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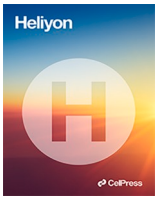

Research article

Hsa_circ_0000092 up-regulates IL24 by SMC1A to induce macrophages M2 polarization

Rihai Ma [a,1], Anmin Wang [b,1], Meng Yang [b], Zihua Huang [b], Guoman Liu [b], Qing Wei [b], Yuan Lu [a], Huamei Wei [c], Jianchu Wang [a], Qianli Tang [a], Jian Pu [a,*]

[a] Department of Hepatobiliary Surgery, Affiliated Hospital of Youjiang Medical University for Nationalities, Guangxi Zhuang Autonomous Region, 533000, China
[b] Graduate College of Youjiang Medical University for Nationalities, Guangxi Zhuang Autonomous Region, 533000, China
[c] Department of Pathology, Affiliated Hospital of Youjiang Medical University for Nationalities, Guangxi Zhuang Autonomous Region, 533000, China

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ABSTRACT

Introduction: Hepatocellular carcinoma (HCC) as the malignant cancers with high morbidity. The EMT of HCC has closely linked to the metastasis and recurrence. Moreover, tumor-associated macrophages (TAMs) can interact with HCC cells in the immune microenvironment; the M2 polarization of TAMs enhance the HCC cells EMT. The mechanism between HCC cells and TAMs is still unclear and our study was aimed to uncover it.

Methods: We performed RT-qPCR and western to detach the RNA and protein expression. The relationship among has_circ_0000092, U2AF2, SMC1A and IL24 were revealed through mechanism experiments. Rescue assays were implemented to determine how circ_0000092 modulates M2 polarization of TAMs.

Results: As detected by RT-qPCR, has_circ_0000092 was with high expression in HCC cells and could recruit U2AF2 to promote transcription of SMC1A. Moreover, circ_0000092 could control macrophage M2 polarization via promoting IL24 expression in HCC cells.

Conclusion: To conclude, hsa_circ_0000092 can up-regulates IL24 by SMC1A to induce macrophages M2 polarization.

1. Introduction

Hepatocellular carcinoma (HCC) as the malignant cancers with high mortality. Its fatality rate ranked 6th and 2nd respectively [1]. Metastases and relapse of HCC often occur, which is the major obstacle for increasing the long-term survival of patients [2]. The EMT of HCC cells is closely linked with metastasis and recurrence [3]. Tumor-associated macrophages (TAMs) interact with HCC cells in the immune microenvironment; additionally, the M2 polarization of TAMs promotes the EMT of HCC [4,5]. The potential mechanism underlying interaction between HCC cells and TAM was still unknown, thus, our project intended to unveil it.

Circular RNAs (circRNAs) has closed loop structure without 3'- or 5'-end, and its malignant regulatory role has been confirmed in

* Corresponding author. Department of Hepatobiliary Surgery, Affiliated Hospital of Youjiang Medical University for Nationalities, No.18 Zhongshan 2 Road, Guangxi Zhuang Autonomous Region, 533000, China.
E-mail address: weidao848042700@163.com (J. Pu).
[1] Co-first author.

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many cancer types [6–8]. circRNA-SORE mediates sorafenib resistance by stabilizing YBX1 in HCC [9]; circRNA-5692 inhibits the HCC progression via miR-328-5p/DAB2IP axis [10]; exosomal circRNA-100338 raises HCC metastasis via enhancing metastasis and angiogenesis [11].

With the help of GEO dataset GSE94508, we found that hsa_circ_0000092 was up-regulated in HCC cells. As a novel circRNA, the hsa_circ_0000092 functions in various cancers remain largely unclear. It was reported that hsa_circ_0000092 could promote the HCC metastasis and EMT through miR-338-3p/HN1 axis [12], but whether it affects TAMs polarization remains to be unveiled. Therefore, we constructed a co-culture system with macrophages and HCC cells for the investigation into the regulation mechanism of hsa_circ_0000092 in HCC.

Structural maintenance of chromosomes protein 1A (SMC1A) has been demonstrated to take critical roles in multiple cancers development. Notably, SMC1A has shown to be implicated in metastasis and modulation of EMT in tumor cells including prostate, colorectal, and breast cancer [13–15]. But the role of SMC1A is not well-understood. In addition, interleukin 24 (IL24) is a multifunctional cytokine that belongs to the IL-20 family and produced by a variety of immune cells, such as B cells, T cells, monocytes, NK cells, and macrophages [16]. Importantly, IL-24 was predicted to be a potential biomarker of macrophage M2 polarization [17].

The current research focused on the role and mechanism of hsa_circ_0000092 on HCC progression. Our study was aimed at finding novel therapeutic strategies for HCC treatment and thus improving the prognosis.

2. Materials and methods

We followed some methods in the report of Wu et al. [18].

2.1. Cell culture

HCC cell lines are useful *in vitro* models for the study of HCC. THP-1, a human leukemia monocytic cell line, is extensively used to study macrophage mechanism. Commercially available HCC cell lines (MHCC97H, Huh7 and HCCLM3) and normal human liver cell line THLE2 were used in this research. MHCC97H, Huh7 and THLE2 cell lines were purchased from Shanghai Yaji Biotechnology Company; HCCLM3 and THP-1 were purchased from China Center for Type Culture Collection Cell Bank. Dulbecco's Modified Eagle Medium (DMEM) with 10 % fetal bovine serum (FBS) was utilized for cell culture (MHCC97H, HCCLM3 and THLE2) in a humid incubator with 5 % CO_2 at 37 °C. Huh7 and THP-1 cell lines were cultured in RPMI-1640 medium plus 10 % FBS. All the cells have been identified by Cellosaurus (https://web.expasy.org/cellosaurus/).

2.2. Plasmid transfection

For the overexpression of circ_0000092, SMC1A and IL24, full-length sequences were separately sub-cloned into pcDNA3.1 vectors, along with pcDNA3.1 empty vector as the negative control (NC). For the knockdown of circ_0000092, U2AF2 and SMC1A, specific siRNAs against them were respectively synthesized as well as non-targeting siRNA (si-NC). Plasmid transfections were carried out with Lipofectamine 2000 following the supplier's protocols.

2.3. qRT-PCR

Total RNA was extracted with TRIzol reagent in HCC cells. RT-qPCR reaction was performed by SYBR Green PCR Kit, followed by $2^{-\Delta\Delta Ct}$ method for calculation of relative gene expression.

2.4. Subcellular fractionation

PARIS™ Kit was utilized to conduct this assay in MHCC97H and HCCLM3 cells. After centrifugation, cells were subjected to cell disruption buffer. Finally, circ_0000092 in cytoplasm/nucleus was examined by qRT-PCR.

2.5. Western blot

Cells were added to RIPA cleavage, detected protein concentration by BCA method, separated protein with SDS-PAGE gel, the first antibody (anti-U2AF2 and anti-SMC1A) was blocked, the second antibody was incubated, and ECL luminescent solution was added. The grayscale value was detected by gel imaging system and analyzed by ImageJ graphic analysis system.

2.6. FISH

MHCC97H and HCCLM3 cells were subjected to 4 % formaldehyde fixation for 18 h before being rinsed by PBS. After dehydration, cells were cultivated with circ_0000092-FISH probe. 3 h after hybridization, DAPI was utilized for nuclear stain. Finally, fluorescence microscope was utilized to acquire images.

2.7. RIP

With the Imprint® RNA Immunoprecipitation Kit and Magna RIP™ RNA-Binding Protein Immunoprecipitation Kit, immunoprecipitation in MHCC97H and HCCLM3 cell was achieved with the specific antibodies against TFs (U2AF2, SRSF1, HNRNPC, IGF2BP3 and TAF15) and normal control anti-IgG antibody. Lysates were obtained from HCC cell lines using RIP lysis buffer. The lysis was incubated with the magnetic beads conjugated with IgG antibody (negative control). The enrichment of circ_0000092 and SMC1A 3' UTR in the immunoprecipitates was measured by qRT-PCR.

2.8. Pull down assays

For RNA pull down, Pierce Magnetic RNA-Protein Pull-Down Kit was utilized according to the provider's suggestions. Protein extracts were mixed with biotin-labeled SMC1A 3′ UTR probes or control probes and magnetic beads. For DNA pull down, DNA pull-down test kit was employed as per the user guide. Protein lysates were cultivated with Biotinylated IL24 promoter probe or control probe Bio-NC. All pulled-down mixtures underwent Western blot analysis.

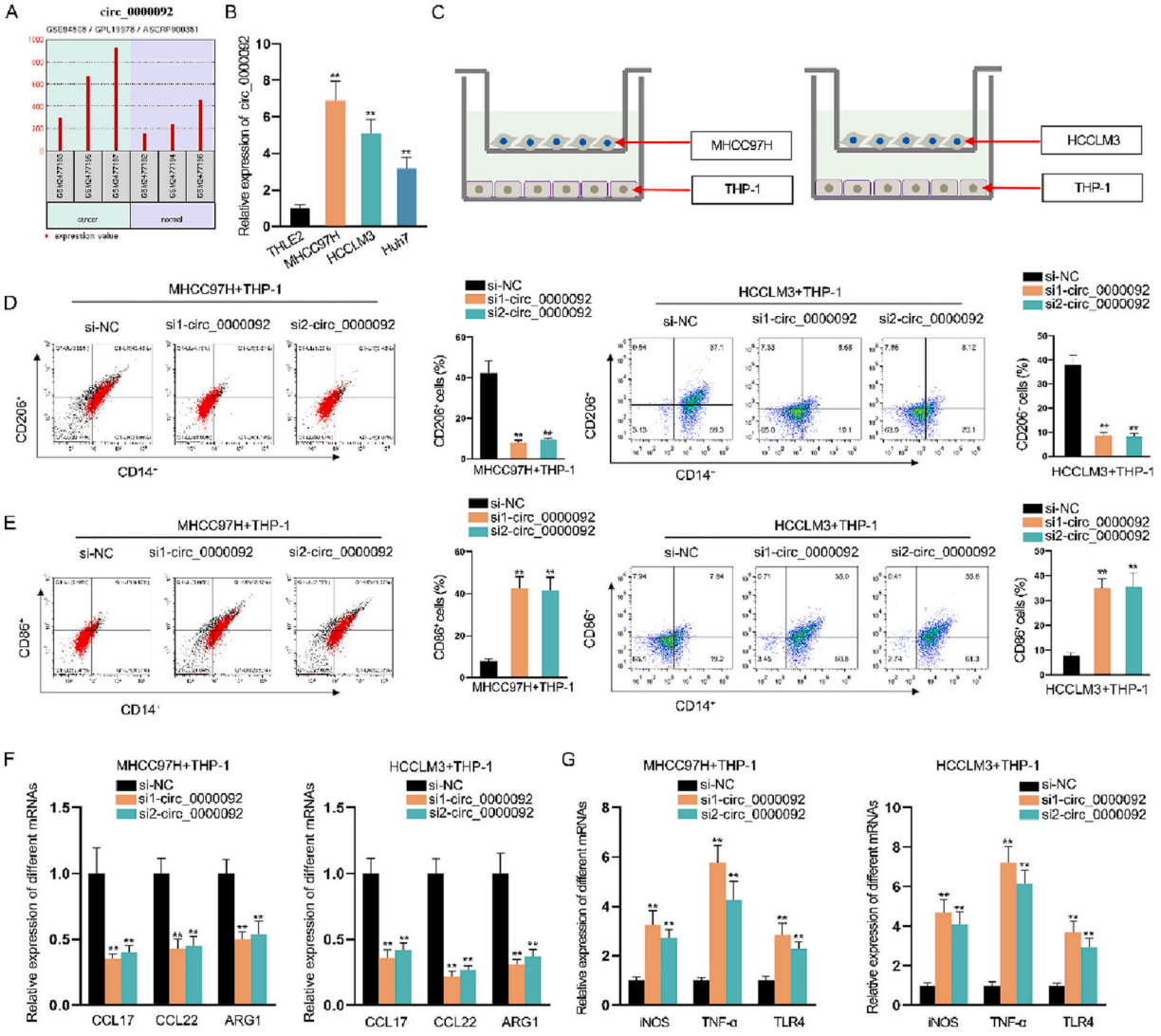

Fig. 1. circ_0000092 promotes macrophages M2 polarization A. GES94508 showed that circ_0000092 was expressed obviously high in HCC. B. The expression of circ_0000092 in normal hepatocyte THLE2 and HCC cell lines (MHCC97H, HCCLM3, Huh7). C. MHCC97H and HCCLM3 were added to the upper compartment, and THP-1 cells were added to the lower compartment in the co-culture system. D-E. Flow cytometry analysis was taken to detect the expression level of macrophage M2 polarization marker CD206 and macrophage M1 polarization marker CD86 under circ_0000092 interference. F. The mRNA expression level of macrophage M2 polarization markers CCL17, CCL22 and ARG1 under circ_0000092 interference. G. The mRNA expression level of macrophage M1 polarization markers iNOS, TNF-α and TLR4 under circ_0000092 interference. $^{**}p < 0.01$.

2.9. ChIP

ChIP assay was implemented in MHCC97H and HCCLM3 cells. Cells were cross-linked in 4 % paraformaldehyde, and then sonicated into chromatin fragments of 200–1000-bp (incubated with the antibodies-SMC1A and anti-IgG), added magnetic beads into it. Subsequently, cell lysates were mixed with bead-antibody complex. At last, the immunoprecipitated DNA was extracted and purified for qRT-PCR quantification.

2.10. Flow cytometry

Annexin V-FITC/PI Apoptosis kit was used in flow cytometry analysis to monitor the apoptotic cells as per the supplier's directions. MHCC97H and HCCLM3 cells were collected from pre-cooled PBS for dual-staining, and subjected to subsequent analysis by flow cytometer after 15 min.

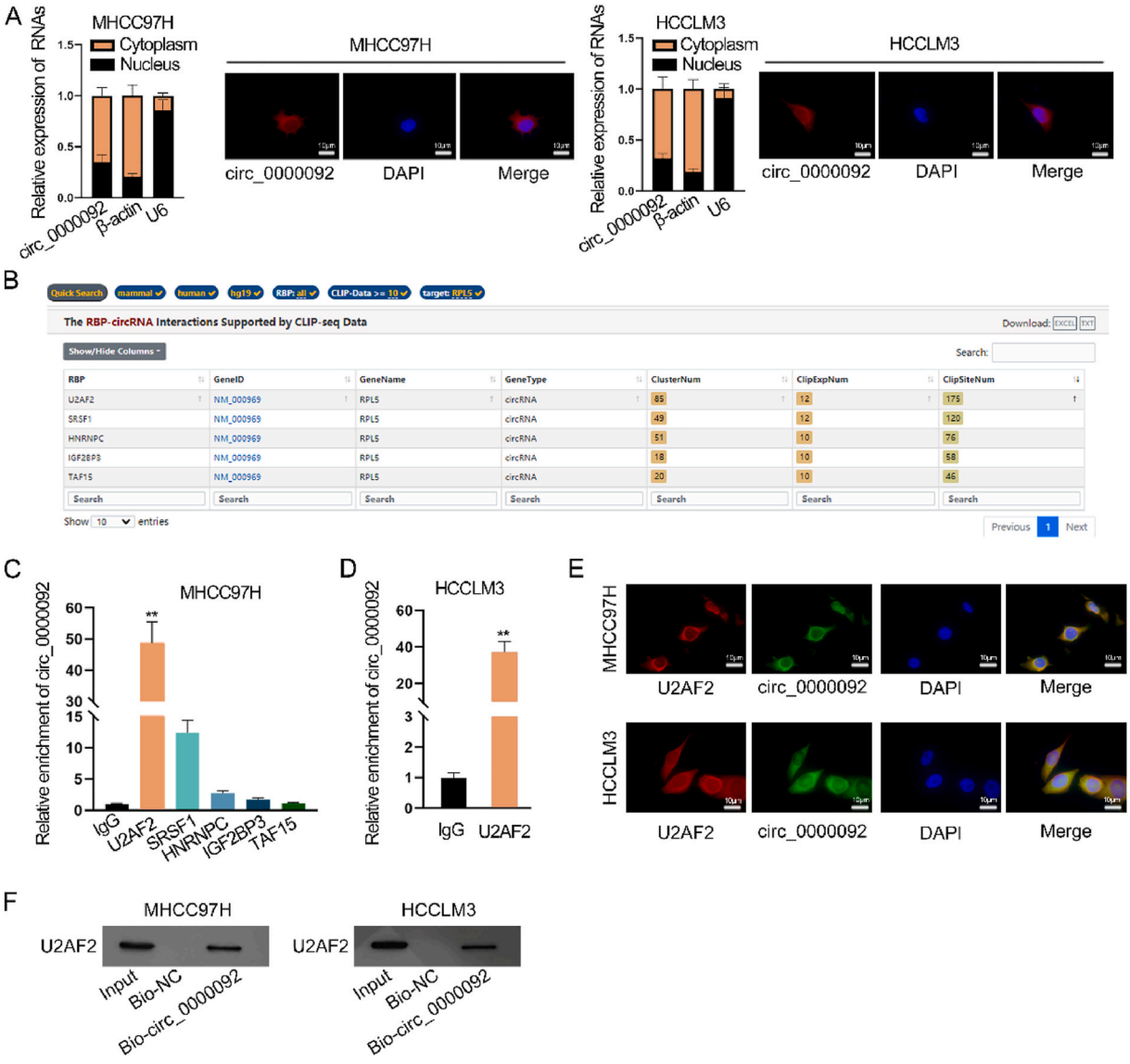

Fig. 2. circ_0000092 binds to U2AF2 A. FISH and subcellular fractionation assays were taken to detect the distribution of circ_0000092 in MHCC97H and HCCLM3 cells. Scale bar = 10 μm. B. StarBase was used to show the RBPs which might bind to circ_0000092. C. RIP assay was used to detect the enrichment of circ_0000092 in the immunoprecipitates bound with different RBPs in MHCC97H cells. D. RIP assay was taken to detect the enrichment of circ_0000092 in the anti-U2AF2 bound immunoprecipitates in HCCLM3 cells. E. FISH-IF assay was utilized to analyze the co-location of circ_0000092 and U2AF2 in MHCC97H and HCCLM3 cells. Scale bar = 10 μm. F. RNA pull down and Western blot were used to detect the enrichment of U2AF2 protein in MHCC97H and HCCLM3 cells after biotinylated circ_0000092. **$p < 0.01$.

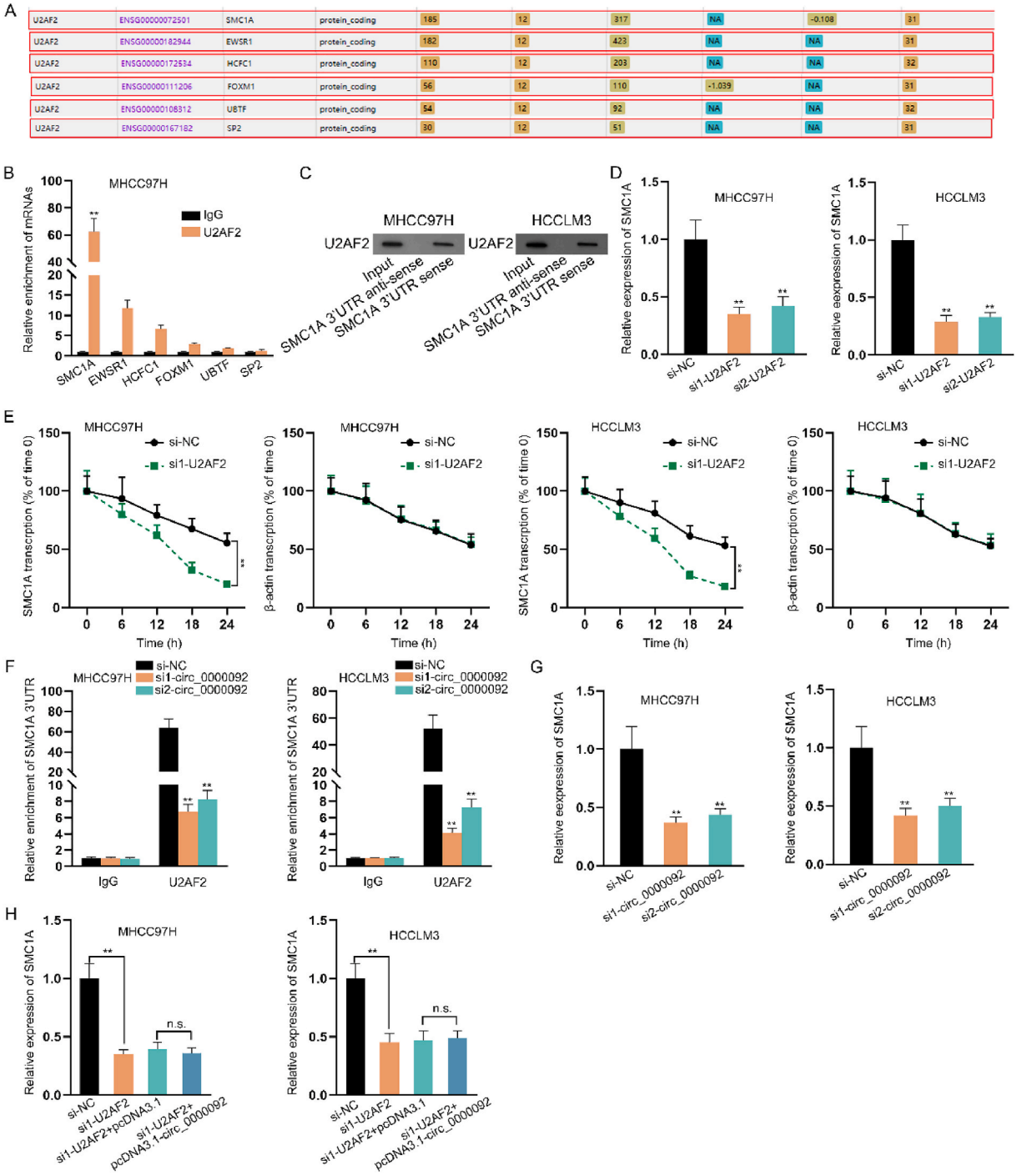

Fig. 3. circ_0000092 stabilizes SMC1A through U2AF2 A. The possible transcription factors binding to U2AF2 was listed in starBase. B. The enrichment of different mRNAs abundantly enriched in the anti-U2AF2 complex was detected by RIP assay. C. After the RNA pull down assay, Western blot assay was taken to detect the U2AF2 enrichment in MHCC97H and HCCLM3 cells with biotinylated SMC1A 3′UTR. D. The expression of SMC1A in HCC cells was detected by qRT-PCR after silencing U2AF2. E. The SMC1A mRNA stability in HCC cells was detected by mRNA stability assay after interfering U2AF2. F. RIP assay in MHCC97H and HCCLM3 cells was taken to detect the effect of interfering circ_0000092 on the binding ability between U2AF2 and SMC1A. G. The regulation of circ_0000092 to SMC1A was evaluated by qRT-PCR. H. The regulation of circ_0000092 to SMC1A after U2AF2 interference was measured by qRT-PCR. **p < 0.01.

2.11. Statistical analyses

Analysis of results from independent experiments (n = 3) was processed by using SPSS version 17.0, and the data were exhibited as mean ± standard deviation (SD). The differences between two groups were determined by Student's t-test, while multiple comparisons were evaluated by ANOVA. Data with a p value below 0.05 exhibited statistical significance.

3. Results

3.1. Circ_0000092 promotes macrophages M2 polarization

According to the former literatures, we found a circRNA which is expressed highly in HCC and related to migration, circ_0000092 [12]. Moreover, GSE94508 datasets showed the up-regulation of circ_0000092 expression in HCC cells (Fig. 1A). Then qRT-PCR was taken to detect the circ_0000092 expression in normal hepatocyte THLE2 and HCC cell lines (MHCC97H, HCCLM3, and Huh7). The result indicated that circ_0000092 was highly expressed in HCC cells (Fig. 1B). MHCC97H and HCCLM3 were chosen for further study as circ_0000092 expressed the most highly in these two cell lines. Next, in the co-culture system, MHCC97H and HCCLM3 were cultured to the upper compartment while THP-1 cells to the lower compartment (Fig. 1C). THP-1 was treated with 100 nM PMA for one day to induce the macrophage cells before adding to the compartment [19]. After testing the interference efficiency of circ_0000092 in HCC cells (Fig. S1A), Flow cytometry analysis was taken to detect the level of macrophage CD206 and macrophage CD86 before and after the circ_0000092 interference. After the silencing of circ_0000092, the expression of CD206 was decreased while that of CD86 was increased (Fig. 1D–E). Moreover, the mRNA expression level of macrophage M2 polarization markers (CCL17, CCL22 and ARG1) and that of macrophage M1 polarization markers (iNOS, TNF-α, and TLR4) was analyzed via qRT-PCR under the circ_0000092 interference. Similarly, the mRNA expression level of macrophage M2 polarization decreased while that of macrophage M1 polarization increased (Fig. 1F–G). To conclude, circ_0000092 promoted macrophage M2 polarization in HCC and macrophage co-culture system.

3.2. Circ_0000092 binds to U2AF2

The next move was to study on the specific molecular mechanism of hsa_circ_0000092 inducing macrophage M2 polarization. At first, FISH and subcellular fractionation assays were taken to detect the circ_0000092 distribution in MHCC97H and HCCLM3 cells. The results showed that circ_0000092 located in both cytoplasm and nucleus while mainly found in the cytoplasm (Fig. 2A). We have known that the regulation and function of circRNAs largely depend on the specificity of RBPs. The interaction between circRNA and RBP is considered to be an important factor in the study of circRNA [20,21]. Therefore, starBase was used to analyze the possible RBP that could bind to circ_0000092. The basic information of circ_0000092 in circBANK was demonstrated in Fig. S2A. The RBP results under the condition of CLIP-Data>10 showed that U2AF2, SRSF1, HNRNPC, IGF2BP3 and TAF15 were the possible RBPs (Fig. 2B). RIP assay shown that U2AF2 had the highest enrichment efficiency, thus U2AF2 was chosen for further study (Fig. 2C). Then RIP assay was taken to detect the enrichment of circ_0000092 on U2AF2 in HCCLM3 cells. The result indicated that U2AF2 could enrich circ_0000092 as well (Fig. 2D). FISH-IF assay was utilized to analyze the co-location of circ_0000092 and U2AF2 in MHCC97H and HCCLM3 cells. We found from the results that circ_0000092 was co-located in the cytoplasm with U2AF2 (Fig. 2E). U2AF2 was reported to be located in cytoplasm before [22]. RNA pull down and Western blot were used to detect the enrichment of U2AF2 protein in the pull-downs of biotinylated circ_0000092. The biotinylated circ_0000092 could enrich U2AF2, which indicated that circ_0000092 could bind to U2AF2 (Fig. 2F). In addition, qRT-PCR results showed that interfering with circ_0000092 had no effect on U2AF2, and interfering with U2AF2 also had no effect on circ_0000092 (Fig. S1B, S2B-C). To sum up, circ_0000092 binds to U2AF2 while does not affect the expression of each other.

3.3. Circ_0000092 stabilizes SMC1A through U2AF2

We have confirmed the binding relation between circ_0000092 and U2AF2. Therefore, we would learn on the regulatory function of circ_0000092 binding to U2AF2 on the downstream factors. Circ_0000092 might regulate the downstream gene expression through U2AF2. Thus we tried to find the possible mRNA binding to U2AF2 in starBase under the condition of CLIP-Data ≥ 10, pan-cancer ≥ 31. There were a lot of target genes which needed further screened. According to the literatures, transcription factors play critual role in regulating cancer progression and inducing macrophage polarization [23–25]. There were also a lot of articles reporting circRNA regulating malignant process of cancer cells through transcription factors [6–8]. Therefore, we wanted to select available transcription factors from the candidate genes. Combined with the mRNAs predicted by GEPIA and hTFtarget binding to U2AF2 (Fig. S3A), the transcription factor was listed in starBase (Fig. 3A). The result indicated that SMC1A, EWSR1, HCFC1, FOXM1, UBTF and SP2 were target transcription factors. According to the UALCAN retrieval results, all the 6 transcription factors were highly expressed in HCC (Fig. S3B). Based on the screening conditions, U2AF2 had the highest possibility of binding to SMC1A, so this gene was selected as a candidate research target. The enrichment of different mRNAs in MHCC97H was detected by RIP assay. Result indicated that SMC1A, EWSR1, HCFC1, FOXM1, UBTF and SP2 all could be enriched by U2AF2. Among them, U2AF2 had the highest enrichment efficiency in SMC1A. Combined with the above results of bioinformatics screening, SMC1A was determined as our object (Fig. 3B). Next, we were to verify the binding ability between U2AF2 and SMC1A, as well as the regulatory function of U2AF2 on SMC1A. It was reported that RBP could affect the mRNA stability through binding to mRNA 3′UTR region, including U2AF2 [22]. After the RNA pull down, Western blot

detected the enrichment of U2AF2 in with the pull-downs of biotinylated SMC1A 3′UTR, indicating that U2AF2 binds to SMC1A 3′ UTR region (Fig. 3C). Moreover, after silencing U2AF2, the expression of SMC1A in HCC cells was detected by qRT-PCR. The result indicated that when U2AF2 was silenced, SMC1A expression was decreased (Fig. 3D). Then the SMC1A mRNA stability in HCC cells was detected after interfering U2AF2. 50 mM α-amanitin was uesd to prevent RNA synthesis [26]. We found that after the interference of U2AF2, the half-life of SMC1A mRNA was shortened, indicating that U2AF2 affected the stability of SMC1A mRNA (Fig. 3E). Results above elucidated that U2AF2 promoted SMC1A stability via targeting SMC1A 3′ UTR region and thus promoted the up-regulation of SMC1A. Following, we came to verify whether circ-0000092 regulated SMC1A through U2AF2. In MHCC97H and HCCLM3 cells, RIP assay was taken to detect the effect of interfering circ_0000092 on the binding ability between U2AF2 and SMC1A. When circ_0000092 was down-regulated, the binding efficiency between U2AF2 and SMC1A 3′ UTR region decreased, indicating that circ_0000092 promoted U2AF2 to regulate the SMC1A expression (Fig. 3F). The regulation of circ_0000092 to SMC1A expression was measured by qRT-PCR in HCC cells. SMC1A expression inhibited when circ_0000092 was interfered (Fig. 3G). Finally, after the interference of U2AF2, SMC1A expression was decreased. There was no significant change in SMC1A expression when interfering with U2AF2 and overexpressing circ_0000092 at the same time, indicating that the regulation of SMC1A expression by circ_0000092 requires the

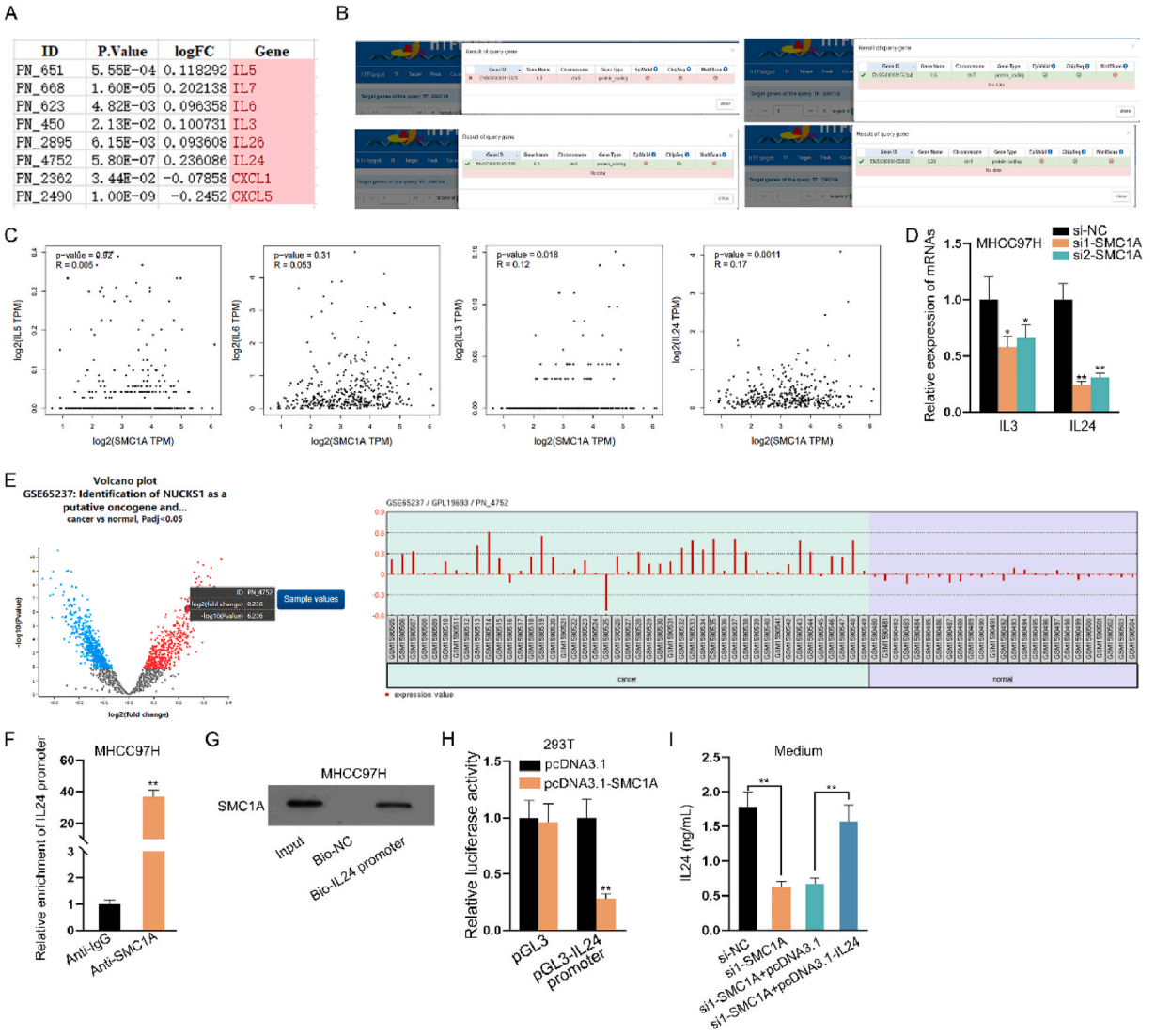

Fig. 4. SMC1A promotes IL24 secretion through transcriptional activation of IL24 expression A. List of abnormal expressed chemokine family and interleukin family genes in the GEO dataset GSE65237. B. HTFtarget predicted the possibility of SMC1A to be the target transcription factor. C. The expression correlation between SMC1A and candidate genes was predicted by GEPIA. D. In MHCC97H cells, the expression of IL3 and IL24 under SMC1A deficiency. E. GSE65237 showed that IL24 was obviously highly expressed in HCC tissues. F. ChIP-qPCR was taken to detect the abundance of IL24 promoter in anti-SMC1A complex. G. The enrichment of SMC1A protein in the pull-downs of biotin-labeled IL24 promoter. H. In 293T cells, luciferase reporter assays were taken to detect the influence of SMC1A to IL24 promoter. I. After the co-transfection of si-SMC1A and pcDNA3.1-IL24, Elisa was used to detect IL24. **p < 0.01.

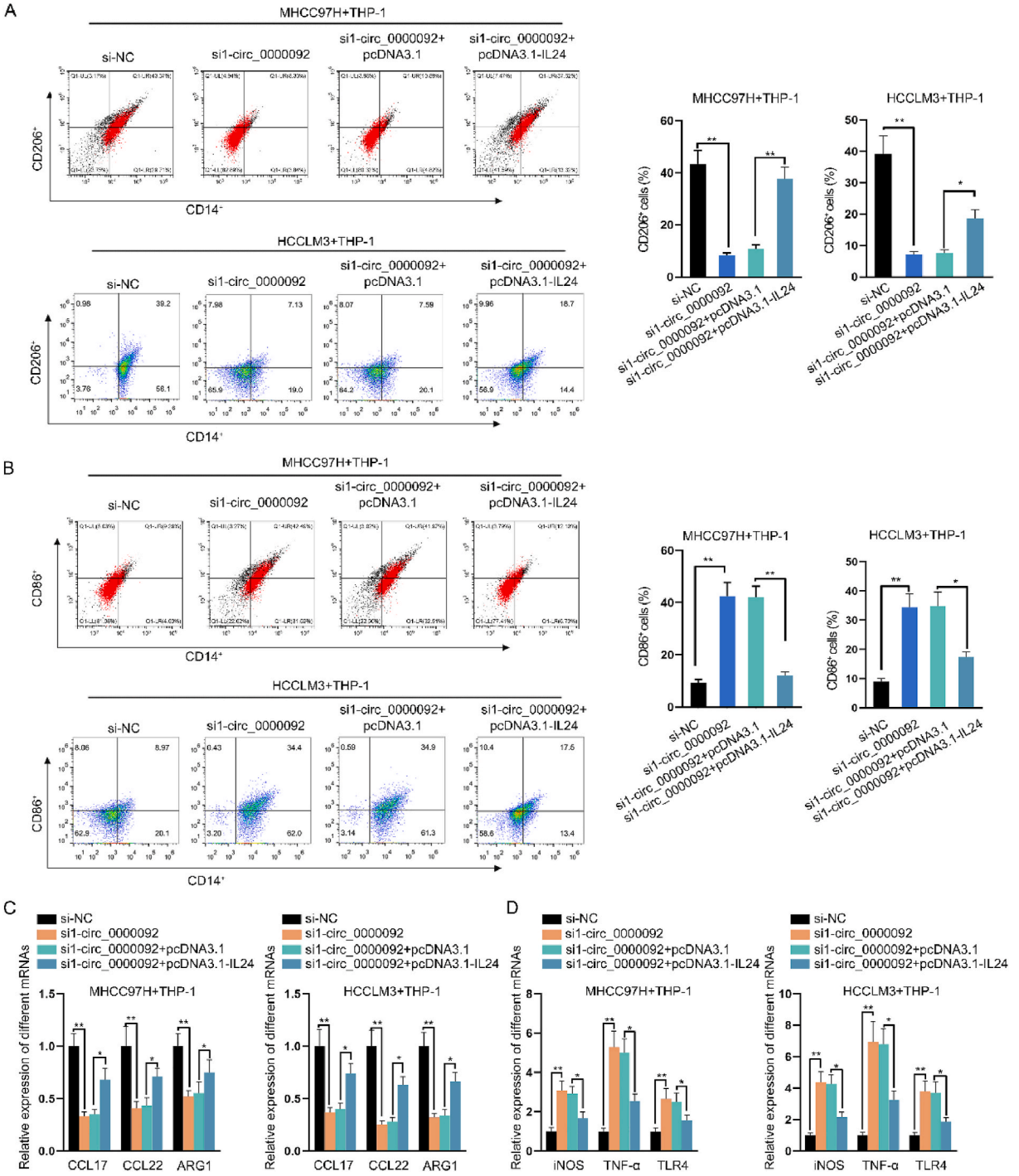

Fig. 5. circ_0000092 induces macrophage M2 polarization through IL24 A. Flow cytometry analysis was taken to detect the expression of CD206 after the co-transfection of si-circ_0000092 and pcDNA3.1-IL24 into HCC cells. B. CD86 expression was detected before and after circ_0000092 was interfered by flow cytometry. C. The mRNA levels of CCL17, CCL22 an ARG1 after the co-transfection of si1-circ_0000092 and pcDNA3.1-IL24. D. qRT-PCR was taken to detect the mRNA expression level of iNOS, TNF-α and TLR4 after the co-transfection of sil-circ_0000092 and pcDNA3.1-IL24. *$p < 0.05$, **$p < 0.01$.

participation of U2AF2 (Figs. S1C and 3H). We learned from the experiments above that circ_0000092 stabilized SMC1A mRNA via U2AF2 and thus promoted its expression.

3.4. SMC1A promotes IL24 secretion through transcriptional activation of IL24 expression

Transcription factors influence phenotypes by regulating the transcription of downstream genes. We looked for the effector factors regulated by SMC1A transcription, which were also required to play a role in inducing the polarization of macrophage M2. Abnormal expression of transcription factors can lead to abnormal expression of downstream regulated genes. Therefore, we used the GEO dataset GSE65237 to initially screen the genes that were abnormally expressed in HCC, and then narrowed the scope by reviewing the literatures. Tumor cells usually overexpress cytokines (including chemokines and interleukins) to regulate tumor development [27], and cytokines play an important role in TAMs polarization [28,29]. Therefore, we focused on the abnormal expression of chemokine family and interleukin family genes in GSE65237. The genes were listed in Fig. 4A. Among them, CXCL1 and CXCL5 expression decreased, which did not fit our expectation; IL7 and IL26 were reported to induce macrophage M1 polarization [30,31]; IL5, IL6, IL3 and IL24 were reported to correlate to macrophage M2 polarization and thus reserved as candidates. HTFtarget predicted the possibility of SMC1A to be the target transcription factor. The result showed that IL5 was not regulated by SMC1A transcription while IL6, IL3 and IL24 might be transcriptionally regulated by SMC1A (Fig. 4B). Then the expression correlation between SMC1A and candidate genes was predicted by GEPIA. It was found that SMC1A had obviously correlation with IL3 and IL24 (Fig. 4C). In MHCC97H cells, IL3 and IL24 level was examined by qRT-PCR after interfering SMC1A (Figs. S1D and 4D). After SMC1A was inhibited, IL3 and IL24 expression both decreased, while IL24 down-regulation was more evident. Combined with the results above, we decided to choose IL24 as the target. Moreover, GSE65237 showed that IL24 was obviously highly expressed in HCC tissues (Fig. 4E). The following experiments were designed to verify the specific mechanism of SMC1A transcriptionally regulate IL24. In MHCC97H cells, ChIP-qPCR was taken to detect the IL24 promoter enriched on SMC1A. SMC1A was confirmed to bind to IL24 promoter (Fig. 4F). DNA pull down assay demonstrated that SMC1A protein could be enriched in IL24 promoter, which further meant that SMC1A could bind to IL24 promoter (Fig. 4G). After verifying the overexpression efficiency of SMC1A (Fig. S1E), we confirmed that SMC1A could bind to IL24 promoter (Fig. 4H). IL24 is a cytokine that is usually secreted by cells into the extracellular environment to produce regulatory effects. Thereafter, we had to verify that SMC1A affected IL24 secretion by regulating IL24 expression. After the co-transfection of si-SMC1A and pcDNA3.1-IL24 into MHCC97H cells, Elisa was used to detect IL24. The result showed that the down-regulation of IL24 caused by SMC1A interference could be totally reversed by overexpressing IL24 (Fig. 4I). It meant that SMC1A indeed influenced IL24 secretion through regulating IL24 expression.

3.5. Circ_0000092 induces macrophage M2 polarization through IL24

In addition to verifying the interference efficiency of circ_0000092, we also verified the overexpression efficiency of IL24(Fig. S1F), followed by rescue assays. Flow cytometry analysis was used detect the expression of CD206 after the co-transfection of si-circ_0000092 and pcDNA3.1-IL24 into HCC cells. From the experimental results we found that the inhibited CD206 expression level caused by circ_0000092 interference could be partially rescued by overexpressing IL24 (Fig. 5A). CD86 expression was detected before and after circ_0000092 was interfered by flow cytometry. Also, the up-regulated CD86 expression level caused by circ_0000092 silencing could be partially countervailed by IL24 overexpression (Fig. 5B). Then qRT-PCR was taken to detect the mRNA expression level of CCL17, CCL22 and ARG1, as well as iNOS, TNF-α and TLR4 after the co-transfection of si1-circ_0000092 and pcDNA3.1-IL24. The influence caused by circ_0000092 down-regulation was partially reversed by IL24 overexpression (Fig. 5C–D). In conclusion, circ_0000092 promoted macrophage M2 polarization through IL24.

4. Discussion

HCC is the major cancer in the world [32], with the increasing deaths over the last several decades [32]. Therefore, it was urgent to explore more reliable targets for HCC to improve its prognosis.

The regulatory role of circRNAs has been affirmed in diverse cancers. Hsa_circRNA_104348 enhances HCC growth through miR-187-3p/RTKN2 axis and raises Wnt/beta-catenin pathway activate [33,34]; N(6)-methyladenosine-modified circRNA-SORE could sustain sorafenib resistance in HCC [35]. Circ_0000092, as a newly found circRNA, its functions in cancer has not been uncovered. Recently, it has been studied to promote HCC progression through up-regulating HN1 by binding to microRNA-338-3p [12]. Moreover, it was reported that TAMs M2 polarization promoted HCC EMT process [4,5]. However, the specific regulatory mechanism of circ_0000092 interacting with TAM was still unclear. In our study, circ_0000092 was known to promote M2 polarization of macrophages and the specific mechanism is also revealed.

Previous investigations have uncovered that circRNAs adjust and control tumor development by regulating miRNAs, and the ceRNA mechanism has been testified to be one of the most common mechanisms in regulating the development of various tumors [36]. Circ_0072995 enhances HCC progression through upregulating EIF4A3 by sponging miR-1253 [37]. Circ_0001955 facilitates HCC progression by sponging miR-516a-5p to upregulate TRAF6 and MAPK11 [38]. Circ_0091579 promotes HCC proliferation through sponging miR-1270 to increase YAP1 [39]. However , through our investigation, circ_0000092 could stabilize SMC1A mRNA through recruiting U2AF2 and thus promote the expression of SMC1A. Besides, SMC1A transcription could activate the expression of IL24. The expression of IL24 increased and induced M2 polarization of macrophages by secretion into extracellular.

The role of SMC1A in other cancers has been widely discussed. For example, SMC1A exerts a promotive effect on the EMT process

by augmenting SNAIL expression to aggravate gastric cancer [40]. Activation of the AKT/FOXM1/STMN1 signaling pathway via SMC1A contributes to the enhanced malignant progression of breast cancer cells [41]. However, there is still room to investigate its role in HCC, and unlike previous studies we also investigated the upstream mechanism of SMC1A. We showed that SMC1A expression is upregulated due to circ_0000092 binding to U2AF2. This is a noteworthy innovation of our study. In addition to the upstream mechanism of SMC1A, this study also explored the downstream mechanism of SMC1A to further reveal the effect of circ_0000092 on M2 polarization in macrophages. We found that SMC1A can transcriptionally activate IL24 to accelerate IL24 secretion, which promotes M2 polarization of macrophages and ultimately promotes the malignant development of HCC.

5. Conclusion

In short, this study validated that hsa_circ_0000092 facilitated macrophage M2 polarization by SMC1A/IL24 axis. and circ_0000092 up-regulates SMC1A through recruitment of transcription factor U2AF2. It is the first time to uncover that SMC1A transcriptionally activates the IL24 expression. Moreover, we found that IL24 plays a role in inducing macrophage M2 polarization. Thus, it was considered that this report might be regarded as a reference for relevant studies on HCC. Besides, further analysis of the correlation between the expression of circ_0000092, U2AF2, SMC1A and IL24 with clinicopathological data and prognosis in HCC patients will be conducted as further planning experiments.

Data availability statement

All data, models, and code generated or used during the study appear in the submitted article.

CRediT authorship contribution statement

Rihai Ma: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Methodology, Investigation, Formal analysis, Data curation. **Anmin Wang:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Data curation. **Meng Yang:** Writing – review & editing, Writing – original draft, Software, Methodology, Formal analysis. **Zihua Huang:** Writing – original draft, Validation, Investigation, Data curation. **Guoman Liu:** Writing – original draft, Validation, Supervision, Formal analysis. **Qing Wei:** Writing – original draft, Validation, Supervision, Resources, Formal analysis. **Yuan Lu:** Validation, Methodology, Formal analysis, Data curation. **Huamei Wei:** Writing – original draft, Resources. **Jianchu Wang:** Software, Investigation, Formal analysis. **Qianli Tang:** Supervision, Investigation. **Jian Pu:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e36517.

References

[1] J. Ferlay, et al., Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012, Int. J. Cancer 136 (5) (2015) E359–E386.
[2] N. Li, Y. Zhu, Targeting liver cancer stem cells for the treatment of hepatocellular carcinoma, Therap Adv Gastroenterol 12 (2019) 1756284818821560.
[3] G. Giannelli, et al., Role of epithelial to mesenchymal transition in hepatocellular carcinoma, J. Hepatol. 65 (4) (2016) 798–808.
[4] G. Lilja, et al., Immunotherapy with cat- and dog-dander extracts. IV. Effects of 2 years of treatment, J. Allergy Clin. Immunol. 83 (1) (1989) 37–44.
[5] O.W. Yeung, et al., Alternatively activated (M2) macrophages promote tumour growth and invasiveness in hepatocellular carcinoma, J. Hepatol. 62 (3) (2015) 607–616.
[6] F. Yang, et al., Cis-acting circ-CTNNB1 promotes β-catenin signaling and cancer progression via DDX3-mediated transactivation of YY1, Cancer Res. 79 (3) (2019) 557–571.
[7] D. Chen, et al., CircRNA hsa_circ_100395 regulates miR-1228/TCF21 pathway to inhibit lung cancer progression, Cell Cycle 17 (16) (2018) 2080–2090.
[8] L. Ding, et al., Circular RNA circ-DONSON facilitates gastric cancer growth and invasion via NURF complex dependent activation of transcription factor SOX4, Mol. Cancer 18 (1) (2019) 45.
[9] J. Xu, et al., CircRNA-SORE mediates sorafenib resistance in hepatocellular carcinoma by stabilizing YBX1, Signal Transduct Target Ther 5 (1) (2020) 298.
[10] Z. Liu, et al., CircRNA-5692 inhibits the progression of hepatocellular carcinoma by sponging miR-328-5p to enhance DAB2IP expression, Cell Death Dis. 10 (12) (2019) 900.
[11] X.Y. Huang, et al., Exosomal circRNA-100338 promotes hepatocellular carcinoma metastasis via enhancing invasiveness and angiogenesis, J. Exp. Clin. Cancer Res. 39 (1) (2020) 20.
[12] J. Pu, et al., hsa_circ_0000092 promotes hepatocellular carcinoma progression through up-regulating HN1 expression by binding to microRNA-338-3p, J. Cell Mol. Med 28 (6) (2024) e15010.
[13] X.W. Pan, et al., SMC1A promotes growth and migration of prostate cancer in vitro and in vivo, Int. J. Oncol. 49 (5) (2016) 1963–1972.
[14] P. Zhou, et al., SMC1A recruits tumor-associated-fibroblasts (TAFs) and promotes colorectal cancer metastasis, Cancer Lett. 385 (2017) 39–45.

[15] X. Zhang, et al., SMC1A regulated by KIAA1429 in m6A-independent manner promotes EMT progress in breast cancer, Mol. Ther. Nucleic Acids 27 (2022) 133–146.
[16] Y. Zhong, X. Zhang, W. Chong, Interleukin-24 immunobiology and its roles in inflammatory diseases, Int. J. Mol. Sci. 23 (2) (2022).
[17] U.M. Zissler, et al., Interleukin-4 and interferon-γ orchestrate an epithelial polarization in the airways, Mucosal Immunol. 9 (4) (2016) 917–926.
[18] C. Wu, et al., Exosome miR-23a-3p from Osteoblast Alleviates Spinal Cord Ischemia/Reperfusion Injury by down-regulating KLF3-activated CCNL2 transcription, Dev. Neurosci. 44 (3) (2022) 121–130.
[19] S. Tao, et al., Linc00514 promotes breast cancer metastasis and M2 polarization of tumor-associated macrophages via Jagged1-mediated notch signaling pathway, J. Exp. Clin. Cancer Res. 39 (1) (2020) 191.
[20] A. Huang, et al., Circular RNA-protein interactions: functions, mechanisms, and identification, Theranostics 10 (8) (2020) 3503–3517.
[21] Y.M. Sun, et al., circMYBL2, a circRNA from MYBL2, regulates FLT3 translation by recruiting PTBP1 to promote FLT3-ITD AML progression, Blood 134 (18) (2019) 1533–1546.
[22] S.K. Gupta, et al., Basal splicing factors regulate the stability of mature mRNAs in trypanosomes, J. Biol. Chem. 288 (7) (2013) 4991–5006.
[23] Y. Jiramongkol, E.W. Lam, FOXO transcription factor family in cancer and metastasis, Cancer Metastasis Rev. 39 (3) (2020) 681–709.
[24] T. Yu, et al., Modulation of M2 macrophage polarization by the crosstalk between Stat6 and Trim24, Nat. Commun. 10 (1) (2019) 4353.
[25] C.S. Lu, et al., Oct4 promotes M2 macrophage polarization through upregulation of macrophage colony-stimulating factor in lung cancer, J. Hematol. Oncol. 13 (1) (2020) 62.
[26] Y.P. Hu, et al., LncRNA-HGBC stabilized by HuR promotes gallbladder cancer progression by regulating miR-502-3p/SET/AKT axis, Mol. Cancer 18 (1) (2019) 167.
[27] J. Candido, T. Hagemann, Cancer-related inflammation, J. Clin. Immunol. 33 (Suppl 1) (2013) S79–S84.
[28] S.C. Funes, et al., Implications of macrophage polarization in autoimmunity, Immunology 154 (2) (2018) 186–195.
[29] C. Atri, F.Z. Guerfali, D. Laouini, Role of human macrophage polarization in inflammation during infectious diseases, Int. J. Mol. Sci. 19 (6) (2018).
[30] C. Bao, et al., Blockade of interleukin-7 Receptor Shapes macrophage alternative activation and promotes functional recovery after spinal cord injury, Neuroscience 371 (2018) 518–527.
[31] Y.H. Lin, et al., Interleukin 26 skews macrophage polarization towards M1 phenotype by activating cJUN and the NF-κB pathway, Cells 9 (4) (2020).
[32] D.W. Kim, C. Talati, R. Kim, Hepatocellular carcinoma (HCC): beyond sorafenib-chemotherapy, J. Gastrointest. Oncol. 8 (2) (2017) 256–265.
[33] G. Huang, et al., CircRNA hsa_circRNA_104348 promotes hepatocellular carcinoma progression through modulating miR-187-3p/RTKN2 axis and activating Wnt/β-catenin pathway, Cell Death Dis. 11 (12) (2020) 1065.
[34] T.S. Han, et al., Epigenetic associations between lncRNA/circRNA and miRNA in hepatocellular carcinoma, Cancers 12 (9) (2020).
[35] J. Xu, et al., N(6)-methyladenosine-modified CircRNA-SORE sustains sorafenib resistance in hepatocellular carcinoma by regulating β-catenin signaling, Mol. Cancer 19 (1) (2020) 163.
[36] J. Zhu, et al., lncRNA/circRNA-miRNA-mRNA ceRNA network in lumbar intervertebral disc degeneration, Mol. Med. Rep. 20 (4) (2019) 3160–3174.
[37] Q. He, et al., Circ_0072995 promotes proliferation and invasion via regulating miR-1253/EIF4A3 signaling in HCC, Cancer Manag. Res. 13 (2021) 5981–5987.
[38] Z. Yao, et al., Circ_0001955 facilitates hepatocellular carcinoma (HCC) tumorigenesis by sponging miR-516a-5p to release TRAF6 and MAPK11, Cell Death Dis. 10 (12) (2019) 945.
[39] M. Liu, et al., Circ_0091579 knockdown inhibited HCC proliferation and Glutamine Metabolism through miR-1270/YAP1 Axis, Biochem. Genet. 62 (1) (2024) 208–228.
[40] Y. Liu, et al., SMC1A facilitates gastric cancer cell proliferation, migration, and invasion via promoting SNAIL activated EMT, BMC Gastroenterol. 23 (1) (2023) 268.
[41] K. Li, et al., AKT/FOXM1/STMN1 signaling pathway activation by SMC1A promotes tumor growth in breast cancer, J. Gene Med. 26 (1) (2024) e3661.