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ORIGINAL RESEARCH

FOXA1 Promotes Cell Proliferation and Suppresses Apoptosis in HCC by Directly Regulating miR-212-3p/FOXA1/AGR2 Signaling Pathway

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Background: Forkhead box protein A1 (FOXA1), acting as a transcriptional activator for liver-specific transcripts, plays a vital part in proliferation, apoptosis and cell cycle.
Methods: The mRNA expression of FOXA1 in 90 HCC tissues and matched adjacent non-tumor tissues was determined by qRT-PCR. The downstream and upstream regulators of FOXA1 were identified by bioinformatics analysis and experimental confirmation.
Results: We found out that the expression of FOXA1 was obviously higher in hepatocellular carcinoma (HCC) tissues than that in matched non-tumor tissues. Similarly, FOXA1 is also highly expressed in HCC cell lines as compared with normal human hepatic cell line L02. Clinical association analysis indicated that high expression of FOXA1 was prominently correlated with high HBV level, large tumor size, high venous infiltration, high Edmondson–Steiner grading, and advanced tumor-node-metastasis tumor stage. Furthermore, the in vitro tests showed that ectopic expression of FOXA1 promoted HepG2 cell proliferation and suppressed apoptosis. In contrast, the downregulation of FOXA1 inhibited cell proliferation, and induced apoptosis in Hep3B cells. To investigate the functional mechanism of FOXA1, anterior gradient 2 (AGR2), an executor in proliferation and apoptosis, was identified as the direct target gene of FOXA1. Meanwhile, we also found the expression of FOXA1 could be inhibited by miR-212-3p, which working as a tumor suppressor downregulated in HCC.
Conclusion: We revealed that FOXA1 exerted its biological function by regulating AGR2 expression, and its ectopic expression may be blamed for low expression of miR-212-3p.
Keywords: FOXA1, AGR2, miR-212-3p, HCC

Background

Hepatocellular carcinoma (HCC) has been one of the cancers with the highest probability of mortality.[1,2] Recently, a lot of proteins have been reported as HCC biomarkers or therapeutic targets, including Forkhead box protein A1 (FOXA1) and Anterior gradient homolog 2 (AGR2). As a transcriptional activator for liver-specific transcripts, FOXA1 plays a key role in proliferation, apoptosis and cell cycle. Many physiology and pathology studies on cancer reported that overexpression of FOXA1 was associated with poor prognosis of urothelial carcinoma,[3] colorectal cancer,[4] prostate cancer,[5] and glioma.[6]

AGR2, also known as secreted cement gland protein XAG-2 homolog, is a suppressor of p53 via preventing DNA damage-induced phosphorylation of p53.[7] Later studies showed that AGR2 was overexpressed in adenocarcinomas of the

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esophagus,[8] pancreatic cancer,[9] breast cancer,[10] prostate cancer,[11] small cell lung cancer[12] and liver cancer.[13] In esophageal adenocarcinoma cells, AGR2 can be used to improve distinguishing adenocarcinoma (99% positive) from squamous cell carcinoma (37% positive, typically in focal areas) in some circumstances[14] and upregulation of AGR2 could promote transform of NIH3T3 cells and tumor growth.[8] In contrast, downregulation of AGR2 was associated with decreased cancer cell proliferation and invasion, and increased gemcitabine sensitivity of resistant cells in vitro and in vivo.[9]

MicroRNAs (miRNAs) are small, noncoding RNAs (20–24 nt). It is well known that miRNAs function in RNA silencing and post-transcriptional regulation of gene expressions by directly interacting with the 3′-UTR or 5′-UTR of target mRNAs.[15,16] Increasing studies have reported that miRNAs participate in the development of tumors by regulating carcinogenesis-related gene expression.[17–19]

In our study, it was investigated that FOXA1 was highly expressed in HCC tissues, which was significantly associated with poor prognosis of the patients with HCC. To explore the biological function of FOXA1 in HCC, cell proliferation and apoptosis array were performed. The results showed that FOXA1 promoted cell proliferation and suppressed apoptosis by regulating AGR2 expression and that miR-212-3p participated in the posttranscriptional regulation of FOXA1. These results indicated a novel insight into the initiation and progression of HCC.

Methods

Tissues and Tissue Microarray

Tissues of 90 formalin-fixed paraffin-embedded (FFPE) tissues including cancerous and corresponding adjacent non-tumor specimens were obtained from HCC patients including 73 males and 17 females in Minhang Branch, Zhongshan Hospital Affiliated to Fudan University. The diagnosis had been done by two pathologists who were blinded to the clinicopathological data. A detail of the patient clinicopathological data is shown in Table 1.

All the patient consent was written informed consent. The study was approved by the Minhang Hospital of Medicine Ethics Committee and that this was conducted in accordance with the Declaration of Helsinki. Ninety pairs of tissues were prepared for tissue microarray (TMA)[20] from the aforementioned FFPE tissues. Each patient's specimen was represented by a single 1 mm core of tissue. The 90 paired TMAs were used for immunohistochemistry (IHC) staining to detect the expression of FOXA1.

Immunohistochemistry (IHC)

IHC was performed following the two-step protocol (Novolink Polymer Detection System) as previously described.[21] Anti-FOXA1 antibody (1:200, Abcam; ab23738) was used. Intensity and percentage of positive staining of the nucleus in the whole view were calculated.[22] The staining intensity was scored and divided into four scales: negative (0), weak (1), medium (2) or strong (3). The staining extent, which was defined as the percentage of positive staining areas of tumor cells relative to the whole tumor area, was scored and divided into five scales: 0 (0%), 1 (1–25%), 2 (26–50%), 3 (51–75%) and 4 (76–100%). The overall protein expression score = staining intensity score × staining extent score. Finally, the overall protein expression score of ≤6 was considered as low protein expression, on the contrary, >8 as high protein expression.

Cell Culture and Transfection

HCC cell lines HepG2, Hep3B, Huh-7 were purchased from the Institute of Biochemistry and Cell Biology of the Chinese Academy of Sciences (Shanghai, China). MHCC97L, MHCC97H and the normal liver cell line Chang's, HL-7702 (L02) were obtained from Zhongshan Hospital Affiliated to Fudan University. The use of these cell lines was approved by the Minhang Hospital of Medicine Ethics Committee. These cell lines were originally purchased from the Committee on Type Culture Collection of Chinese Academy of Sciences (Shanghai, China). Chang's and HL-7702 cell lines were authenticated by STR profile. All the cell lines were cultured in a 5% CO_2 incubator at 37°C with high glucose DMEM (Hyclone, USA) and 10% fetal bovine serum (Gibco, USA).

Gene expression plasmids, including pcDNA3.1-FOXA1 and pcDNA3.1-AGR2, were maintained in our lab. The miRNA vectors for miR-212-3p expression vector (HmiR 0269-MR04) and miR-212-3p inhibitor (HmiR-AN0319-AM04) were purchased from Fulengen (Guangzhou, China). The vector for miR-212-3p (CmiR0001-MR04) and for miR-212-3p inhibitor (CmiR-AN0001-AM04) were used as negative control. The targeted sequences for FOXA1 siRNA duplex (5′-GCACUGCAAUAC UCGCCUU-3′), AGR2 siRNA duplex (5′- CCUCAAUCUGGUUUA UGAA-3′) or negative control were all synthesized in Sangon Biotech (Shanghai) Co., Ltd. First, cells seeded 10^6 every well, next day transfected with plasmid using Lipofectamine 2000 (Thermo Fisher Scientific) according

Table 1 Clinicopathologic Characteristics of miR-212 and FOXA1 Expression in HCC

Characteristics	Total No. of Patients	No. of Patients		*p* value	No. of Patients		*p* value
	n=90	FOXA1^{low}	FOXA1^{high}		miR-212-3p^{low}	miR-212-3p^{high}	
Age (y)							
<50	25	15	10	0.787	13	12	0.579
≥50	65	41	24		38	27	
Sex							
Male	73	46	27	0.748	40	33	0.458
Female	17	10	7		11	6	
HBV							
Absent	15	13	2	0.032*	9	6	0.775
Present	75	43	32		42	33	
Serum AFP level (mg/mL)							
<400	31	22	9	0.215	12	19	0.013*
≥400	59	34	25		39	20	
Tumor size (cm)							
<5	37	28	9	0.028*	11	26	<0.001*
≥5	53	28	25		40	13	
No. of tumor nodules							
1	55	29	24	0.115	34	21	0.216
≥2	35	25	10		17	18	
Cirrhosis							
Absent	33	18	15	0.253	19	14	0.895
Present	57	38	19		32	25	
Venous infiltration							
Absent	47	36	11	0.035*	22	25	0.049*
Present	53	30	23		29	14	
Edmondson–Steiner grading							
I+II	24	19	5	0.046*	11	13	0.211
III+IV	66	37	29		40	26	
TNM tumor stage							
I+II	54	40	14	0.005*	24	30	0.004*
III+IV	36	16	20		27	9	

Note: *p<0.05, statistically significant.
Abbreviations: HCC, hepatocellular carcinoma; HBV, hepatitis B virus; AFP, alpha-fetoprotein; TNM, tumor-node-metastasis.

to the manufacturer's instructions. After 8 h incubation, cells were replaced with fresh medium.

RNA Extraction and Quantitative Reverse-Transcription Real-Time PCR Analysis

Total RNA was extracted from tumor samples or HCC cell lines by Trizol Reagent (Invitrogen), and then reverse transcription was performed using first-strand cDNA synthesis kit (Fermentas). For miR-212-3p, the reverse-transcription PCR procedure was performed using miRNA cDNA Synthesis Kit (D350A, TaKaRa). The expression of miR-212-3p, FOXA1 and AGR2 was determined using SYBR Premix Ex Taq (TaKaRa). GAPDH and U6 were used as an endogenous control. The relative expression of genes was calculated and expressed as $2^{-\Delta\Delta Ct}$. The primers were as follows: FOXA1: forward 5′- GCAATACTCGCCTTACGGCT-3′, reverse 5′- TAC ACACCTTGGTAGTACGCC-3′; AGR2: forward 5′- GTCAGCATTCTTGCTCCTTGT-3′, reverse 5′- GGG TCGAGAGTCCTTTGTGTC-3′; GAPDH: forward 5′- GAAGGTGAAGGTCGGAGTC-3′, reverse 5′-GA AGATGGTGATGGGATTTC −3′. The primers for

miR-212-3p (HmiRQP0319) and U6 (HmiRQP9001) were purchased from Fulengen (Guangzhou, China).

Cell Proliferation and Apoptosis Array

Detecting of cell proliferation was performed by Cell Counting Kit-8 (CCK-8; Dojindo, Japan) assays. In brief, HepG2 and Hep3B cells were seeded in 96-well plates. Twenty-four hour later, the two cells were transfected with gene expression vector and siRNA, respectively. Four wells were settled for each group and there were approximately 3–5×10^4 cells were seeded in each well. Then, 10 μL CCK-8 reagent was added to each well after 0, 24, 48, 72 h, respectively. The absorbance at 450 nm was measured using a microplate reader. For apoptosis array, cells were treated with 5-fluorouracil (5-FU, 50 μM) for 24 h and harvested in PBS. Annexin-V-FLUOS Staining Kit (11858777001, Roche) was used and the percentage of apoptotic cells were calculated by flow cytometry.

Western Blotting Analysis

To obtain the whole cell lysates, cells were processed by RIPA buffer. Then, the BCA kit (Pierce) was used to measure protein concentrations. Fifty milligrams of total cell lysates was loaded onto SDS-PAGE. Resolved proteins were transferred onto PVDF membranes (Millipore) and immunoblotted with anti-FOXA1 antibody (Ab23738) or anti-AGR2 antibody (AB76473). β-Actin was used as the endogenous control. Finally, the results were analyzed by ImageJ software (National Institutes of Health, Bethesda, MD, USA).

Luciferase Reporter Assay

It is predicted that the 3′- UTR of FOXA1 may be combined with miR-212-3p. The WT and mutated sequences (control) were synthesized and inserted into pGL3 vector to construct miRNA vectors, pGL3-FOXA1-3′UTR (WT) and pGL3-FOXA1-3′UTR (Mut). For the reporter assays, HepG2 cells were seeded into 24-well plates and transfected with pGL3-FOXA1-3′UTR (WT) or pGL3-FOXA1 -3′UTR (Mut) combining with miR-212-3p expression vector, miR-212-3p inhibitor, control vector, or negative control, respectively. Forty-eight hours later, the cells were harvested, and Renilla luciferase activity was measured using the Dual-Luciferase® Reporter Assay System (Promega Corporation), according to the manufacturer's instructions. Three independent experiments were performed.

AGR2 promoter regulated by FOXA1 was assayed as above. The primers for pGL3-AGR2 were as follows: forward 5′-AAGGTACCGCATCCCATTCATTAACTTTC -3′ and reverse 5′- TCCAAGCTTCTGAGTGTGCCAGC-3′ for pGL3-AGR2 (−823/+129).

Chromatin Immunoprecipitation (ChIP)

ChIP was performed according to the manufacturer's recommendations (Active Motif, Carlsbad, CA). Anti-FOXA1 antibody (Ab23738, Abcam) was used to IP the TF/chromatin complex. DNA was purified from the IPed TF/chromatin complex using a PCR purification kit (Qiagen). The purified products were analyzed by real-time PCR. The primers against AGR2 promoter were as follows: sense 5′-GGA AGGTTCGTTTCTGAGTT and anti-sense 5′- AGCTGT GCTGGCACACTCAGACAGCT-3′.

Statistical Analysis

Results were described with mean ± SD. Statistical significance was performed SPSS 20.0 (SPSS, Chicago, IL, USA) and GraphPad Prism 6 software (GraphPad Software, Inc, San Diego, CA, USA). Pearson chi-squared test, multivariant Cox regression analysis, Kaplan–Meier plot, Log-rank test, Spearman's rank correlation coefficient, or a two-tailed Student's *t*-test were used for significance analysis, in which $p<0.05$ was set as the threshold.

Results

FOXA1 Was Highly Expressed in HCC and Correlated with Poor Prognosis

To measure the mRNA expression levels of FOXA1, qRT-PCR was performed in 90 HCC tissues and matched adjacent non-tumor tissues. The result showed that the mRNA levels of FOXA1 were higher in HCC tissues than that in matched non-tumor tissues (Figure 1A). To conform the above results, the TCGA liver cancer dataset was used, and the mRNA levels of FOXA1 were higher in 50 paired HCC samples, as shown in our data (Figure 1B). Like the pattern of mRNA, the protein expression of FOXA1 was higher in HCC tissues by IHC (Figure 1C). Moreover, FOXA1 expression was also higher in HCC cell lines than in L02 cell line (Figure 1D). Clinical data analysis revealed that higher expression of FOXA1 was significantly associated with HBV infection ($p=0.032$), large tumor size ($p=0.028$), venous infiltration ($p=0.035$), high Edmondson–Steiner grading ($p=0.046$) and advanced TNM stage ($p=0.005$) (Table 1). It was demonstrated that

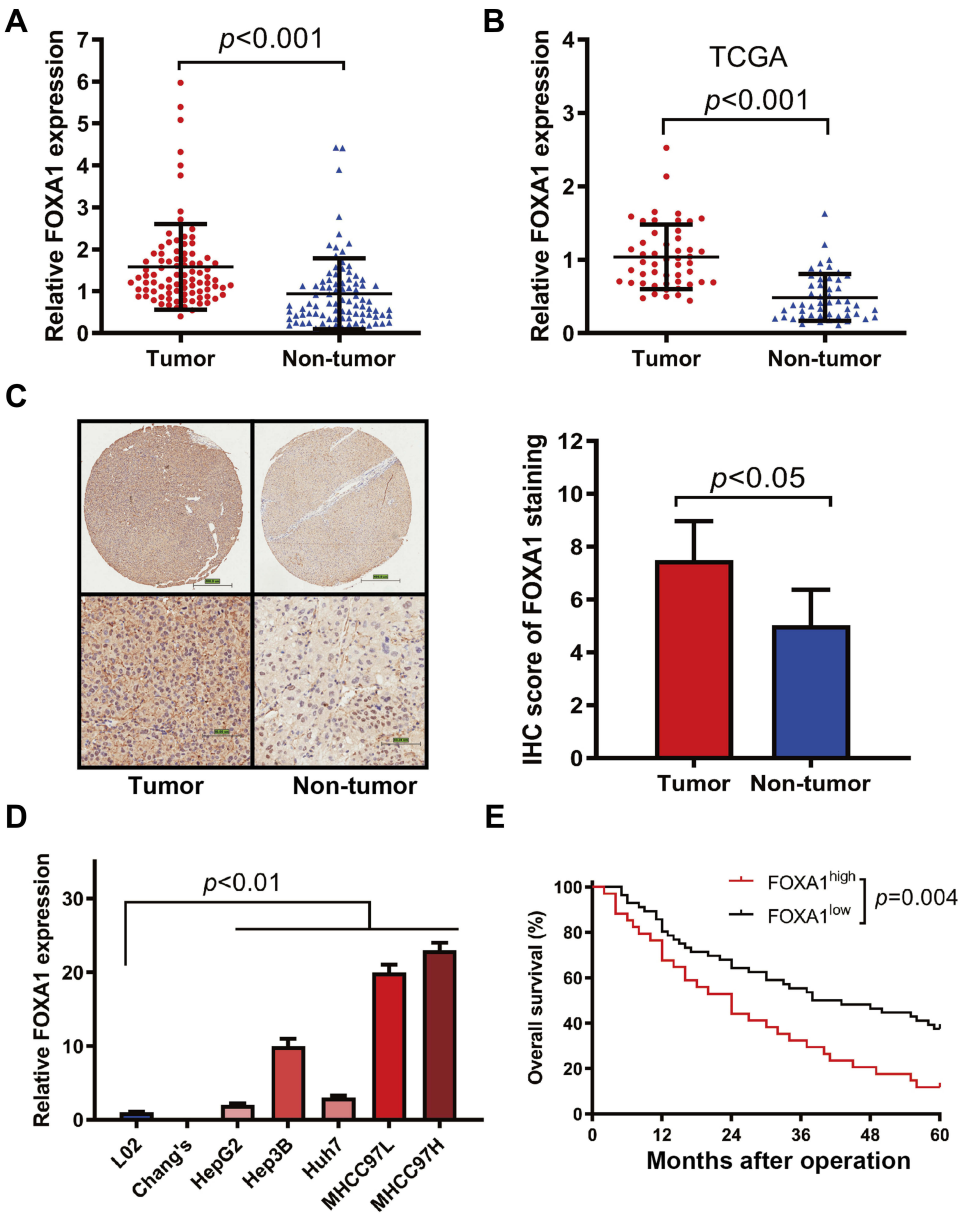

Figure 1 FOXA1 was upregulated in HCC and correlated with poor prognosis. (**A**) FOXA1 mRNA was overexpressed in tumor tissue compared with corresponding adjacent livers in 90-paired HCC patients via quantitative RT-PCR analysis. (**B**) FOXA1 expression in 50 pairs of HCC samples from TCGA. (**C**) Representative images of immunohistochemistry (IHC) in 90 pairs of HCC tissues showing the protein levels of FOXA1 in HCC and adjacent non-tumor tissue. (**D**) Differential expression of FOXA1 in HCC cell lines (HepG2, Hep3B, Huh7 and MHCC97L and MHCC97H) compared with the immortalized normal human hepatic cell Chang's and L02 cell line. (**E**) Kaplan–Meier analysis of OS was compared between patients with high and low expression of FOXA1.

patients with higher expression of FOXA1 had shorter overall survival (OS) (p=0.004, Figure 1E) using Kaplan–Meier and Log-rank tests.

FOXA1 Regulated AGR2 Expression Through Directly Binding to AGR2 Promotor in HCC Cells

As a transcription factor, FOXA1 functions depending on the regulation of target genes. Using Gene Transcription Regulation Database (GTRD, http://gtrd.biouml.org/), we found AGR2 was a potential target gene regulated by FOXA1. Furthermore, at least five FOXA1 binding sites were predicted in AGR2 promoter region using alibaba2 web tools (http://gene-regulation.com/pub/programs/alibaba2/index.html). To investigate whether AGR2 is a target gene of FOXA1 in HCC cells, we examined the mRNA expression of FOXA1 and AGR2 in 90 HCC samples using qRT-PCR. It was confirmed that the expression level of

AGR2 was significantly correlated with that of FOXA1 in HCC (Figure 2A).

Moreover, the low FOXA1-expressing HepG2 cells were transiently transfected with pcDNA3.1-FOXA1, and the mRNA level of AGR2 was measured by Western blotting. In a complementary experiment, we down-regulated FOXA1 expression in high FOXA1-expressing Hep3B cells using FOXA1-specific siRNA and measured AGR2 expression level. The results demonstrated that regulation of FOXA1 expression directly results in a corresponding change of AGR2 expression in HCC cell lines (Figure 2B).

To investigate whether AGR2 was directly regulated by FOXA1, luciferase reporter assay and ChIP were performed. In luciferase reporter assay, HepG2 cells were transiently transfected with pGL3-AGR2 (−823/+129) and pcDNA3.1-FOXA1 (or pcDNA3.1 as control). In this assay, FOXA1 up-regulated AGR2 promoter activity in a 4.7-fold manner compared with control (Figure 2C). For ChIP, FOXA1-DNA complexes were immunoprecipitated from Hep3B cells. The IPed gDNA was collected and detected by pPCR using primers targeting *AGR2* gene promoter region. The ChIP-qPCR result showed that AGR2 gene promoter was about 5-fold enriched (Figure 2D). Thus, it was verified that FOXA1 bound to AGR2 promoter in intact cells, consistent with our hypothesis that AGR2 is a bona fide FOXA1 target gene.

FOXA1 Promoted Proliferation and Suppressed Apoptosis in HCC Cell Lines Depending on AGR2

To explore the potential biological function of FOXA1, knock-down assay was performed in Hep3B cells (Figure 2B). Previous research has shown that FOXA1 participated in cell proliferation.[23] Our results not only confirmed that but also

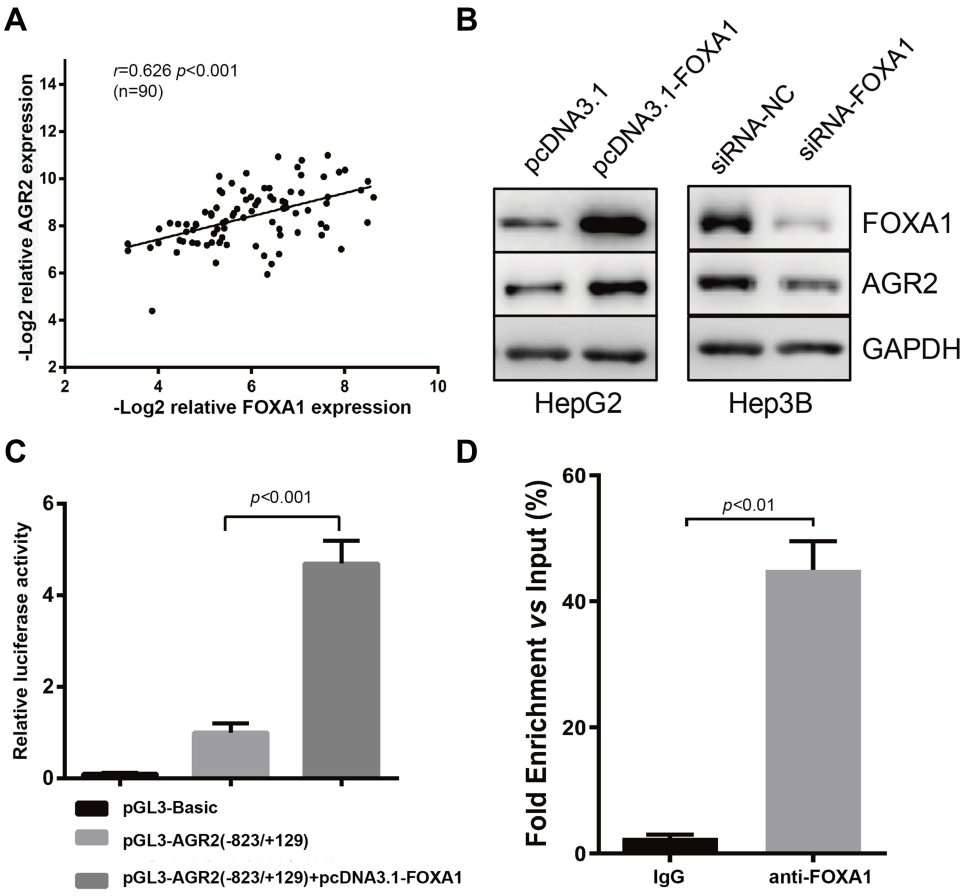

Figure 2 FOXA1 regulated AGR2 expression through directly binding to AGR2 promotor in HCC cells. (**A**) Total RNA was extracted from 90 HCC samples and analyzed by quantitative RT-PCR using FOXA1- or AGR2-specific primers. The relative abundance of mRNA was calculated by normalization to GAPDH mRNA. $-\log_2$ (relative AGR2 mRNA expression) was plotted against $-\log_2$ (relative WT1 mRNA expression). Standard linear regression analysis was performed to quantify the correlation between these values, and the coefficient of correlation was found to be r=0.626. (**B**) FOXA1 overexpression induced the protein level of AGR2, and siRNA-FOXA1 reduced the protein level of AGR2. (**C**) Luciferase reporter assay showing that overexpression of FOXA1 induced luciferase activity of wild-type FOXA1 3′UTR but not that of the mutant. (**D**) Chromatin immunoprecipitation was performed using antibodies against FOXA1, or a control IgG. Precipitated DNA was analyzed by quantitative PCR using primers for AGR2. The graph shows the enrichment in DNA immunoprecipitated by the IgG or anti-FOXA1 antibody compared with the input.

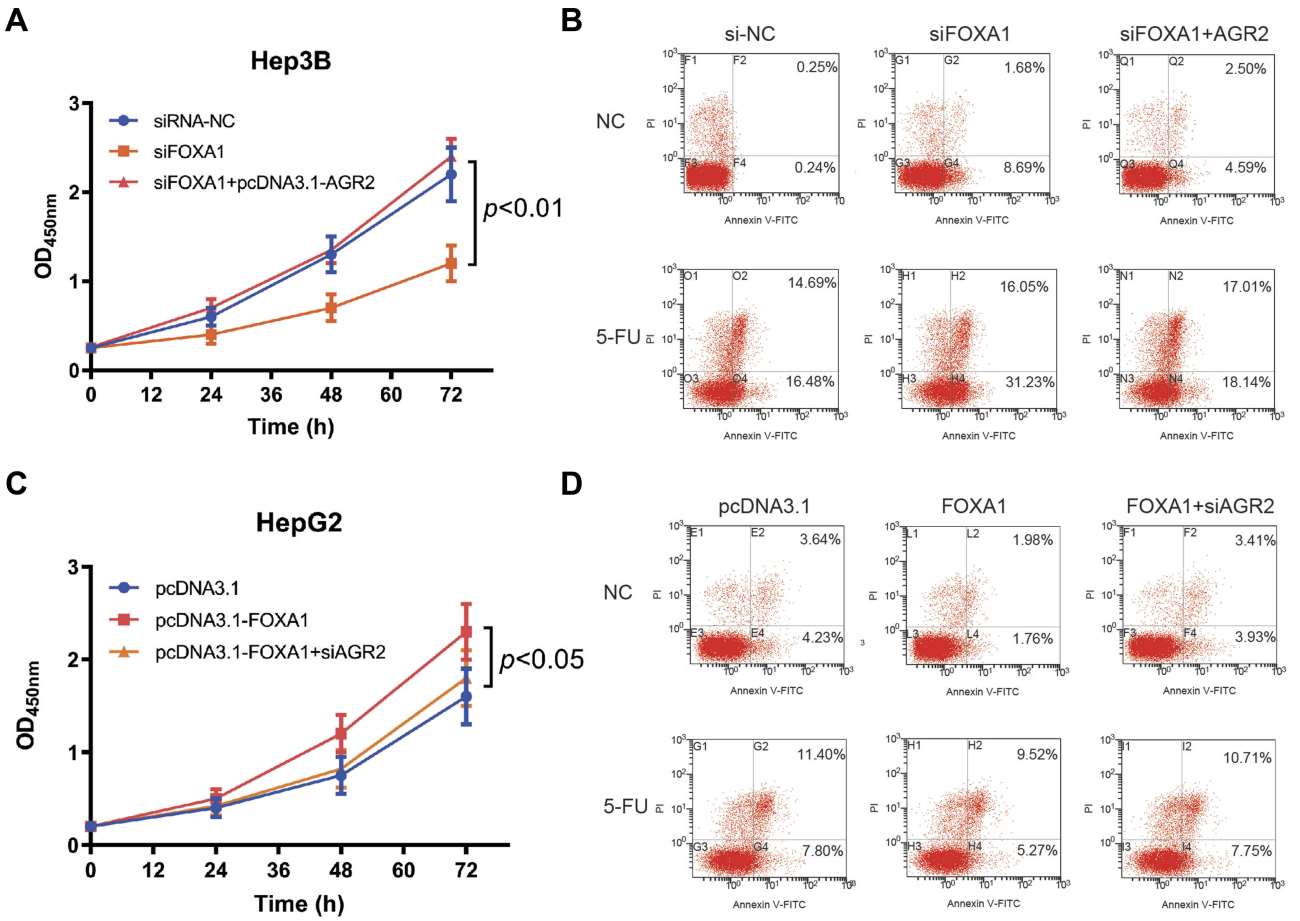

Figure 3 FOXA1 promoted proliferation and suppressed apoptosis in HCC cell lines depending on AGR2. (**A**) CCK8 assay showing that downregulation of FOXA1 inhibited cell proliferation in Hep3B cell lines, rescue experiments further confirmed that overexpression of AGR2 could recover the pro-proliferation ability of FOXA1-knockdown Hep3B cells. (**B**) Apoptosis assay showing that downregulation of FOXA1 promoted cell apoptosis in Hep3B cell lines, rescue experiments further confirmed that overexpression of AGR2 could recover the anti-apoptosis ability of FOXA1-knockdown Hep3B cells. (**C**–**D**) Overexpression of FOXA1 promoted HepG2 cell proliferation and suppressed its apoptosis, and AGR2 served as the functional protein in pro-proliferation and anti-apoptosis process of FOXA1.

showed that AGR2 was a mediator in the anti-proliferation process (Figure 3A). An apoptosis assay showed that FOXA1 knockdown also induced Hep3B cells apoptosis and attenuate anti-apoptosis ability of Hep3B cells induced by 5-FU, an anti-metabolic drug. Meanwhile, it was further confirmed that overexpression of AGR2 could recover the anti-apoptosis ability of FOXA1-knockdown Hep3B cells in rescue experiments (Figure 3B).

In the complementary experiment, HepG2 cells were transfected with pcDNA3.1-FOXA1, and cell proliferation array and apoptosis array were performed as above. The results showed that ectopic expression of FOXA1 promoted HepG2 cell proliferation and suppressed its apoptosis, and AGR2 served as the functional protein in pro-proliferation and anti-apoptosis process of FOXA1 (Figure 3C and D).

miR-212-3p Regulated FOXA1 in HCC

It was well known that miRNAs participated in the posttranscriptional regulation of oncogenes. To find out the miRNA regulating FOXA1 expression, the bioinformatics analysis was executed via TargetScan, miRDB and microRNA. miR-212-3p was predicted as the regulator interacting with the 3′-UTR of FOXA1 mRNA (Figure 4A). Same to previous studies reported,[19,24] miR-212-3p was downregulated in HCC in our study (Figure 4B), and the decreased level of miR-212-3p was associated with high serum AFP level (p=0.013), large tumor size (p<0.001), venous infiltration (p=0.049) and advanced TNM tumor stage (p=0.004) (Table 1). Further, Kaplan–Meier estimation was used to analyze the OS in 90 HCC patients. Low expression of miR-212-3p was associated with reduced overall survival of HCC patients (p=0.013, Figure 4C). Meanwhile, we

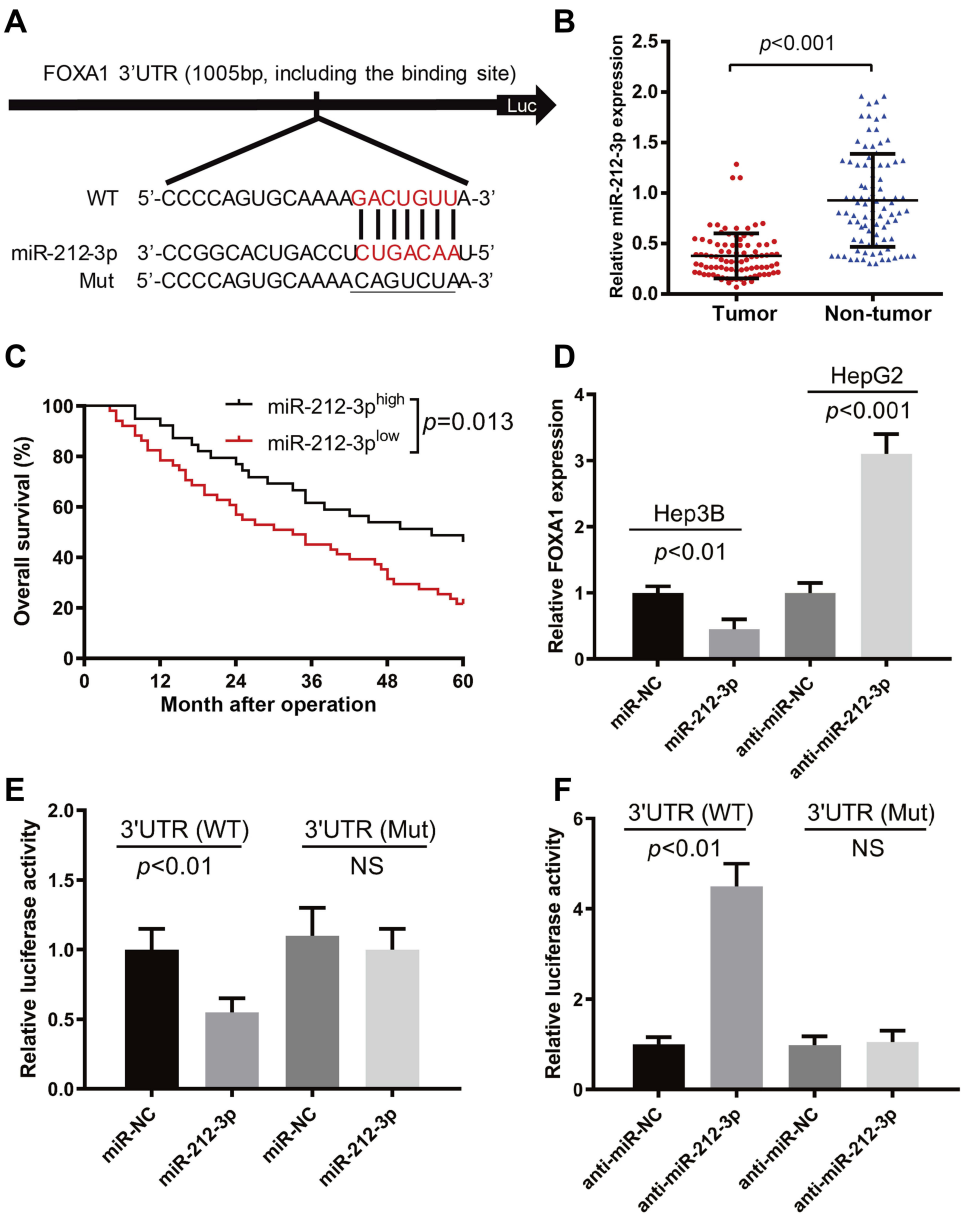

Figure 4 Downregulation of miR-212-3p in HCC contributed to FOXA1 upregulation. (**A**) miR-212-3p most likely binding site within FOXA1 3′UTR and its mutated version. (**B**) miR-212-3p was downregulated in tumor tissue compared with corresponding adjacent livers in 90-paired HCC patients via quantitative RT-PCR analysis. (**C**) Kaplan–Meier analysis of OS was compared between patients with high and low expression of miR-212-3p. (**D**) Overexpression of miR-212-3p reduced FOXA1 mRNA expression in Hep3B cells, and miR-212-3p inhibition induced FOXA1 mRNA expression in HepG2 cells. (**E**) Luciferase reporter assay showing that overexpression of miR-212-3p reduced luciferase activity of wild-type FOXA1 3′UTR but not that of the mutant in Hep3B cells. (**F**) Luciferase reporter assay showing that inhibition of miR-212-3p induced luciferase activity of wild-type FOXA1 3′UTR but not that of the mutant in HepG2 cells.

found that the upregulated expression of miR-212-3p in Hep3B cells remarkably reduced the mRNA level of FOXA1 and inhibition of miR-212-3p in HepG2 cells induced mRNA expression of FOXA1 (Figure 4D). To further confirm that miR-212-3p regulated FOXA1 expression, luciferase reporter array was performed using the wild type (WT) or mutant (Mut) miR-212-3p interacting sequences of FOXA1. The result showed that overexpression of miR-212-3p in Hep3B cells significantly reduced the luciferase activity of FOXA1 promotor (Figure 4E); inhibition of miR-212-3p in HepG2 cells significantly induced the luciferase activity of FOXA1 promotor (Figure 4F). As a control, there was no significant change when WT luciferase vector was replaced with Mut luciferase vector (Figure 4E and F). These results strongly proved that FOXA1 was a target gene of miR-212-3p.

Discussion

As reported in many studies, the expression of FOXA1 was increased in various types of cancers, including urothelial carcinoma,[3] colorectal cancer,[4] prostate cancer,[5] and glioma.[6] It was confirmed that FOXA1 regulated the expression of lots of carcinogenesis-related genes,[25–27] including HSP72,[28] BCL2,[29] HOXB13,[30] UGT2B15,[31] UGT2B17.[32] In our study, we found a significantly higher expression of FOXA1 in HCC tissues than that in adjacent non-tumor tissues. Furthermore, the association analysis in clinical indicated that high expression of FOXA1 was dominantly correlated with high HBV level, large tumor size, high venous infiltration, high Edmondson-Steiner grading, and advanced tumor-node-metastasis tumor stage. The difference in prognostic of FOXA1 in human malignancies stimulates us to explore the biological functions and the internal mechanisms. Firstly, gain- and loss-of-function experiments were performed to evaluate the biological functions of FOXA1 and the results demonstrated that FOXA1 exerted a vital catalytic role in HCC cell growth by inducing cell proliferation and suppressing apoptosis. As FOXA1 was a transcriptional factor, we supposed that FOXA1 exerted its biological functions via regulating its target genes. Fortunately, we found that AGR2, upregulated in liver cancer cells,[13] was a direct target gene of FOXA1. Importantly, downregulation of FOXA1 resulted in decreased cell proliferation and increased apoptosis, and upregulation of AGR2 in FOXA1-downregulating Hep3B cells recovered cell proliferation and anti-apoptosis ability. Altogether, our results suggest that AGR2 is a downstream target of FOXA1, which may induce cell proliferation and suppress apoptosis by regulating AGR2 expression in HCC.

To further investigate our study on FOXA1, we explored the cause for the overexpression of FOXA1 in HCC. Because of the key roles of miRNAs in the initiation and progression of human cancers through targeting oncogenes, we focused on the potential miRNA targeting FOXA1. It was reported that FOXA1 was a direct target of miR-212-3p.[19,24] In our study, the upregulation of miR-212-3p significantly reduced the expression of FOXA1 in Hep3B cells, whereas the downregulation of miR-212-3p increased the expression of FOXA1 in HepG2 cells. The luciferase reporter further demonstrated that miR-212-3p could interact with FOXA1 3′-UTR but not mutant 3′-UTR.

Conclusion

We found that FOXA1 is highly expressed in HCC tissues. The high expression of FOXA1 is correlated with poor prognostic features and reduced survival of HCC patients. It was demonstrated that FOXA1 may function in cell proliferation and apoptosis by regulating AGR2 expression, and that FOXA1 is regulated by miRNA-212-3p.

In Conclusion, we found a purported mechanism of miR-212-3p/FOXA1/AGR2 axes in HCC proliferation and apoptosis, suggesting a promising prognostic biomarker for HCC patients.

Data Sharing Statement

The datasets supporting the conclusions of the current study are available from the corresponding author (Tao Ye, E-mail: ye_tao@fudan.edu.cn) on reasonable request. Please contact the corresponding author, if you want to request the dataset.

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Disclosure

The authors declare that there are no conflicts of interest.

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