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WJH mainly publishes articles reporting research results and findings obtained in the field of hepatology and covering a wide range of topics including chronic cholestatic liver diseases, cirrhosis and its complications, clinical alcoholic liver disease, drug induced liver disease autoimmune, fatty liver disease, genetic and pediatric liver diseases, hepatocellular carcinoma, hepatic stellate cells and fibrosis, liver immunology, liver regeneration, hepatic surgery, liver transplantation, biliary tract pathophysiology, non-invasive markers of liver fibrosis, viral hepatitis.

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ORIGINAL ARTICLE

Basic Study

Hypoxia upregulates hepatic angiopoietin-2 transcription to promote the progression of hepatocellular carcinoma

Jun-Ling Yang, Jie Yang, Rong-Fei Fang, Wen-Li Sai, Deng-Fu Yao, Min Yao

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Abstract

BACKGROUND

Angiopoietin-2 (Ang-2) level is related to hepatocellular carcinoma (HCC) progression. However, the dynamic expression and regulatory mechanism of Ang-2 remain unclear.

AIM

To investigate Ang-2 levels in chronic liver diseases and validate early monitoring value with a dynamic model in hepatocarcinogenesis.

METHODS

Sprague-Dawley rats in hepatocarcinogenesis were induced with diet 2-fluorenylacet-amide, and grouped based on liver histopathology by hematoxylin and eosin staining. Differently expressed genes or *Ang-2* mRNA in livers were analyzed by whole-genome microarray. Ang-2 levels in chronic liver diseases were detected by an enzyme-linked immunosorbent assay.

RESULTS

Clinical observation reveled that the circulating levels of Ang-2 and hypoxia-inducible factor-1α (HIF-1α) in patients with chronic liver diseases were progressively increased from benign to HCC ($P < 0.001$). Dynamic model va-

lidated that the up-regulated Ang-2 in liver and blood was positively correlated with HIF-1α in hepatocarcinogenesis ($P < 0.001$). Mechanistically, Ang-2 was regulated by HIF-1α. When specific HIF-1α- microRNAs transfected into HCC cells, the cell proliferation significantly inhibited, HIF-1α and Ang-2 down-regulated, and also affected epithelial-mesenchymal transition *via* increasing E-cadherin to block cell invasion or migration with reducing of snail, twist and vimentin.

CONCLUSION

Hypoxia-induced Ang-2 up-regulating expression might serve as a sensitive early monitoring biomarker for hepatocarcinogenesis or HCC metastasis.

Key Words: Angiopoietin-2; Hypoxia-inducible factor-1α; Hepatocarcinogenesis; Dynamic model; Metastasis

Core Tip: In this study, the expression of angiopoietin-2 (Ang-2) in the malignant progression of chronic benign liver diseases was investigated, and its value in monitoring hepatocellular carcinoma (HCC) progression in a dynamic rat model was validated. In a hypoxic environment, Ang-2 activation is associated with the malignant transformation of hepatocytes or the metastasis of HCC cells. Mechanistically, the down-regulation of hypoxia-inducible factor-1α transcription inhibited Ang-2 expression and the epithelial-mesenchymal transition in HCC. These results suggest that there are positive correlations between hypoxia-inducible factor-1α and Ang-2 in benign liver diseases and hepatocyte malignant progression, suggesting that abnormal Ang-2 expression might be a sensitive early biomarker for monitoring the occurrence or metastasis of HCC.

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INTRODUCTION

Up to now, hepatocellular carcinoma (HCC) remains one of the most common cancers in the world[1]. Also, it is still one of the most malignancy in the inshore area of the Yangtze River, Qidong, China, because of patients with high hepatitis B virus (HBV) infection-related chronic liver diseases[2]. In clinic, advanced liver cancers are no other effective treatments beyond sorafenib or multitarget tyrosine kinase inhibitors due to liver tissue hypoxia enhancing cell proliferation with angiogenesis or suppressing cell differentiation or apoptosis, leading to multi-drug resistance (MDR) to transarterial chemoembolization[3,4]. Hypoxia-inducible factor-1 (HIF-1) is a heterodimer from α- and β-subunits, the former tight regulation with O_2 alterations, and the latter constitutive expression in tissues. Although HIF-1α primarily involves protein ubiquitination, it in liver is an important role in angiogenesis transcriptional response *via* different molecular mechanisms such as genes of growth factors, cancer suppressors, oncogenes and epithelial-mesenchymal transition (EMT) formation[5,6]. After HIF-1α activation, it might regulate a repertoire of vascular endothelial growth factor (VEGF), signalling pathways[7] and angiopoietin-2 (Ang-2) for tumor angiogenic formation[8]. Those data indicated that hepatic HIF-1α might play important roles in expressions of angiogenic factors and might be prime molecular targeted for HCC anti-angiogenesis therapy[9,10].

Recently, abnormal HIF-1α expression at early stages of hepatocyte malignant transformation has been reported, and the up-regulated HIF-1α level might be related to angiogenesis in hepatocarcinogenesis[11,12]. HIF-1α and VEGF play important roles in the dynamic changes and correlation of HCC after transarterial chemoembolization[13]. Anti-angiogenic therapy is beneficial for patients with HCC following surgical resection of the tumor. Hepatic HIF-1α overexpression under hypoxic microenvironment in HCC tissues might affect the biological behaviors of cancer cells, synergize with related-signaling pathways, and endogenously stimulate angiogenic growth factors required, therefore, HCC treatments has not achieved satisfactory results[14-16]. However, the exact relationship between HIF-1α and angiogenesis in HCC occurrence remains to be explored. The aims of this study were to investigate the levels of circulating HIF-1α and Ang-2 in the progression of patients with chronic benign liver disease to HCC, their dynamic alterations in a rat model of hepatocarcinogenesis, and their regulatory mechanism and clinical values for HCC formation or metastasis.

MATERIALS AND METHODS

Patient recruitment

This study was approved by the Ethics Committee of the Affiliated Hospital of Nantong University, China (No. 2018-

L015). A total of 80 patients with HCC were included (Table 1) from May 2019 to October 2021 at the Affiliated Hospital of Nantong University in China. The other cases studied included 50 patients with liver cirrhosis (LC), 50 patients with chronic hepatitis (CH), and 50 healthy controls who were serum negative for HBV markers (hepatitis B surface antigen, hepatitis B core antibody, and HBV-DNA) or anti-hepatitis C virus antibodies and had normal liver function from the Nantong Central Blood Bank, China. Written informed consent was obtained from all patients, with complete medical records and follow-up data. The diagnosis of HCC was made according to the standards of the National Cancer Cooperative Group[17] and confirmed by B-ultrasonography, computed tomography or magnetic resonance imaging, α-fetoprotein detection and postoperative histopathology. Diagnosis of viral hepatitis was based on the guidelines of prevention and treatment for chronic hepatitis B (2019 version)[18].

Table 1 Serum angiopoietin-2 and hypoxia-inducible factor-1α levels in patients with chronic liver diseases

Group	*n*	Ang-2		HIF-1α	
		mean ± SD (μg/L)	> 35 μg/L, *n* (%)	mean ± SD (μg/L)	> 100 μg/L, *n* (%)
HCC	80	42.9 ± 5.1	162 (90.0)	145.6 ± 32.6	163 (90.6)
LC	50	26.2 ± 6.1[a]	3 (6.0)[a]	79.5 ± 28.4[b]	14 (28.0)[b]
CH	50	21.8 ± 6.9[b]	2 (4.0)[b]	60.1 ± 18.8[b]	1 (2.0)[b]
NC	50	16.9 ± 2.8[b]	0 (0.0)[b]	23.9 ± 4.2[b]	0 (0.0)[b]

[a]$P < 0.05$, compared with the hepatocellular carcinoma group.
[b]$P < 0.01$, compared with the hepatocellular carcinoma group.
Ang-2: Angiopoietin-2; HIF-lα: Hypoxia-inducible factor-1α; NC: Normal control; HCC: Hepatocellular carcinoma; LC: Liver cirrhosis; CH: Chronic hepatitis.

Hepatocarcinogenesis model

The use of the rat model was approved by the guidelines of the Animal Care and Use Committee of Nantong University (No. 20190304-001), China. A total of 48 4-week-old Sprague-Dawley rats obtained from the Animal Center of Nantong University were generated for the hepatocarcinogenesis model[11] in a clean environment with a 12-hour light/dark cycle and 55% humidity. The control rats (n = 12) were fed a normal diet, and the model rats (n = 36) were fed a 0.05% 2-fluoreneacetylamino (Sigma) diet and checked every day. After the rats were sacrificed at different times, the livers and blood were collected for analysis. Livers were diagnosed by pathological examination with hematoxylin and eosin (H&E) or Ang-2 and HIF-1α immunohistochemical (IHC) staining and were grouped by two independent pathologists.

Differentially expressed genes

Dynamic alterations in whole gene expression profiles were detected with an Affymetrix GeneChip® Rat Genome 230 2.0 Array (28000 genes, YESLAB, Shanghai, China), and the levels of Ang-2 and HIF-1α in the liver tissue supernatant and serum were quantitatively detected by enzyme-linked immunosorbent assays (ELISAs).

Liver IHC analysis

Rat liver tissues used for IHC analysis were deparaffinized. Peroxidase was quenched with methanol and 3% H_2O_2 for 15 minutes. For antigen retrieval, liver sections were boiled under pressure in citrate buffer (pH = 6.0) for 3 minutes. Then, the tissues were incubated with primary rabbit anti-rat HIF-1α (NeoMarkers, United Kingdom) or anti-rat Ang-2 (Abcam, United States) antibodies, which were diluted in 1% bovine serum albumin (1:300), for 1 hour. Following washing with phosphate-buffered saline, the sections were incubated with peroxidase-conjugated goat anti-rabbit antibody (Dako Cytomation, United States) for 15 minutes and then washed again with phosphate-buffered saline. Color was developed by 15 minutes of incubation with diaminobenzidine solution (Kem-En-Tec Diagnostics, Denmark), and the sections were weakly counterstained with hematoxylin. Primary antibodies were omitted for the negative controls.

HIF-1α miRNA plasmid DNA

According to human *HIF-1α* gene sequence[19], short hairpin-expressing plasmid DNAs were constructed from pcDNA™6.2-GW/EmGFPmiR vector and BLOCK-iT™ Pol II miR RNAi Expression Vector Kit (Invitrogen, United States). Targeting *HIF-1α* genes are as follows: 5′-TGCTGTAAAGCATCAGGTTCCTTCTTGTTTTGGCCACTGACT-GACAAGAAG GACTGATGCTTTA-3′ and 5′-CCTGTAAAGCATCAGTCCTTCTTGTCAGTCAGTGGCCAAAAC-AAGAAGGAACCTGATGCTTTAC-3′. *HIF-1α* gene fragments were purified and confirmed by the MegaBACE 1000 Sequencing and Analysis System using DYEnamic™ ET Dye Terminator Cycle Sequencing Kit (Amersham Biosciences, Little Chalfont, United Kingdom) according to manufacturer's instructions.

Cell culture and transfection

Based on the previous reported[20], the strongest HIF-1α expressions in HepG2 cell lines were compared with the average

ratios from HIF-1α to β-actin, and both from the Chinese Academy of Sciences (Shanghai, China) were chosen for further investigation. HCC cell lines were cultured in RPMI-1640 (Gibco BRL, Gaithersburg, MD, United States) containing 10% FCS (Gibco BRL, United States), 2.0 mmol/L L-glutamine, 100 U/mL penicillin and 100 μg/mL streptomycin in a constant environment (37 °C, 10% CO_2 and 10% humidity).

Plasmid transfection

Cells were cultured on 6-well plates and divided into blank control (Con), negative microRNA (Neg) and microRNA (MiR) groups. Cells (5×10^3) in the MiR or Neg group were transfected with HIF-1α microRNA or negative microRNA according to the instructions of related-reagent kit (Roche, Germany).

Cell migration or invasion assay

Quantitative and qualitative analysis of HepG2 cells migration were assessed by *in vitro* Transwell assay with modified Boyden Chambers and Transwell-coated Matrigel membrane filter (BD Biosciences, Bedford, MA, United States). Cells (5×10^3) from Con, Neg, and MiR groups (n = 3/group) were plated onto the upper compartment in without foetal bovine serum or 10% foetal bovine serum in the lower chamber as a chemoattractant. Fluorescent images of nuclear Hoechst staining (10 μg/mL) were captured at 24 hours of incubation in a 5% CO_2 humidified at 37 °C. Percentages of migrated cells in each group were counted from 10 random microscope fields for each sample in 3 independent experiments. For cell migration analysis, the modified Boyden Chambers without the Transwell-precoated Matrigel membrane filter in above method was performed.

Quantitative real-time polymerase chain reaction

Total RNA (1 μg) that was extracted using Trizol reagent (MRC, Cincinnati, OH) were reverse-transcripted into cDNA using the RevertAid™ First Strand cDNA Synthesis Kit (Fermentas, Vilnius, Lithuania). Each reaction well in a 25-μL final volume contains 2 μL of template DNA, 9.5 μL of ddH_2O, 12.5 μL of SYBR (TaKaRa, Jpn), 0.5 μL of HIF-1α forward primer: 5′-CCACTGCCACCACTGATGAA-3′ (nt 2254-2273), and 0.5 μL of reverse primer: 5′-TTGGTGAGGCT-GTCCGACTT-3′ (nt 2412-2431) to generate amplified product of 178 bp. Cycling program of quantitative real-time polymerase chain reaction was 2 minutes at 95 ℃ for hot-start, followed by 40 cycles of denaturation at 95 °C for 10 seconds, annealing at 60 °C for 30 seconds and elongation at 72 °C for 45 seconds in ICycler (BIO-RAD, United States).

Western blotting

Total proteins from HCC cells were lysed in RIPA buffer with protease and phosphatase inhibitors (Roche), and the concentrations were quantified with a Bicinchoninic Acid Protein Assay Kit (Beyotime Institute of Biotechnology, Shanghai, China). Equal amounts of 50 μg protein were separated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis, transferred onto polyvinylidine difluoride membranes (Millipore, Billerica, MA, United States), blocked with 5% bovine serum albumin in blocking buffer (Solarbio, China) for 2 hours at 25 °C, and incubated with specific primary rabbit anti-human antibodies overnight at 4 °C. β-actin (CST, United States) was used as a protein loading control.

EMT assay

HIF-1α primary antibodies were obtained from Santa Cruz (Univ-Bio., Shanghai, China), and antibodies against Ang-2, vimentin, E-cadherin, Twist, and Snail were obtained from Abcam (Cambridge, MA, United States). The membranes were subsequently incubated with a horseradish peroxidase-conjugated secondary goat anti-rabbit antibody (Abbkine, China). Detection was performed with an enhanced chemiluminescence kit (Beyotime Institute of Biotech., Shanghai, China). All images were taken with Quantity One software (Bio-Rad, Laboratories, Inc., United States).

ELISA

The concentrations of Ang-2 and HIF-1α in the serum or tissue supernatant were quantitatively detected using ELISA kits according to the manufacturer's instructions. Their levels were calculated according to a standard curve generated with specific standards provided by the manufacturer with inter- and intra-assay variances under 10%. ELISA kits for Ang-2 and HIF-1α detection were purchased from R&D Systems (Abingdon, United Kingdom; ADL Biotech Dev Co., United States; and Abcam Co., Shanghai, China, respectively).

Statistical analysis

The data are expressed as the means ± SDs and were analyzed in SPSS 19.0. Model rats were grouped according to liver pathological examination (H&E staining) and were divided into four groups: Rat HCC (rHCC), precancerosis (PC), Deg, and normal control (NC). Pearson's χ^2 test, ANOVA and the q test were performed to analyze the differences between different groups. A P value < 0.05 was considered statistically significant.

RESULTS

Ang-2 upregulation in HCC

Comparison of circulating HIF-1α and Ang-2 expressing levels in cases with chronic liver diseases (HCC, CH or LC) is shown in Table 1. The average levels of HIF-1α or Ang-2 in the HCC group were 1.8 or 1.6, 2.4 or 2.0, and 6.1 or 2.5 times

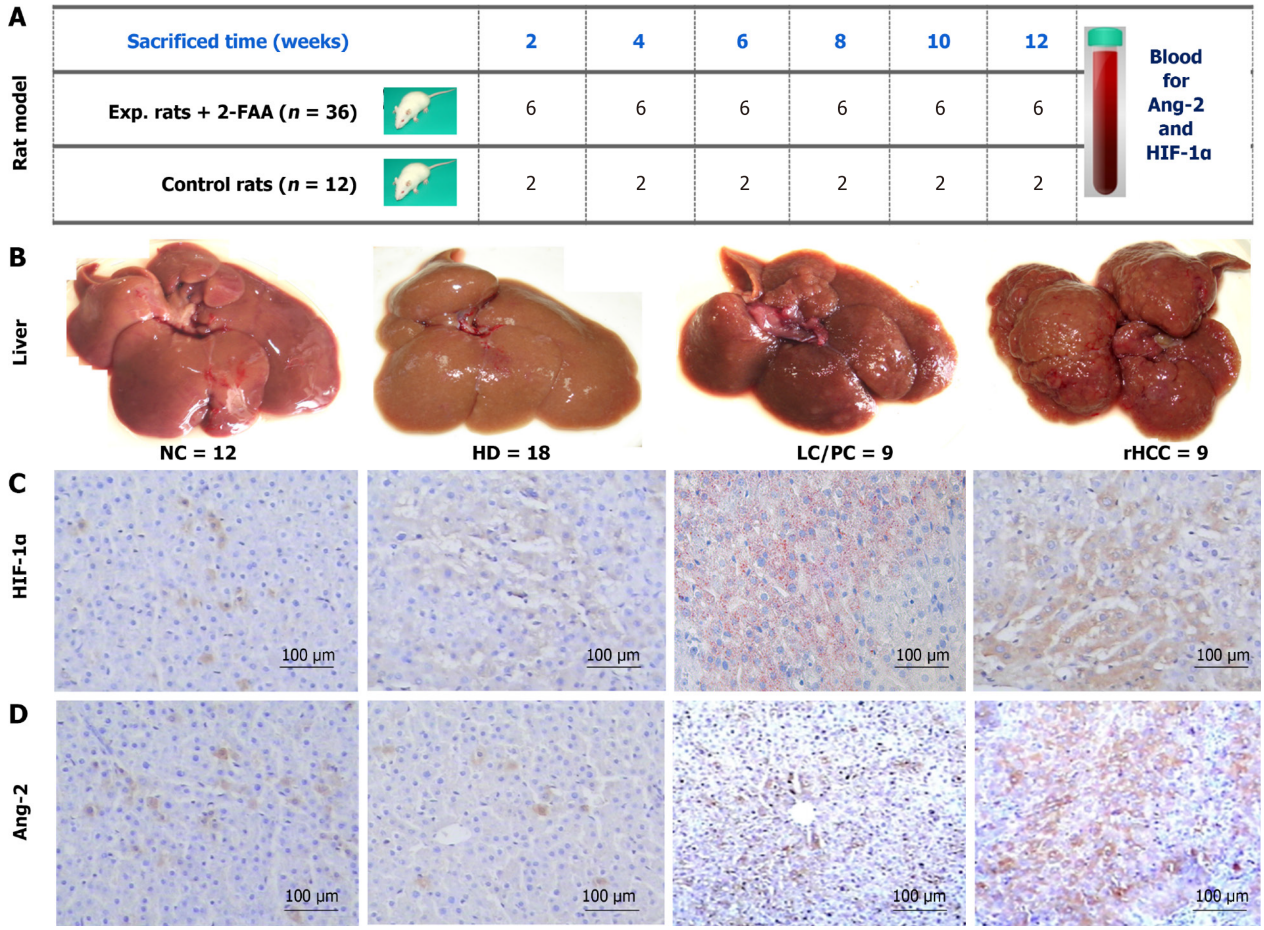

Figure 1 Dynamic expression of hypoxia-inducible factor-1α and angiopoietin-2 during hepatocarcinogenesis in rats. A: Sprague-Dawley rats (n = 48) were used to establish dynamic hepatocarcinogenesis models; B: Model livers subjected to morphological changes in liver sections were examined by hematoxylin and eosin staining and divided into normal control (n = 12), hepatocyte degeneration (n = 18) at the early stage, liver cirrhosis or precancerosis (n = 9) at the middle stage, and rat hepatocellular carcinoma (n = 9) at the later stage; C: Corresponding liver hypoxia-inducible factor-1α expression by immunohistochemical (SP × 100); D: Corresponding liver angiopoietin-2 expression by immunohistochemical (SP × 100). 2-FAA: 2-fluorenylacet-amide; Ang-2: Angiopoietin-2; HIF-1α: Hypoxia-inducible factor-1α; NC: Normal control; HD: Hepatocyte degeneration; LC: Liver cirrhosis; PC: Precancerosis; rHCC: Rat hepatocellular carcinoma.

higher than those in the LC, CH and NC groups, respectively. The incidences of HIF-1α (more than 100 μg/L) or Ang-2 (more than 35 μg/L) were 90.6% or 90.0%, 28.0% or 6.0%, 2.0% or 4.0% and 0% or 0% in the HCC, LC, CH, and NC groups, respectively. In addition, the circulating levels of Ang-2 and HIF-1α were significantly positively correlated (r = 0.933, P < 0.001), indicating that the Ang-2 and HIF-1α levels synchronously increased with the progression of chronic liver disease from benign to malignant transformation.

Clinicopathological features of high Ang-2 in HCC

The clinicopathological characteristics of high Ang-2 levels in the sera of patients with HCC are shown in Table 2. Significant differences in Ang-2 expression were found in relation to tumor size (≥ 5 cm, P < 0.001), differentiation degree (well/moderate *vs* poor, P < 0.001), gross classification (unifocal *vs* multifocal, P < 0.001), α-fetoprotein level (≥ 25 μg/L, P < 0.001), LC (P < 0.001), HBV infection (P < 0.001), portal vein invasion (P < 0.001), lymph node metastasis (P < 0.001) and tumor-node-metastasis staging (I-II *vs* III-IV, P < 0.001) but not in patients' sex (male *vs* female, P = 0.359) or age (≥ 50 years *vs* < 50 years, P = 0.087), suggesting that high Ang-2 is associated with the malignant progression of chronic liver diseases.

Validation of the role of Ang-2 in hepatocarcinogenesis

A schematic representation of the rat HCC model groupings according to pathological alterations in the liver caused by Ang-2 or HIF-1α upregulation is shown in Figure 1. The morphological changes in the liver sections were examined by H&E staining, and model livers were divided into the following groups: NC, hepatocyte degeneration (HD), PC/LC, and rHCC formation group. The increased expression levels of liver HIF-1α and Ang-2 were verified in liver sections. The dynamic alterations in HIF-1α and Ang-2 expression are shown in Table 3. The specific concentrations (ng/per mg liver tissues) of HIF-1α or Ang-2 or their serum levels (μg/L) were quantitatively detected from the NC group to the HD group at the early stage, the LC/PC ratio at the middle stage, and rHCC formation at the later stage, with significant positively correlated (r = 0.971, P < 0.001) between liver Ang-2 and HIF-1α levels at different stages. In liver and blood samples, dynamically upregulated HIF-1α and Ang-2 were found in hepatocarcinogenesis to promote neovascular supply of O_2,

Table 2 Clinicopathological features associated with serum angiopoietin-2 levels in patients with hepatocellular carcinoma

Parameter	*n*	Ang-2 (mean ± SD, μg/L)	*t* value	*P* value
AFP (μg/L)			4.386	< 0.001
< 50	29	40.1 ± 8.1		
≥ 50	51	45.2 ± 7.3		
Portal vein invasion			10.11	< 0.001
With	49	48.2 ± 6.3		
Without	31	39.4 ± 4.4		
HBsAg			4.933	< 0.001
Negative	15	38.9 ± 6.1		
Positive	65	45.8 ± 7.6		
Tumor size			8.979	< 0.001
< 5 cm	38	40.3 ± 4.9		
≥ 5 cm	42	48.7 ± 7.3		
Liver cirrhosis			8.121	< 0.001
With	56	49.3 ± 6.1		
Without	24	40.2 ± 8.4		
Differentiation degree			9.408	< 0.001
Well/moderate	57	40.9 ± 4.1		
Poor	23	48.7 ± 6.9		
Gross classification			4.700	< 0.001
Unifocal	27	41.8 ± 6.3		
Multifocal	53	47.7 ± 8.7		
TNM			7.838	< 0.001
I-II	24	39.4 ± 4.9		
III-IV	56	49.1 ± 8.5		

AFP: Alpha-fetoprotein; Ang-2: Angiopoietin-2; HBsAg: Hepatitis B surface antigen; TNM: Tumor node metastasis.

Table 3 Dynamic alterations in angiopoietin-2 and hypoxia-inducible factor-1α during hepatocarcinogenesis in rats

Group	*n*	Ang-2		HIF-1α	
		Liver (ng/mg)[1]	Serum (μg/L)	Liver (ng/mg)[1]	Serum (μg/L)
NC	12	69.7 ± 6.0	105.2 ± 19.2	9.7 ± 2.8	206.3 ± 9.6
HD	18	73.2 ± 17.0	140.2 ± 25.2	12.6 ± 3.2	270.2 ± 48.0
LC/PC	9	93.7 ± 11.2[a]	203.8 ± 23.8[a]	16.9 ± 2.6[b]	401.6 ± 89.4[b]
rHCC	9	107.6 ± 9. 8[b]	222.4 ± 23.3[b]	23.5 ± 8.9[b]	445.9 ± 69.5[b]

[1]ng/per mg liver tissue.
[a]$P < 0.05$, compared with the normal control group.
[b]$P < 0.01$, compared with the normal control group.
Ang-2: Angiopoietin-2; HIF-lα: Hypoxia-inducible factor-1α; NC: Normal control; rHCC: Rat hepatocellular carcinoma; HD: Hepatocyte degeneration; LC: Liver cirrhosis; PC: Precancerosis.

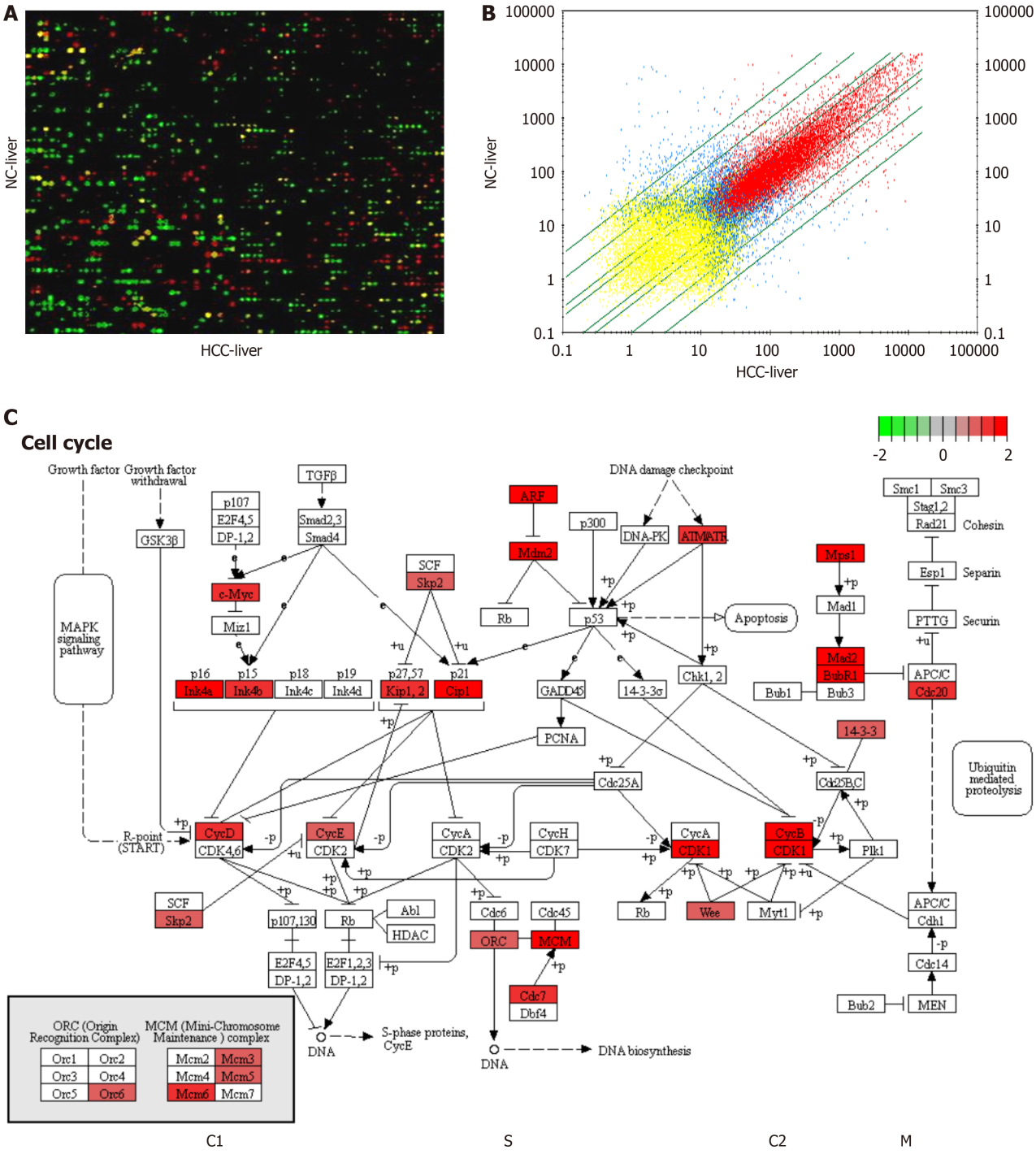

Figure 2 Alterations in whole gene expression profiling. A: Microarray analysis of the expression levels of a total of 28000 genes between rat hepatocellular carcinoma and normal control liver samples by false color imaging; B: The scatter plot of gene alterations between rat hepatocellular carcinoma and normal control livers is shown in a volcano plot; C: Gene upregulation in the cell cycle in hepato- carcinogenesis model rats. NC: Normal control; HCC: Hepatocellular carcinoma.

particularly in the LC/PC or rHCC group.

Differentially expressed genes in hepatocarcinogenesis

The original protocol for establishing a rat hepatocarcinogenesis model and many of the genes related to alterations in HCC formation in rats are shown in Figure 2. The identification of differentially expressed genes (DEGs) at different stages of hepatocarcinogenesis with established models simulated the clinical course and dynamic alterations in whole-gene transcriptomics by false color imaging (Figure 2A) or volcano plots (Figure 2B), including HIF-1α and Ang-2. Compared with those in the NC group, 70 and 93 DEGs were upregulated and downregulated, respectively, at the HD stage, featuring mainly oxidoreductase, acid mercaptoenzyme activities, peptide antigens, cofactor binding, the endoplasmic reticulum and the endoplasmic reticulum membrane; 1015 and 437 DEGs were upregulated and downregulated, respectively, at the LC/PC stage, including growth factors, calcium-dependent phospholipids, alloproteins,

insulin-like growth factor (IGF) binding, oxidoreductases, acid-mercaptoenzymes, the microtubule cytoskeleton in the centromeric region, the plasma membrane, and the extracellular and spindle as the main components; and 1234 and 504 DEGs were upregulated and downregulated, respectively, at the rHCC stage, including organic sodium transporter, microtubule movement, protein dimerization activity, alloprotein, calcium ion and cytoskeleton protein binding in the extracellular fraction, the microtubule cytoskeleton, the plasma membrane, cellular components, and genes involved in the cell cycle (Figure 2C).

Downregulation of Ang-2 affected biological behaviors

The effects of HIF-1α levels in HCC cell lines and specific microRNA transfection on biological behaviors are shown in Figure 3. High HIF-1α expression in HepG2 cells was detected (Figure 3A), and gradually decreased expression ($P < 0.01$) at the protein (Figure 3B) and mRNA levels ($2^{-\triangle\triangle Ct}$, Figure 3C) was detected after specific HIF-1α miRNA (150 nmol/L) transfection. Cell proliferation (Figure 3D) was significantly inhibited ($P < 0.01$) and the Ang-2 level (Figure 3E) was significantly lower than those in the negative and control groups at 48 or 72 hours. Additionally, the HCC cell invasion and migration abilities in the MiR group at 72 hours were markedly lower ($P < 0.001$, Figure 3F and G) than those in the control and negative groups, indicating that interfering with HIF-1α transcription could significantly affect Ang-2 expression and the biological behaviors of HCC cells, especially invasion and migration.

HIF-1α regulated Ang-2 or EMT and a possible mechanism

The changes in *HIF-1α* mRNA transcription in HCC cells and alterations in EMT- related epithelial E-cadherin, mesenchymal vimentin, and the transcription factors snail and twist in the culture medium, as determined by western blotting, are shown in Figure 4. No significant differences in these proteins were found between the Neg and Con groups. Compared with those in the NC group, the levels of E-cadherin significantly ($P < 0.001$) increased in HepG2 cells in which HIF-1α transcription was stably silenced in the MiR group (Figure 4A); however, the levels of vimentin (Figure 4B), snail (Figure 4C) and twist (Figure 4D) were markedly lower ($P < 0.001$) in the MiR group than in the NC group. A possible regulatory mechanism (Figure 4E) involved the upregulation of Ang-2 under hypoxia *in vitro*, and an *in vivo* hepatocarcinogenesis model promoted HCC progression or metastasis *via* EMT.

DISCUSSION

HCC is still one of the most common malignant tumors in the world, including the shore area of the Yangtze River. HCC tissues under a hypoxic microenvironment results in a high degree of vascular and traditional therapy with MDR[3,7]. HIF-1α is a transcriptional regulator of many genes and promotes tumorigenicity *via* upregulated expressing target genes involved in the cell apoptosis, cell proliferation, angiogenesis, cell invasion and metastasis of HCC, such as VEGF[21], Ang-2[22], Wnt3a[23], IGF-II and IGF-1R[24,25]. This study investigated the relationship between HIF-1α in liver or blood and HCC- associated angiogenesis or EMT, and confirmed the abnormal mechanism of Ang-2 expression by specific HIF-1α microRNA transfected into HCC cells, and the down-regulated HIF-1α in HCC inhibited the proliferation, angiogenesis and EMT of HCC cells.

The maintenance of adequate oxygen and angiogenic factors is essential for the growth of HCC or other solid tumors based on basic or clinical studied. Hepatic HIF-1α is involved in the regulation of hepatocyte inflammatory progression and is associated with the activation of multiple inflammation-associated cancers or the activation of the signalling pathways in HBV- or hepatitis C virus- infected associated HCC[26,27]. The excessive growth of HCC cells is limited under a hypoxic microenvironment in liver tissues, as tissue HIF-1α regulates immune escape of anti-cancer, cellular lipid metabolism, tumor angiogenesis and distal metastasis of HCC cells[28], extracellular matrix remodeling, and activation of liver cancer stem cells[29,30], and the MDR formation by regulating the phosphatidylinositol 3-kinase/protein kinase B/HIF-1α/MDR-1 signalling pathway for affecting HCC therapy[31,32]. In addition, in HIF-1α-deficient cells, markedly reduced anchorage- independent growth was exhibited and increased sensitivity to chemo- therapeutic agents in HCC cells[4,33,34]. Basic and clinical studies on angiogenesis in HCC have demonstrated that the growth or control of HCC is dependent on the perfect balance between the positive and negative regulators of endogenous HIF-1α. An interesting study about HIF-1α and Ang-2 levels in the sera of cases with benign or malignant liver diseases suggested that both levels simultaneously increased compared with those of normal controls and patients with CH and LC in the progression to HCC[35].

Abnormalities in angiogenic factors are associated with hepatocarcinogenesis in rats. HIF-1α binds to an evolutionarily conserved HRE located in the first intron of Ang-2[36,37]. All Ang-2 and VEGF expression levels tended to increase with histo- pathological changes, with the highest levels in HCC, followed by precancerosis, degeneration, and the controls. The levels of Ang-2 and HIF-1α transcriptions in the rHCC or LC/PC group were significantly higher than those in the HD or NC group. Also, the levels of circulating HIF-1α and Ang-2 expressions in the rHCC or LC/PC group were higher than those in the HD or NC group. There was a positive correlation between them in blood and them in liver tissues. Furthermore, abnormal HIF-1α expression was associated with the malignant transformation of hepatocytes in hepatocarcinogenesis, as HIF-1α regulates the transcription of many downstream genes, including those involved in angiogenesis or proliferation, to establish the basis for HCC growth and metastasis[38,39]. However, specific HIF-1α microRNAs could decrease Ang-2 expression and inhibit angiogenesis in HCC, suggesting that HIF-1α could regulate angiogenesis during HCC progression[40,41].

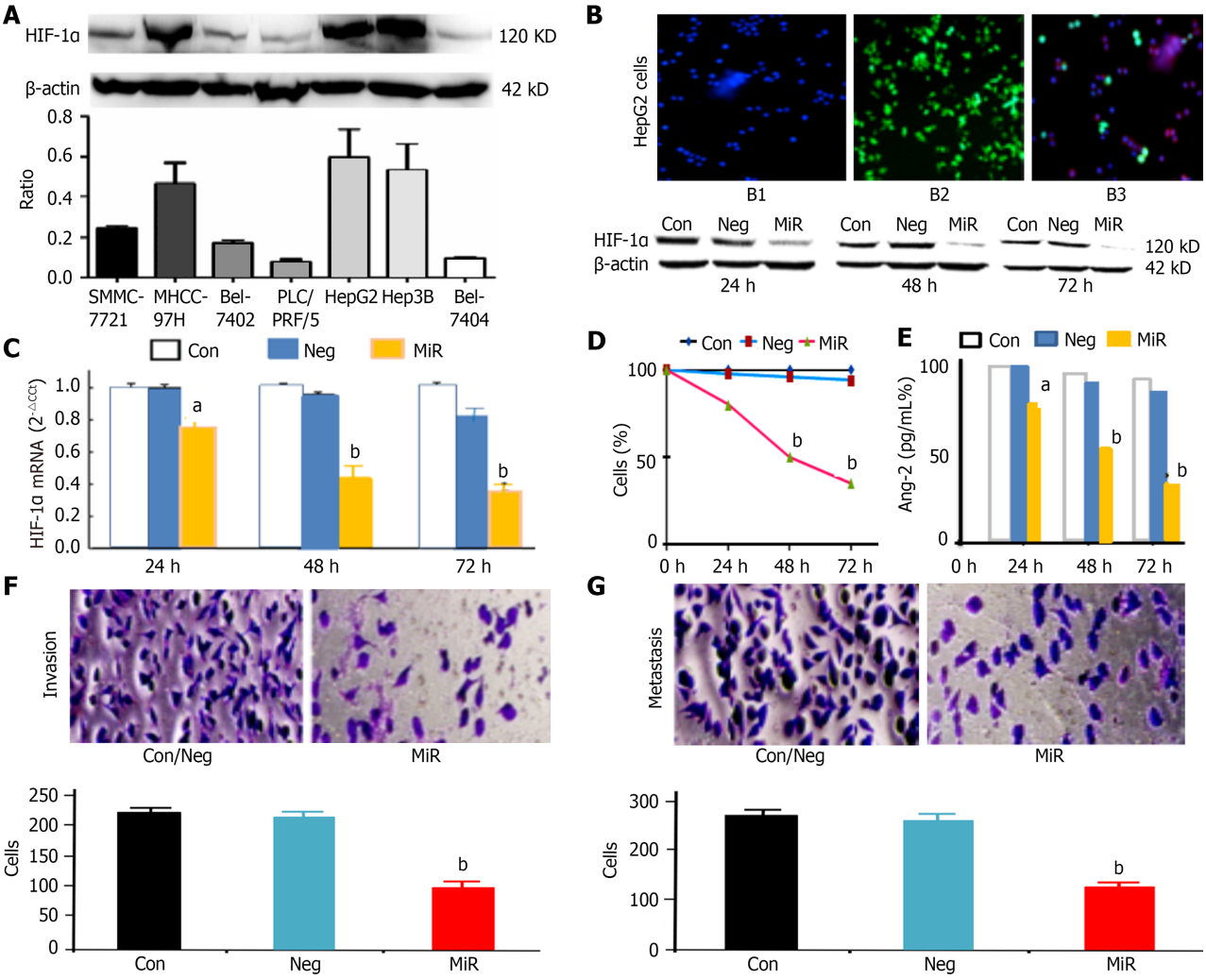

Figure 3 Silencing of hypoxia-inducible factor-1α affects angiopoietin-2 and the biological behavior of hepatocellular carcinoma cells. A: Hypoxia-inducible factor-1α (HIF-1α) expression among different hepatocellular carcinoma cell lines; B: Fluorescence micrographs of HepG2 cells (× 40): Control group (B1, no fluorescence), negative microRNA group (B2), and microRNA group (B3) at 48 hours, with HIF-1α (120 kDa) downregulation illustrated at the protein level by western blotting (B4) with β-actin (42 kDa) as a control; C: Downregulation of *HIF-1α* mRNA after microRNA (150 mmol/Lol/L) transfection by quantitative real-time polymerase chain reaction; D: Cell proliferation inhibition; E: Angiopoietin-2 downregulation; F: Crystal violet staining and invasion of cells (× 200); G: Crystal violet staining and migration of cells (× 200). HIF-1α: Hypoxia-inducible factor-1α; Con: Control group; Neg: Negative microRNA group; MiR: MicroRNA group; Ang-2: Angiopoietin-2. $^{a}P < 0.05$ or $^{b}P < 0.01$, compared with the microRNA or control group.

EMT is a cellular program critical for hepatocyte malignant progression that increases cancer cell invasive or metastatic potential by up-regulated the levels of mesenchymal indicators[42,43] or HCC-related transcription factors and downregulating the expressions of epithelial markers[44,45]. In the case of HCC, EMT promotes tumorigenesis or metastatic potential and increases elimination resistance to HCC treatment regimens. Vimentin may increase HCC cell migration or invasion, decrease E-cadherin and upregulate N-cadherin, whereas high vimentin expression is related to poor prognosis of HCC patients[46-48]. In the present study, the relationship between EMT-related E-cadherin, vimentin, twist or snail markers and HIF-1α expression were investigated in HepG2 cells. The E-cadherin in the HepG2 cells was significantly increased at the protein level after stable silencing HIF-1α transcription in the MiR group compared with the NC or Neg group, whereas the levels of vimentin, snail and twist expression were significantly downregulated, suggested that HIF-1α might promote HCC cell metastasis by enhancing EMT capacity[15,49] and be a potential effective target for HCC treatment[50-53].

CONCLUSION

In conclusion, HIF-1α activation might promote the malignant transformation of hepatocytes and the invasion or metastasis of HCC cells by regulating Ang-2 expression and EMT. Abnormal Ang-2 expression might be a sensitive early biomarker for monitoring the occurrence or metastasis of HCC. When silencing HIF-1α transcription could inhibit Ang-2 expression and EMT in HCC, and there was a certain correlation between HIF-1α and Ang-2. These findings provide a potential regulatory mechanism for HCC progression. However, more *in vitro* and *in vivo* studies are needed to confirm

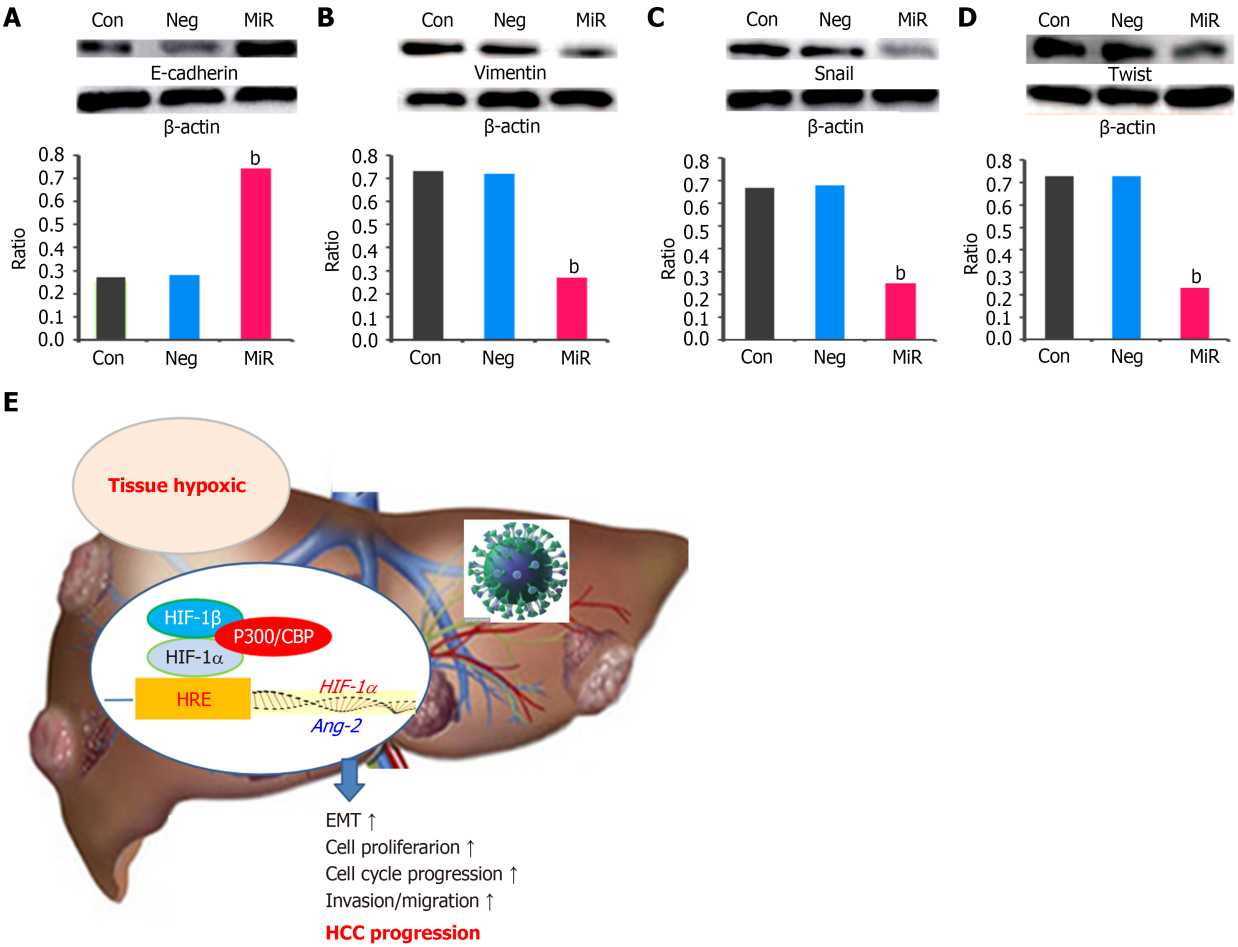

Figure 4 Silencing of hypoxia-inducible factor-1α affected epithelial-mesenchymal transition in HepG2 cells (*n* = 6). A: E-cadherin and the relative ratio; B: Vimentin and the relative ratio; C: Snail and the relative ratio; D: Twist and the relative ratio; E: Hypoxia upregulated angiopoietin-2, promoting hepatocellular carcinoma progression. Ang-2: Angiopoietin-2; Con: Control group; Neg: Negative microRNA group; MiR: MicroRNA group; HRE: Hypoxia response element; EMT: Epithelial-mesenchymal transition; HBV: Hepatitis B virus; HIF-1α: Hypoxia-inducible factor-1α; HCC: Hepatocellular carcinoma. $^{b}P < 0.01$, compared with the control group.

the exact mechanism involved. Future work should explore the feasibility of silencing HIF-1α or Ang-2 in combination with treatments with anticancer drugs or multitarget strategies for the effective treatment of HCC.

FOOTNOTES

Author contributions: Yang JL and Yang J contributed equally to this study, they are co-first authors of this manuscript. Yang JL and Yang J conceived the study and analyzed and interpreted the data; Yang J, Fang RF, and Yao M performed the experiments; Sai WL and Yao DF performed the statistical and bioinformatics analyses; Yang J, Fang RF, and Yao M acquired the materials and data; Yao DF and Yao M acquired the funding and wrote the manuscript, they contributed equally to this study and are the co-corresponding authors of this manuscript. All the authors have read and approved the final manuscript.

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REFERENCES

1 **Sankar K**, Gong J, Osipov A, Miles SA, Kosari K, Nissen NN, Hendifar AE, Koltsova EK, Yang JD. Recent advances in the management of hepatocellular carcinoma. *Clin Mol Hepatol* 2024; **30**: 1-15 [PMID: 37482076 DOI: 10.3350/cmh.2023.0125]
2 **Chen JG**, Zhu J, Zhang YH, Zhang YX, Yao DF, Chen YS, Lu JH, Ding LL, Chen HZ, Zhu CY, Yang LP, Zhu YR, Qiang FL. Cancer survival in Qidong between 1972 and 2011: A population-based analysis. *Mol Clin Oncol* 2017; **6**: 944-954 [PMID: 28588795 DOI: 10.3892/mco.2017.1234]
3 **Giannitrapani L**, Di Gaudio F, Cervello M, Scionti F, Ciliberto D, Staropoli N, Agapito G, Cannataro M, Tassone P, Tagliaferri P, Seidita A, Soresi M, Affronti M, Bertino G, Russello M, Ciriminna R, Lino C, Spinnato F, Verderame F, Augello G, Arbitrio M. Genetic Biomarkers of Sorafenib Response in Patients with Hepatocellular Carcinoma. *Int J Mol Sci* 2024; **25** [PMID: 38396873 DOI: 10.3390/ijms25042197]
4 **Jacquemin C**, El Orch W, Diaz O, Lalande A, Aublin-Gex A, Jacolin F, Toesca J, Si-Tahar M, Mathieu C, Lotteau V, Perrin-Cocon L, Vidalain PO. Pharmacological induction of the hypoxia response pathway in Huh7 hepatoma cells limits proliferation but increases resilience under metabolic stress. *Cell Mol Life Sci* 2024; **81**: 320 [PMID: 39078527 DOI: 10.1007/s00018-024-05361-6]
5 **Ting KKY**. Revisiting the role of hypoxia-inducible factors and nuclear factor erythroid 2-related factor 2 in regulating macrophage inflammation and metabolism. *Front Cell Infect Microbiol* 2024; **14**: 1403915 [PMID: 39119289 DOI: 10.3389/fcimb.2024.1403915]
6 **Jing L**, Ruan Z, Sun H, Li Q, Han L, Huang L, Yu S, Wang Y, Guo H, Jiao M. Epithelial-mesenchymal transition induced cancer-stem-cell-like characteristics in hepatocellular carcinoma. *J Cell Physiol* 2019; **234**: 18448-18458 [PMID: 30908631 DOI: 10.1002/jcp.28480]
7 **Chen Z**, Wang M, Lv X, Xu Y, Wang X, Li B, Ling C, Du J. Sanshimao formula inhibits the hypoxia-induced pro-angiogenesis of hepatocellular carcinoma cells partly through regulating MKK6/p38 signaling pathway. *J Pharm Pharmacol* 2024; **76**: 426-434 [PMID: 38290061 DOI: 10.1093/jpp/rgad086]
8 **Morse MA**, Sun W, Kim R, He AR, Abada PB, Mynderse M, Finn RS. The Role of Angiogenesis in Hepatocellular Carcinoma. *Clin Cancer Res* 2019; **25**: 912-920 [PMID: 30274981 DOI: 10.1158/1078-0432.CCR-18-1254]
9 **Petrillo M**, Patella F, Pesapane F, Suter MB, Ierardi AM, Angileri SA, Floridi C, de Filippo M, Carrafiello G. Hypoxia and tumor angiogenesis in the era of hepatocellular carcinoma transarterial loco-regional treatments. *Future Oncol* 2018; **14**: 2957-2967 [PMID: 29712486 DOI: 10.2217/fon-2017-0739]
10 **Vogel A**, Saborowski A. Current strategies for the treatment of intermediate and advanced hepatocellular carcinoma. *Cancer Treat Rev* 2020; **82**: 101946 [PMID: 31830641 DOI: 10.1016/j.ctrv.2019.101946]
11 **Yao DF**, Jiang H, Yao M, Li YM, Gu WJ, Shen YC, Qiu LW, Wu W, Wu XH, Sai WL. Quantitative analysis of hepatic hypoxia-inducible factor-1alpha and its abnormal gene expression during the formation of hepatocellular carcinoma. *Hepatobiliary Pancreat Dis Int* 2009; **8**: 407-413 [PMID: 19666411]
12 **Tirpe AA**, Gulei D, Ciortea SM, Crivii C, Berindan-Neagoe I. Hypoxia: Overview on Hypoxia-Mediated Mechanisms with a Focus on the Role of HIF Genes. *Int J Mol Sci* 2019; **20** [PMID: 31817513 DOI: 10.3390/ijms20246140]
13 **Liu K**, Min XL, Peng J, Yang K, Yang L, Zhang XM. The Changes of HIF-1α and VEGF Expression After TACE in Patients With Hepatocellular Carcinoma. *J Clin Med Res* 2016; **8**: 297-302 [PMID: 26985249 DOI: 10.14740/jocmr2496w]
14 **Mortezaee K**. Hypoxia induces core-to-edge transition of progressive tumoral cells: A critical review on differential yet corroborative roles for HIF-1α and HIF-2α. *Life Sci* 2020; **242**: 117145 [PMID: 31816327 DOI: 10.1016/j.lfs.2019.117145]
15 **Chen Y**, Huang F, Deng L, Yuan X, Tao Q, Wang T, Li D, Fan Y, Peng Q, Tang D. HIF-1-miR-219-SMC4 Regulatory Pathway Promoting Proliferation and Migration of HCC under Hypoxic Condition. *Biomed Res Int* 2019; **2019**: 8983704 [PMID: 31828143 DOI: 10.1155/2019/8983704]
16 **Guo Y**, Xiao Z, Yang L, Gao Y, Zhu Q, Hu L, Huang D, Xu Q. Hypoxiainducible factors in hepatocellular carcinoma (Review). *Oncol Rep* 2020; **43**: 3-15 [PMID: 31746396 DOI: 10.3892/or.2019.7397]
17 **Cheng S**, Chen M, Cai J; National Research Cooperative Group for Diagnosis and Treatment of Hepatocellular Carcinoma with Tumor Thrombus. Chinese expert consensus on multidisciplinary diagnosis and treatment of hepatocellular carcinoma with portal vein tumor thrombus: 2016 edition. *Oncotarget* 2017; **8**: 8867-8876 [PMID: 27780939 DOI: 10.18632/oncotarget.12817]
18 **Chinese Society of Infectious Diseases**; Chinese Medical Association; Chinese Society of Hepatology, Chinese Medical Association. [The guidelines of prevention and treatment for chronic hepatitis B (2019 version)]. *Zhonghua Gan Zang Bing Za Zhi* 2019; **27**: 938-961 [PMID: 31941257 DOI: 10.3760/cma.j.issn.1007-3418.2019.12.007]
19 **Wang P**, Tong K, Li Y, Li X, Zhang Y, Gu J, Lei P, Yan S, Hu P. The role and mechanism of HIF-1α-mediated glypican-3 secretion in hypoxia-induced tumor progression in hepatocellular carcinoma. *Cell Signal* 2024; **114**: 111007 [PMID: 38081444 DOI: 10.1016/j.cellsig.2023.111007]
20 **Dong ZZ**, Yao M, Wang L, Wu W, Gu X, Yao DF. Hypoxia-inducible factor-1alpha: molecular-targeted therapy for hepatocellular carcinoma.

Mini Rev Med Chem 2013; **13**: 1295-1304 [PMID: 22512580 DOI: 10.2174/1389557511313090004]
21 **Pinto E**, Pelizzaro F, Cardin R, Battistel M, Palano G, Bertellini F, Kitenge MP, Peserico G, Farinati F, Russo FP. HIF-1α and VEGF as prognostic biomarkers in hepatocellular carcinoma patients treated with transarterial chemoembolization. *Dig Liver Dis* 2024; **56**: 872-879 [PMID: 37783655 DOI: 10.1016/j.dld.2023.09.019]
22 **Abdel Ghafar MT**, Elkhouly RA, Elnaggar MH, Mabrouk MM, Darwish SA, Younis RL, Elkholy RA. Utility of serum neuropilin-1 and angiopoietin-2 as markers of hepatocellular carcinoma. *J Investig Med* 2021; **69**: 1222-1229 [PMID: 33833047 DOI: 10.1136/jim-2020-001744]
23 **Zheng W**, Yao M, Fang M, Pan L, Wang L, Yang J, Dong Z, Yao D. Oncogenic Wnt3a: A Candidate Specific Marker and Novel Molecular Target for Hepatocellular Carcinoma. *J Cancer* 2019; **10**: 5862-5873 [PMID: 31737122 DOI: 10.7150/jca.31599]
24 **Qian J**, Yao D, Dong Z, Wu W, Qiu L, Yao N, Li S, Bian Y, Wang Z, Shi G. Characteristics of hepatic igf-ii expression and monitored levels of circulating igf-ii mRNA in metastasis of hepatocellular carcinoma. *Am J Clin Pathol* 2010; **134**: 799-806 [PMID: 20959664 DOI: 10.1309/AJCPTFDSE2V3LCZP]
25 **Tai BJ**, Yao M, Zheng WJ, Shen YC, Wang L, Sun JY, Wu MN, Dong ZZ, Yao DF. Alteration of oncogenic IGF-II gene methylation status associates with hepatocyte malignant transformation. *Hepatobiliary Pancreat Dis Int* 2019; **18**: 158-163 [PMID: 30692043 DOI: 10.1016/j.hbpd.2019.01.003]
26 **Yang SL**, Ren QG, Zhang T, Pan X, Wen L, Hu JL, Yu C, He QJ. Hepatitis B virus X protein and hypoxiainducible factor-1α stimulate Notch gene expression in liver cancer cells. *Oncol Rep* 2017; **37**: 348-356 [PMID: 27840976 DOI: 10.3892/or.2016.5211]
27 **Lu Y**, Sui J, Liu Y, Li R, Ma L, Zhang X, Qin X, Li S. Association between hypoxia-inducible factor-1α gene polymorphisms and risk of chronic hepatitis B and hepatitis B virus-related liver cirrhosis in a Chinese population: a retrospective case-control study. *Gene* 2015; **564**: 96-100 [PMID: 25824382 DOI: 10.1016/j.gene.2015.03.053]
28 **Tong Y**, Yang H, Xu X, Ruan J, Liang M, Wu J, Luo B. Effect of a hypoxic microenvironment after radiofrequency ablation on residual hepatocellular cell migration and invasion. *Cancer Sci* 2017; **108**: 753-762 [PMID: 28182306 DOI: 10.1111/cas.13191]
29 **Kang Q**, Gong J, Wang M, Wang Q, Chen F, Cheng KW. 6-C-(E-Phenylethenyl)Naringenin Attenuates the Stemness of Hepatocellular Carcinoma Cells by Suppressing Wnt/β-Catenin Signaling. *J Agric Food Chem* 2019; **67**: 13939-13947 [PMID: 31769973 DOI: 10.1021/acs.jafc.9b05733]
30 **Cui CP**, Wong CC, Kai AK, Ho DW, Lau EY, Tsui YM, Chan LK, Cheung TT, Chok KS, Chan ACY, Lo RC, Lee JM, Lee TK, Ng IOL. SENP1 promotes hypoxia-induced cancer stemness by HIF-1α deSUMOylation and SENP1/HIF-1α positive feedback loop. *Gut* 2017; **66**: 2149-2159 [PMID: 28258134 DOI: 10.1136/gutjnl-2016-313264]
31 **Zhong T**, Zhang W, Guo H, Pan X, Chen X, He Q, Yang B, Ding L. The regulatory and modulatory roles of TRP family channels in malignant tumors and relevant therapeutic strategies. *Acta Pharm Sin B* 2022; **12**: 1761-1780 [PMID: 35847486 DOI: 10.1016/j.apsb.2021.11.001]
32 **Xie Y**, Zhong DW. AEG-1 is associated with hypoxia-induced hepatocellular carcinoma chemoresistance *via* regulating PI3K/AKT/HIF-1alpha/MDR-1 pathway. *EXCLI J* 2016; **15**: 745-757 [PMID: 28337106 DOI: 10.17179/excli2016-694]
33 **Jiao M**, Nan KJ. Activation of PI3 kinase/Akt/HIF-1α pathway contributes to hypoxia-induced epithelial-mesenchymal transition and chemoresistance in hepatocellular carcinoma. *Int J Oncol* 2012; **40**: 461-468 [PMID: 21922131 DOI: 10.3892/ijo.2011.1197]
34 **Li S**, Yang Y, Ding X, Yang M, She S, Peng H, Xu X, Ran X, Li S, Hu P, Hu H, Zhang D, Ren H. LHBs can elevate the expression of MDR1 through HIF-1α in patients with CHB infection: a comparative proteomic study. *Oncotarget* 2017; **8**: 4549-4562 [PMID: 27999186 DOI: 10.18632/oncotarget.13941]
35 **Wang D**, Zhang X, Lu Y, Wang X, Zhu L. Hypoxia inducible factor 1α in hepatocellular carcinoma with cirrhosis: Association with prognosis. *Pathol Res Pract* 2018; **214**: 1987-1992 [PMID: 30274686 DOI: 10.1016/j.prp.2018.09.007]
36 **Wang Z**, Jiang L, Wang J, Chai Z, Xiong W. Morphine promotes angiogenesis by activating PI3K/Akt/HIF-1α pathway and upregulating VEGF in hepatocellular carcinoma. *J Gastrointest Oncol* 2021; **12**: 1761-1772 [PMID: 34532126 DOI: 10.21037/jgo-20-394]
37 **Wang Z**, Yan M, Li J, Long J, Li Y, Zhang H. Dual functions of STAT3 in LPS-induced angiogenesis of hepatocellular carcinoma. *Biochim Biophys Acta Mol Cell Res* 2019; **1866**: 566-574 [PMID: 30521872 DOI: 10.1016/j.bbamcr.2018.11.016]
38 **Zhao H**, Yao P, Fu N, Chen L. deSUMOylation signaling: a novel mechanism of liver CSC properties and hepatocarcinogenesis in hypoxia. *Acta Biochim Biophys Sin (Shanghai)* 2017; **49**: 1135-1137 [PMID: 29040348 DOI: 10.1093/abbs/gmx109]
39 **Cheng S**, Zhang X, Xu Y, Dai X, Li J, Zhang T, Chen X. Krüppel-like factor 8 regulates VEGFA expression and angiogenesis in hepatocellular carcinoma. *Sci Rep* 2018; **8**: 17415 [PMID: 30479372 DOI: 10.1038/s41598-018-35786-6]
40 **Matsuura Y**, Wada H, Eguchi H, Gotoh K, Kobayashi S, Kinoshita M, Kubo M, Hayashi K, Iwagami Y, Yamada D, Asaoka T, Noda T, Kawamoto K, Takeda Y, Tanemura M, Umeshita K, Doki Y, Mori M. Exosomal miR-155 Derived from Hepatocellular Carcinoma Cells Under Hypoxia Promotes Angiogenesis in Endothelial Cells. *Dig Dis Sci* 2019; **64**: 792-802 [PMID: 30465177 DOI: 10.1007/s10620-018-5380-1]
41 **Wen Y**, Zhou X, Lu M, He M, Tian Y, Liu L, Wang M, Tan W, Deng Y, Yang X, Mayer MP, Zou F, Chen X. Bclaf1 promotes angiogenesis by regulating HIF-1α transcription in hepatocellular carcinoma. *Oncogene* 2019; **38**: 1845-1859 [PMID: 30367150 DOI: 10.1038/s41388-018-0552-1]
42 **Sengez B**, Carr BI, Alotaibi H. EMT and Inflammation: Crossroads in HCC. *J Gastrointest Cancer* 2023; **54**: 204-212 [PMID: 35020133 DOI: 10.1007/s12029-021-00801-z]
43 **Dongre A**, Weinberg RA. New insights into the mechanisms of epithelial-mesenchymal transition and implications for cancer. *Nat Rev Mol Cell Biol* 2019; **20**: 69-84 [PMID: 30459476 DOI: 10.1038/s41580-018-0080-4]
44 **Gurzu S**, Kobori L, Fodor D, Jung I. Epithelial Mesenchymal and Endothelial Mesenchymal Transitions in Hepatocellular Carcinoma: A Review. *Biomed Res Int* 2019; **2019**: 2962580 [PMID: 31781608 DOI: 10.1155/2019/2962580]
45 **Giannelli G**, Koudelkova P, Dituri F, Mikulits W. Role of epithelial to mesenchymal transition in hepatocellular carcinoma. *J Hepatol* 2016; **65**: 798-808 [PMID: 27212245 DOI: 10.1016/j.jhep.2016.05.007]
46 **Lei HW**, Cai J, Li CM, Yang F, Shi WQ, Shi WQ, Wang LP, Feng YY. Rapamycin Combi with TAE on the Growth, Metastasis, and Prognosis of Hepatocellular Carcinoma in Rat Models. *Ann Hepatol* 2018; **17**: 645-654 [PMID: 29893708 DOI: 10.5604/01.3001.0012.0948]
47 **Feng B**, Zhu Y, Sun C, Su Z, Tang L, Li C, Zheng G. Basil polysaccharide inhibits hypoxia-induced hepatocellular carcinoma metastasis and progression through suppression of HIF-1α-mediated epithelial-mesenchymal transition. *Int J Biol Macromol* 2019; **137**: 32-44 [PMID: 31252022 DOI: 10.1016/j.ijbiomac.2019.06.189]
48 **Wang M**, Zhao X, Zhu D, Liu T, Liang X, Liu F, Zhang Y, Dong X, Sun B. HIF-1α promoted vasculogenic mimicry formation in hepatocellular carcinoma through LOXL2 up-regulation in hypoxic tumor microenvironment. *J Exp Clin Cancer Res* 2017; **36**: 60 [PMID:

28449718 DOI: 10.1186/s13046-017-0533-1]

49 **Shao S**, Duan W, Xu Q, Li X, Han L, Li W, Zhang D, Wang Z, Lei J. Curcumin Suppresses Hepatic Stellate Cell-Induced Hepatocarcinoma Angiogenesis and Invasion through Downregulating CTGF. *Oxid Med Cell Longev* 2019; **2019**: 8148510 [PMID: 30800209 DOI: 10.1155/2019/8148510]

50 **Zhang JG**, Zhou HM, Zhang X, Mu W, Hu JN, Liu GL, Li Q. Hypoxic induction of vasculogenic mimicry in hepatocellular carcinoma: role of HIF-1 α, RhoA/ROCK and Rac1/PAK signaling. *BMC Cancer* 2020; **20**: 32 [PMID: 31931758 DOI: 10.1186/s12885-019-6501-8]

51 **Luo J**, Sun P, Zhang X, Lin G, Xin Q, Niu Y, Chen Y, Xu N, Zhang Y, Xie W. Canagliflozin Modulates Hypoxia-Induced Metastasis, Angiogenesis and Glycolysis by Decreasing HIF-1α Protein Synthesis *via* AKT/mTOR Pathway. *Int J Mol Sci* 2021; **22** [PMID: 34948132 DOI: 10.3390/ijms222413336]

52 **Chen Y**, Chen X, Ding X, Wang Y. Afatinib, an EGFR inhibitor, decreases EMT and tumorigenesis of Huh7 cells by regulating the ERKVEGF/MMP9 signaling pathway. *Mol Med Rep* 2019; **20**: 3317-3325 [PMID: 31432165 DOI: 10.3892/mmr.2019.10562]

53 **Safri F**, Nguyen R, Zerehpooshnesfchi S, George J, Qiao L. Heterogeneity of hepatocellular carcinoma: from mechanisms to clinical implications. *Cancer Gene Ther* 2024; **31**: 1105-1112 [PMID: 38499648 DOI: 10.1038/s41417-024-00764-w]

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