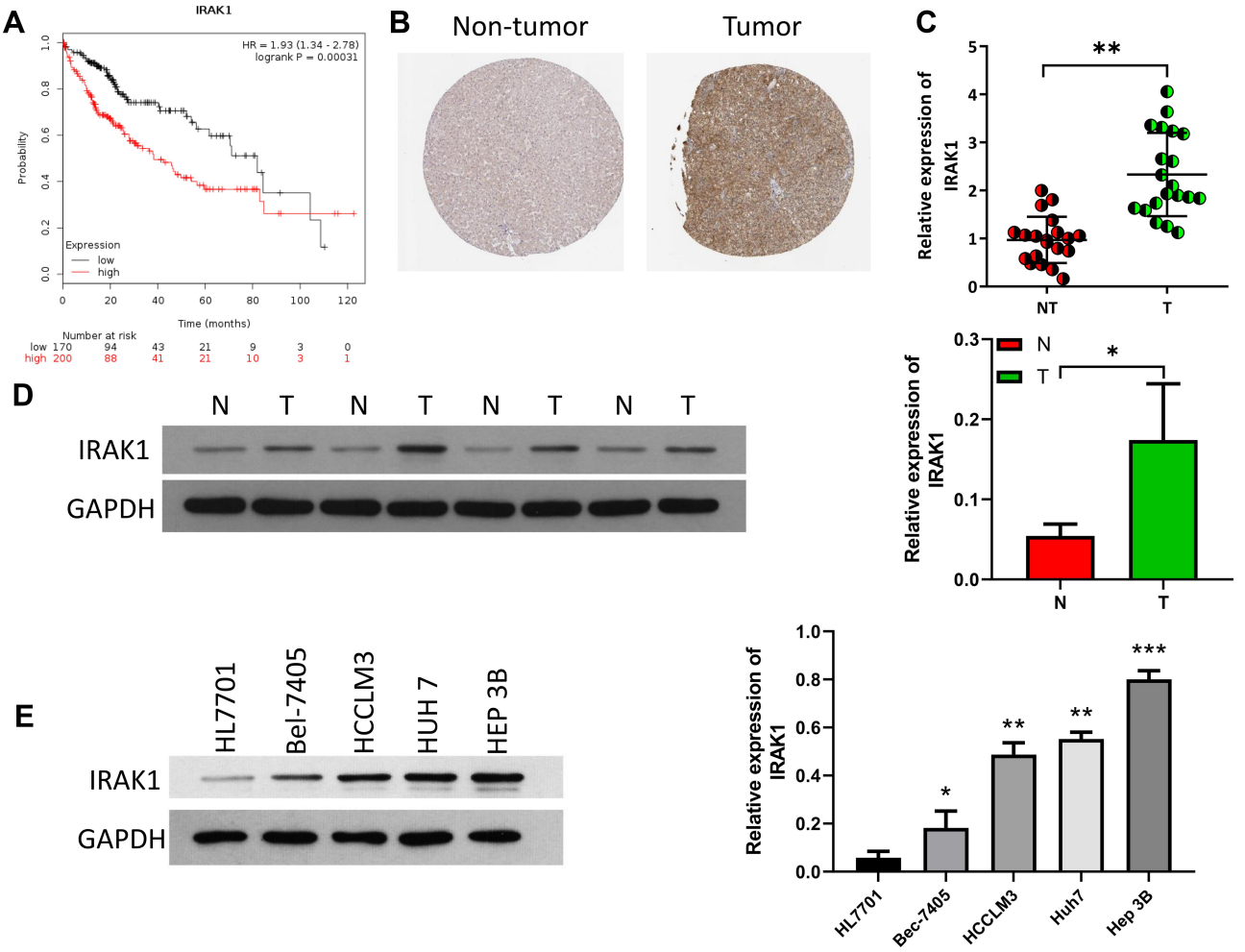

Figure 1 IRAK1 was significantly upregulated in HCC cells and tissues. (**A**) Kaplan–Meier Plotter was used to analyze how IRAK1 expression affected the prognosis of HCC. (**B**) IHC assays were performed to confirm IRAK1 expression in HCC and normal tissues based on information in an online database. (**C**) The levels of IRAK1 mRNA in 20 pairs of HCC tissue were determined by qRT-PCR. (**D**) Western blotting analysis of IRAK1 expression in HCC tissues. (**E**) IRAK1 expression in HCC cells was monitored by Western blotting. *P < 0.05, **P < 0.01, ***P < 0.001.

Human Protein Atlas (https://www.proteinatlas.org/). Those data revealed that the levels of IRAK1 were significantly higher in HCC tissues than in normal tissues (Figure 1B). Second, our qRT-PCR results showed that IRAK1 was more highly expressed in HCC tissues than in adjacent tissues ($P < 0.01$, Figure 1C). In agreement with those findings, our Western blot analysis showed that the levels of IRAK1 protein were strikingly elevated in HCC tissues (Figure 1D). Furthermore, there was a remarkable increase in IRAK1 expression in HCC cell lines (Bel-7405, HCCLM3, Huh7 and Hep3B), and especially in Huh7 and Hep3B cells, when compared to HL-7701 cells (Figure 1E). Taken together, these data proved that IRAK1 was upregulated in HCC.

Knockdown of IRAK1 Repressed the Proliferation, Invasion, Migration, and EMT of HCC Cells, and Induced the Apoptosis of HCC Cells

We next examined the possible functions of IRAK1 in HCC. We first used two siRNAs to knockdown *IRAK*1 in Huh7 and Hep3B cells, and transfection efficiency was verified via Western blotting. When compared to cells transfected with the NC, transfection with IRAK1 siRNAs significantly inhibited IRAK1 expression in Huh7 and Hep3B cells, and especially transfection with siRNA#1 (Figure 2A). Meanwhile, immunofluorescence assays showed that knockdown of IRAK1 by siRNAs markedly downregulated IRAK1 expression, and that IRAK1 protein was mainly located in the cytoplasm of Huh7 and Hep3B cells (Figure 2B). Furthermore, flow cytometry studies showed that knockdown of IRAK1 facilitated the apoptosis of Huh7 and Hep3B cells ($P < 0.05$, Figure 2C). EdU assays indicated that knockdown of *IRAK1* reduced the proliferation of both Huh7 and Hep3B cells (Figure 2D). Moreover, results of Transwell assays showed that the numbers of migrated and invaded Huh7 cells were significantly decreased following transfection with IRAK1 siRNAs, and particularly after transfection with siRNA#1; simultaneously, the migration and invasion capabilities of Hep3B cells were also significantly reduced by IRAK1 knockdown ($P < 0.05$, Figure 2E–H). We next explored the influence of IRAK1 knockdown on the EMT process. As expected, knockdown of IRAK1 significantly upregulated the level of E-cadherin expression (an epithelial marker) and significantly downregulated the levels of N-cadherin and vimentin expression (mesenchymal markers) (Figure 2I). Thus, our results showed that downregulation of IRAK1 could significantly inhibit HCC progression.

Knockdown of *IRAK1* Suppressed Activation of the NLRP3 Inflammasome Pathway in HCC

Western blot assays were performed to further examine the latent molecular mechanisms by which IRAK1 was involved in HCC progression. As shown in Figure 2J, we found that knockdown of *IRAK1* by siRNAs notably downregulated IL-1β, NLRP3, p-ERK1/2, and p-JNK1/2 expression in Huh7 and Hep3B cells. Also, expression of Caspase 3 was also upregulated various to IRAK1 knockdown. As a consequence, we speculated that downregulation of IRAK1 might inhibit the MAPKs/NLRP3/IL-1β pathway in HCC cells.

NLRP3 Reversed the Antitumor Effect of IRAK1 Knockdown in HCC Cells

Next, we investigated whether IRAK1 knockdown-mediated inhibition of malignant behavior in HCC cells requires NLRP3. After co-transfecting Huh7 and Hep3B cells with IRAK1 siRNAs and the NLRP3 plasmid, our Western blot results showed that overexpression of NLRP3 could dramatically attenuate the downregulation of IRAK1 expression that was mediated by IRAK1 siRNAs in Huh7 and Hep3B cells (Figure 3A). Subsequently, a series of functional verification experiments were carried out with Huh7 and Hep3B cells. First, flow cytometry data showed that downregulation of IRAK1 significantly increased the apoptosis rates of Huh7 and Hep3B cells, and overexpression of NLRP3 could attenuate the effect of IRAK1 downregulation ($P < 0.05$, Figure 3B). Second, downregulation of IRAK1 expression in Huh7 and Hep3B cells resulted in the inhibition of cell proliferation as detected by EdU staining, and that effect could also be blocked by NLRP3 overexpression (Figure 3C). Next, we found that downregulation of IRAK1 significantly repressed the migration and invasion of Huh7 and Hep3B cells, while that repressive effect could be partially reversed by NLRP3 overexpression ($P < 0.05$, Figure 3D-3G). Moreover, knockdown of IRAK1 upregulated E-cadherin levels and downregulated N-cadherin and vimentin levels in Huh7 and Hep3B cells, and those changes in EMT-related markers could also be partially inhibited by NLRP3 overexpression (Figure 3H). These findings strongly suggested that the anti-cancer effect of IRAK1 knockdown might be achieved by regulating NLRP3.

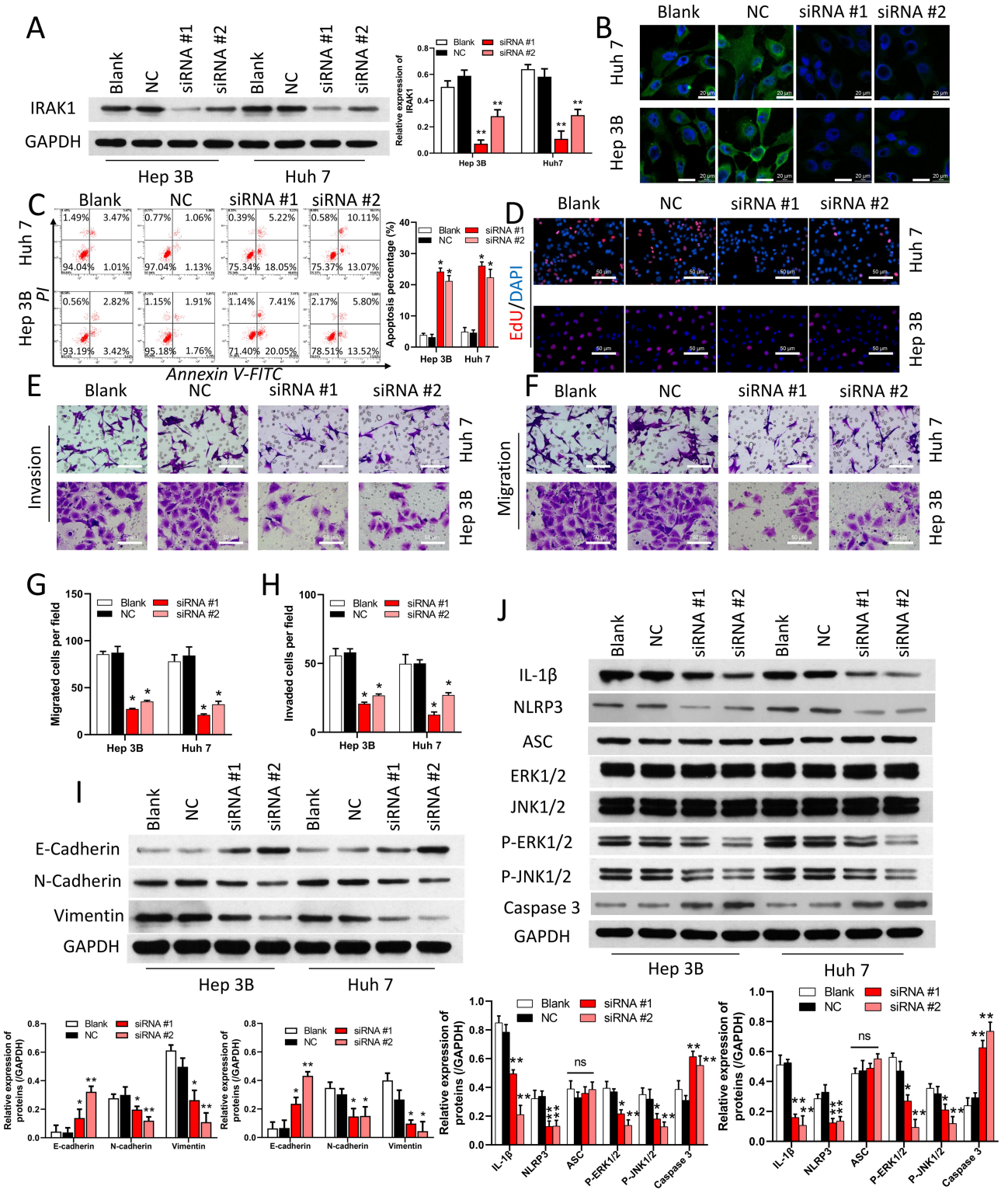

Figure 2 Knockdown of IRAK1 inhibited the proliferation, invasion, migration, and EMT of HCC cells, activation of the NLRP3 inflammasome pathway in HCC cells. Huh7 and Hep3B cells were transfected with specific siRNAs targeting IRAK1 and a NC siRNA, respectively. (**A**) The effect of transfection with IRAK1 siRNAs was determined by Western blotting. (**B**) IRAK1 expression in Huh7 and Hep3B cells was also confirmed by IF assays. Magnification, x200; Scale bar = 50 μm. (**C** and **D**) EdUstaining showed that IRAK1 knockdown resulted in a reduction in the ability of cells to proliferate. Magnification, x100; Scale bar = 100 μm. (**E** and **F**) Transwell assay results are displayed as the numbers of invaded and migrated cells per microscopic field. Magnification, x200; Scale bar = 50 μm. (**G** and **H**) The numbers of invaded and migrated cells were calculated based on results from 5 different microscopic fields. (**I**) Western blot assays were performed to monitor the effect of IRAK1 knockdown on the expression of EMT marker proteins (E-cadherin, N-cadherin, and vimentin). (**J**) Western blot assays were performed to detect how IRAK1 knockdown affected the levels of IL-1β, NLRP3, ASC, ERK1/2, JNK1/2 and Caspase 3 expression in Huh7 and Hep3B cells. *$P < 0.05$, **$P < 0.01$, vs NC. ns, no significance.

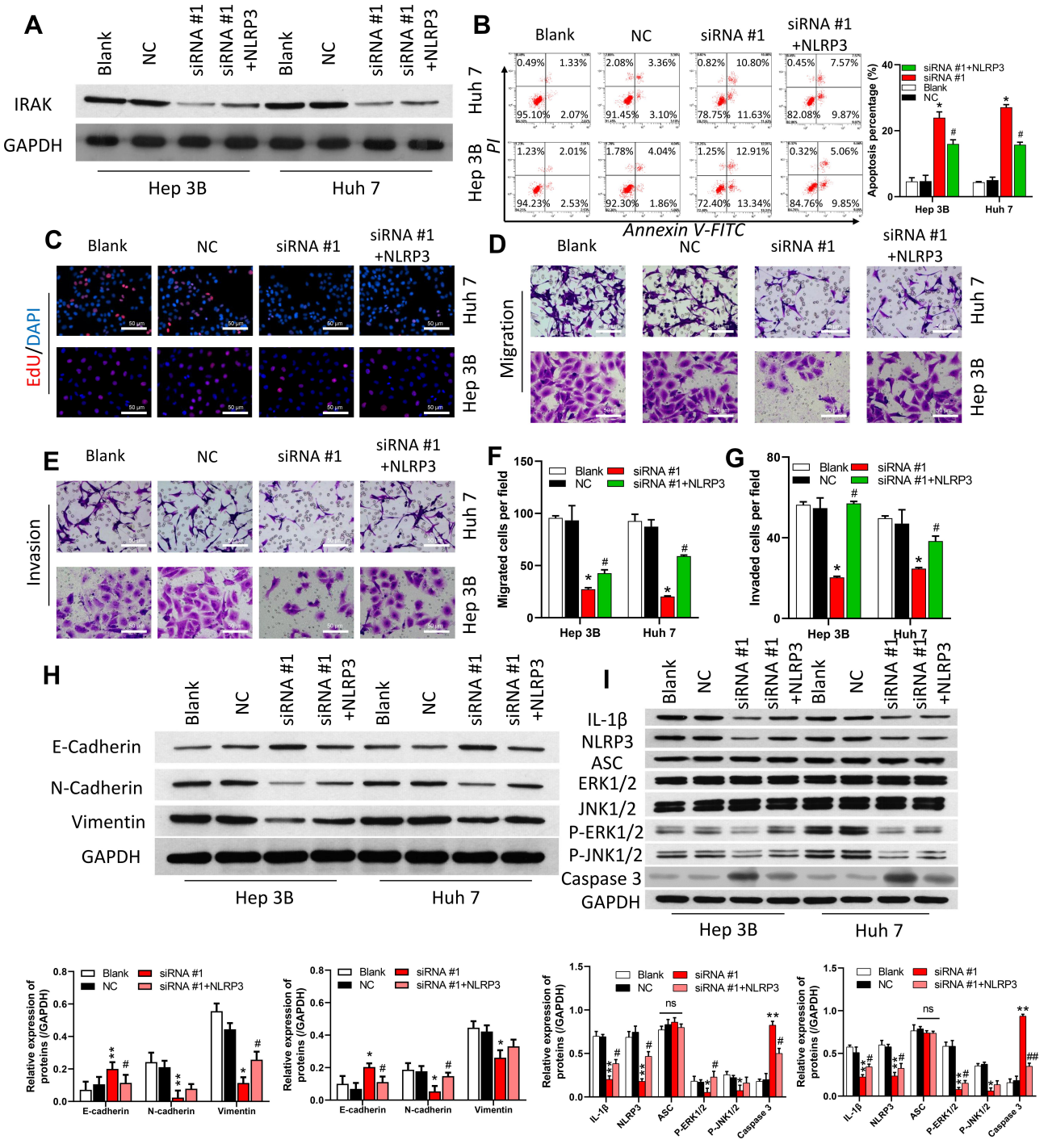

Figure 3 NLRP3 reversed the antitumor and downstream pathway inhibitory effects of IRAK1 knockdown in HCC cells. Specific IRAK1 siRNAs and a NLRP3 plasmid were co-transfected into Huh7 and Hep3B cells. (**A**) Western blot analyses were performed to evaluate IRAK1 expression in the co-transfected Huh7 and Hep3B cells. (**B** and **C**) Representative images showing cell proliferation as detected by EdU staining. Magnification, x100; Scale bar = 100 μm. (**D** and **E**) Transwell assays were performed to assess changes in the invasion and migration capabilities of co-transfected HCC cells. (**F** and **G**) We counted the numbers of invaded and migrated cells in each group as determined by Transwell assays. (**H**) Western blot analyses of E-cadherin, N-cadherin, and vimentin expression in Huh7 and Hep3B cells. (**I**) The levels of IL-1β, NLRP3, ASC, ERK1/2, JNK1/2 and Caspase 3 expression were confirmed by Western blot assays. $^{*}P < 0.05$, $^{**}P < 0.01$, vs NC; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, vs siRNA #1. ns, no significance.

Knockdown of *IRAK1* Blocked the MAPKs/IL-1β Pathway in HCC Cell via NLRP3

A Western blot analysis was performed to further investigate the regulatory effect of IRAK1-mediated NLRP3 on the MAPKs/IL-1β pathway in HCC cells. As shown in Figure 3I, the levels of IL-1β, NLRP3, p-ERK1/2, and p-JNK1/2 expression were significantly downregulated in the IRAK1 siRNAs group when compared to their levels in the NC group, while the expression levels of those proteins were significantly upregulated in the IRAK1 siRNAs plus NLRP3 group when compared to the IRAK1 siRNAs group. However, we also found that Caspase 3 expression was promoted by IRAK1 knockdown, and was reversed by NLRP3 overexpressing based on IRAK1 knockdown. Above all, we demonstrated that the apoptosis, proliferation, migration, invasion, and EMT of Huh7 and Hep3B cells might be affected by the regulatory axis of IRAK1/NLRP3/MAPKs/IL-1β.

Discussion

IRAK1 is a key molecule in the TLR-mediated signaling pathway.[13] The activation of TLR molecules can induce the synthesis of MYD88, which results in activation of IRAK1 and its downstream signaling pathways.[13] As a receptor protein kinase, IRAK1 significantly affects the inflammatory response by regulating multiple genes associated with inflammation.[25] Inflammation has been confirmed to play two opposing roles in cancer progression; as it promotes an anti-tumor immune response, but also increases the proliferation and metastasis of cancer cells.[26–28] Several recent studies have found that IRAK1 plays a significant role in promoting cancer progression.[11,25] For example, IRAK1 signaling was shown to promote the proliferation and inhibit the apoptosis of non-small cell lung cancer cells.[17] Other studies have suggested that high levels of IRAK1 might be associated with cancer cell metastasis and an increased risk for developing lung cancer.[29] Furthermore, another study showed that IRAK1 might promote the growth, metastasis, and paclitaxel resistance of triple-negative breast cancer.[30] More importantly, research has also verified that a high level of IRAK1 expression is associated with a poor survival rate of HCC patients.[16] We further discovered that IRAK1 expression was obviously upregulated at both the mRNA and protein levels in HCC cells and tissues.

Uncontrolled cell proliferation and inhibition of apoptosis are the most crucial features of malignant cancers.[31–33] Additionally, the ability to migrate and invade normal tissues is an important characteristic of metastatic cancer cells, and also reasons for the poor prognosis of patients with malignant tumors.[34] EMT, as a frequent pathological phenomenon, is relevant to the process of cancer invasion and metastasis.[35] Activation of the EMT process is a key step that must be taken for epithelial tumor cells to acquire a malignant phenotype.[36] Furthermore, EMT is a reversible process, meaning that EMT reversal (MET) can restore epithelial polarity to cells and may prevent the migration and metastasis of cancer cells.[37] In our study, we revealed that knockdown of IRAK1 could abolish the aggressive proliferation, invasion, migration, and EMT of HCC cells, and enhance their rate of apoptosis. Therefore, we showed that downregulation of IRAK1 could significantly repress the malignant behavior of HCC.

As a multiprotein complex, an inflammasome can be activated by a variety of different exogenous pathogens and endogenous molecules, and then induce the processing and maturation of IL-1β, IL-18, and IL-33 in cells via caspase-1. That mechanism allows inflammasomes to participate in multiple types of immune responses.[38,39] Inflammasomes are protein complexes that play important roles in regulating inflammation and cell death, both of which play critical roles in the development of immune inflammatory diseases.[40,41] The NLRP3 inflammasome is one of the most well-studied inflammasomes, and can recruit ASC and procaspase-1 to form inflammatory complexes through PYD domains.[42] Researchers have reported that NLRP3 can mediate inflammatory responses in a variety of diseases, including atherosclerosis, type 2 diabetes, arthrolithiasis, obesity-related kidney disease, and cancers.[43–46] Recent studies have also demonstrated that the NLRP3 inflammasome contributes to the development of HCC, and that NLRP3 can be considered as a potential target for treating HCC.[47,48] Our study further demonstrated that knockdown of *IRAK1* could significantly downregulate both NLRP3 and IL-1β expression in Huh7 and Hep3B cells. Given the vital role of NLRP3 and its dependency on IRAK1 in HCC progression, a series of recovery experiments were performed. Those results showed that the antitumor effect of IRAK1 knockdown in HCC was achieved by regulating NLRP3.

Another major finding of our research was the potential regulatory mechanism of IRAK1 in HCC. MAPKs comprise a family of serine/threonine protein kinases that regulate many basic biological processes and the cellular immune response.[49] MAPKs mainly include p38,

extracellular signal-regulated kinase (ERK1/2), and several c-Jun N terminal kinases (JNKs).[50] During inflammatory signal transduction, MAPKs can transfer extracellular signals to cells and nuclei, and conduct signal transduction via cascade reactions.[51] MAPKs are also involved in cell proliferation, differentiation, apoptosis, and other physiological responses.[52,53] In our study, we first showed that knockdown of IRAK1 could significantly downregulate p-ERK1/2 and p-JNK1/2 expression in HCC cells, and that effect was realized by regulation of NLRP3. Therefore, we speculated that the promoting effect of IRAK1 on malignant HCC behaviors might be related to the MAPKs/NLRP3/IL-1β pathway.

In conclusion, our study suggests that knockdown of IRAK1 can inhibit the activation of ERK/JNK-NLRP3 inflammasomes, and thereby prevent the malignant progression of HCC. Therefore, IRAK1, NLRP3, and MAPKs can be considered as possible biological targets for treating HCC.

Acknowledgment

This study was supported by Guangzhou Science and Technology Project (2021-02-08-14-1008-0004).

Disclosure

All authors declare no conflicts of interest in this work.

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