

Expression of FOXM1 and Aurora-A predicts prognosis and sorafenib efficacy in patients with hepatocellular carcinoma

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Abstract.

BACKGROUND: Effective prognostic biomarkers and powerful target-therapeutic drugs are needed for improving the treatment of Hepatocellular carcinoma (HCC).

OBJECTIVE: This study aimed to evaluate the expression of FOXM1 and Aurora-A and their prognostic value in HCC.

METHODS: We determined the differentially expressed genes signature in HCC using the Gene Set Enrichment Analysis (GSEA), and then evaluated the expression of FOXM1 and Aurora-A in TCGA and KMUH cohort. Associations between co-expression of FOXM1 and Aurora-A and clinical variables were calculated. Overall survival (OS) and recurrence-free survival (RFS) were estimated with different FOXM1 and Aurora-A expression status.

RESULTS: FOXM1-related gene sets were mostly associated with cell cycle regulation in HCC tissues. We found a positive correlation between the expression of FOXM1 and Aurora-A. Overexpression of FOXM1 and Aurora-A was associated with larger tumor size, advanced stage, higher grade, and double-positive for HBV and HCV. The coordinated overexpression of FOXM1 and Aurora-A was the most significant independent prognostic factor for OS and RFS. Furthermore, the concomitant high expression of FOXM1 and Aurora-A predicted the worst OS of sorafenib-treated patients with HCC.

CONCLUSIONS: The co-expression of FOXM1 and Aurora-A could be a reliable biomarker to predict the sorafenib response and prognosis of HCC patients.

Keywords: FOXM1, Aurora-A, hepatocellular carcinoma (HCC), sorafenib

1. Introduction

Hepatocellular carcinoma (HCC) usually results in a high mortality rate, especially in South-Eastern Asia [1]. Some therapeutic strategies such as curative resection, liver transplantation, and target therapy may

be promising for the treatment of HCC, whereas its prognosis remains unsatisfactory due to frequent recurrence and metastasis rates. HCC is a highly heterogeneous malignancy with robust clinical and molecular diversities. Many HCC patients are diagnosed with advanced-stage due to the lack of precise early diagnosis and effective treatment [2]. HCC treatment failures are primarily caused by frequent recurrence and lead to poor prognosis. New reliable biomarkers for survival prognosis and prediction of drug response are needed because they are crucial for developing more effective diagnostic and therapeutic strategies.

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Many genome-wide expression profiling studies have revealed genetic alterations during tumorigenesis in the past decade. The combination of transcriptome sequencing and bioinformatics analysis has allowed the identification of differentially expressed genes involved in the development and progression of HCC. Although serum alfa-fetoprotein (AFP) can be a diagnostic marker to detect HCC, normal AFP levels are present in 30% of patients at the time of diagnosis, even with advanced HCC [3,4]. However, there is still no reliable biomarker to predict the survival prognosis of HCC by liver biopsy in clinical practice. Thus, it is essential to find new putative biomarkers that may predict the prognosis of HCC and guide personalized therapy for HCC patients.

Forkhead box protein M1 (FOXM1) plays an important role in regulating cell cycle progression, differentiation of cancer, and drug resistance [5]. Recent studies demonstrated that FOXM1 is a key transcription factor associated with HCC [6] and that elevated FOXM1 expression is associated with poor prognosis of HCC [7], colorectal cancer [8] and lung cancer [9]. FOXM1 also is related to drug resistance in breast and lung cancer [10,11]. These results clearly show the critical role of FOXM1 in tumorigenesis and therapeutic response to chemotherapy. Although FOXM1 is up-regulated in numerous cancers, it is unclear whether the differentially expressed genes signature, including FOXM1, can be prognostic biomarkers and whether they contribute to alternative signaling pathways for the tumor progression of HCC.

Here, we identify specific genes that are significantly co-deregulated with FOXM1 in HCC. We analyzed the expression data of the HCC cohort from The Cancer Genome Atlas (TCGA) and a validation (KMUH) cohort and evaluated an association between the gene expression signature and clinicopathological characteristics, including patient's survival. FOXM1 can predict the worst overall survival (OS) of sorafenib-treated patients with HCC. This work will provide further insight into developing prognostic biomarkers and potential molecular targets for new therapeutic direction.

2. Materials and methods

2.1. The Cancer Genome Atlas (TCGA) dataset

We downloaded TCGA Liver Cancer (LIHC) level 3 processed mRNA expression data and clinicopathological data from the UCSC Xena (<http://xena.ucsc.edu>).

Table 1
Clinical variables of the HCC patients

Variables	No. (%) (n = 341)
Age	
< 60	151 (44.3%)
≥ 60	190 (55.7%)
Gender	
Male	231 (67.7%)
Female	110 (32.3%)
TNM stage	
T1-2	253 (74.6%)
T3-4	86 (25.4%)
N0	231 (98.3%)
N1	4 (1.7%)
M0	245 (98.8%)
M1	3 (1.2%)
Stage	
1-2	237 (74.3%)
3-4	82 (25.7%)
Grade	
1-2	213 (63.4%)
3-4	123 (36.6%)
Child pugh	
A	201 (91.0%)
B	20 (9.0%)
Vascular invasion	
No	191 (65.6%)
Yes	100 (34.4%)
Etiology	
HBV	48 (32.2%)
HCV	20 (13.4%)
HBV + HCV	81 (54.4%)

The mRNA expression profile was transformed in RNA-Seq by Expectation Maximization (RSEM) values from the Illumina HiSeq 2000 RNA Sequencing platform and calculated as $\log_2(x + 1)$ transformed RSEM normalized counts. A total of 341 patients in the TCGA dataset were included in the analysis. Patients with incomplete mRNA expression, OS, or recurrence-free survival (RFS) information were excluded. The median age of the patients was 61.0 years (range, 16 to 90 years). Other clinicopathological information is shown in Table 1. The median expression of FOXM1 and Aurora-A were 8.80 and 8.71, respectively. We used the cut-off finder online tool [12] (<http://molpath.charite.de/cutoff/>) to determine the optimal cut-off value of gene expression with prognostic significance. The cut-off values were 8.80 and 9.02 for FOXM1 and Aurora-A, respectively.

2.2. Patient specimens

A total of 40 diagnosed HCC specimens with adjacent non-cancerous tissues were used according to the ethical guidelines of the Declaration of Helsinki and was approved by the Institutional Review Board

Table 2
Gene sets enrichment of FOXM1-related GO biological process gene sets in 341 HCC from TCGA dataset

Gene sets	Size	ES	NES	NOM <i>p</i> -value	FDR <i>q</i> -value
GO_CELL_CYCLE_PHASE_TRANSITION	242	0.608	1.986	0.000	0.001
GO_DNA_REPAIR	461	0.503	1.956	0.004	0.001
GO_SIGNAL_TRANSDUCTION_BY_P53_CLASS_MEDIATOR	123	0.488	1.925	0.000	0.001
GO_CELL_CYCLE_G2_M_PHASE_TRANSITION	132	0.552	1.917	0.000	0.001
GO_SIGNAL_TRANSDUCTION_IN_RESPONSE_TO_DNA_DAMAGE	95	0.518	1.887	0.000	0.001
GO_REGULATION_OF_CELL_CYCLE_ARREST	107	0.504	1.830	0.000	0.004
GO_REGULATION_OF_CELL_AGING	32	0.570	1.788	0.008	0.005
GO_NEGATIVE_REGULATION_OF_CELL_AGING	16	0.631	1.646	0.021	0.021

ES, enrichment score; NES, normalized enrichment score; NOM, nominal; FDR, false discovery rate.

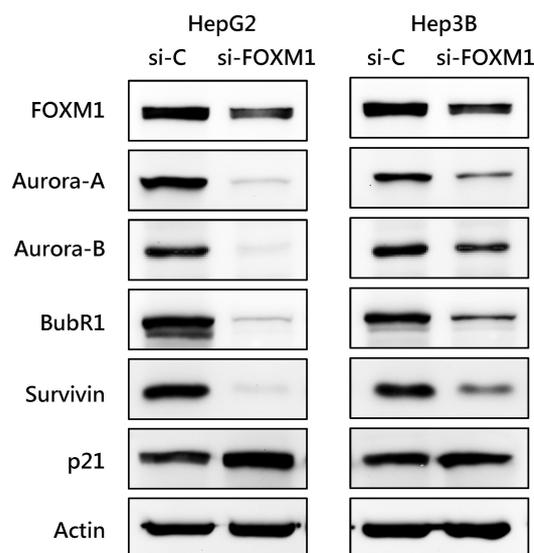
(IRB) of Kaohsiung Medical University Hospital (KMUHIRB-F(I)-20170093). The median age of the patients was 60 years (range, 38 to 80 years). The follow-up period was from 45 to 2,402 days, with a median of 1,572 days.

2.3. Gene set enrichment analysis (GSEA)

For Gene Ontology (GO) biological process gene sets rank analysis, we used the Gene Set Expression Analysis (GSEA) tool [13,14] to estimate the difference of FOXM1-related gene sets between all primary HCC and adjacent tissues. Each gene set would get an enrichment score (ES) that represented the degree of the overexpressed gene set. The nominal (NOM) *p*-value estimated the statistical significance of the enrichment score for a single gene set. The false discovery rate (FDR) was adjusted for gene set size, and $FDR < 0.25$ were considered statistically significant.

2.4. Quantitative real-time PCR (RT-qPCR)

Total RNA was isolated from fresh-frozen tissues derived from HCC patients using Trizol reagent (Invitrogen) according to the manufacturer's instructions. Reverse transcription was performed using a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems). Diluted cDNA (10 ng) was used as a template, and the relative expression of each mRNA was evaluated using the StepOne Real-Time PCR Systems (Applied Biosystems). GAPDH mRNA was used as an internal control. The following primer sequences were used: for FOXM1, forward, 5'-ATACGTGGATTGAGGACCACT-3', and reverse, 5'-TCCAATGTCAAGTAGCGGTTG-3'; Aurora-A, forward, 5'-GCTGGAGAGCTTAAAATTGCAG-3' and reverse, 5'-TTTTGTAGGTCTCTTGATATGTG-3'; GAPDH, forward, 5'-AGCCACA TCGCTCAGACAC-3', and reverse, 5'-GCCCAATAC GACCAAATCC-3'



Supplementary Fig. 1. Effect of FOXM1 knockdown on the expression of cell cycle-regulating proteins in HCC cells. HepG2 and Hep3B cells were transfected with control siRNA or with FOXM1 siRNA. The expression of the indicated cell cycle-regulating proteins was analyzed by western blotting.

2.5. siRNA and transfection

The FOXM1 siRNA was purchased from Santa Cruz (sc-43769), and siRNAs were transfected into cells by jetPRIME® (Polyplus transfection) according to the user instruction.

2.6. Western blot analysis

Western blot analysis was performed as we previously described [15]. The used antibodies are listed below. FOXM1 (sc-376471) and p21 (sc-6246) were purchased from Santa Cruz. Aurora-A (610404), Aurora-B (611083), BubR1 (612502) were purchased from BD Biosciences. Survivin (GTX100052) was purchased from GENETEX. Actin (A2228) was purchased from Sigma-Aldrich.

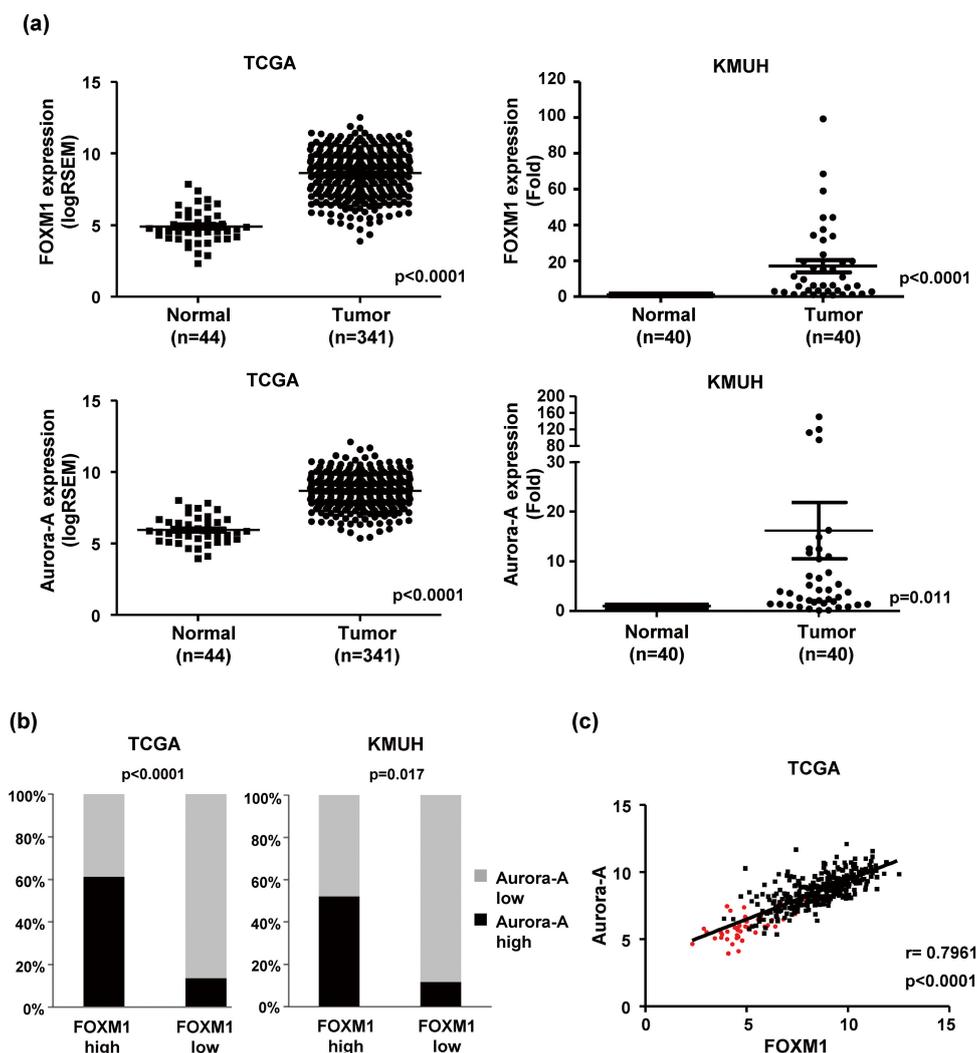


Fig. 1. FOXM1 expression is positively correlated with Aurora-A expression in human HCC. (a) The expression of FOXM1 and Aurora-A in HCC and adjacent liver tissues by transcriptome sequencing from the TCGA dataset (Left). The expression levels of the FOXM1 and Aurora-A mRNAs were validated in 40 paired HCC from KMUH using RT-qPCR (Right). The horizontal lines depict the mean \pm S.D. (b) The level of FOXM1 and Aurora-A in HCC tissues from the TCGA and KMUH datasets and the correlation between their expression levels was analyzed using Fisher's exact test ($p < 0.001$). (c) The relationship between FOXM1 and Aurora-A expression in HCC (black dot, $n = 341$) and adjacent liver tissues (red dot, $n = 44$) was analyzed using Pearson's correlation. RSEM, RNA-seq by Expectation Maximization.

2.7. Statistical analysis

All statistical analyses were performed using GraphPad Prism 5 (GraphPad Software, La Jolla, CA, USA) and SPSS 20.0 software (IBM, Chicago, USA). The two-tailed t -test was used to compare means between two groups. Pearson's chi-square test analyzed categorical data. Pearson's correlation coefficient was used to analyze the correlation between FOXM1 and Aurora-A. Survival curves were estimated by Kaplan-Meier and compared by the log-rank test. Cox regression analyses were conducted to assess the association between can-

didate genes and survival. For all the tests, significance was accepted at $p < 0.05$.

3. Results

3.1. FOXM1 overexpression is correlated with Aurora-A upregulation in human HCC

We analyzed the expression pattern of FOXM1-related pathways in HCC from the TCGA dataset using the Gene Set Enrichment Analysis (GSEA). A total of

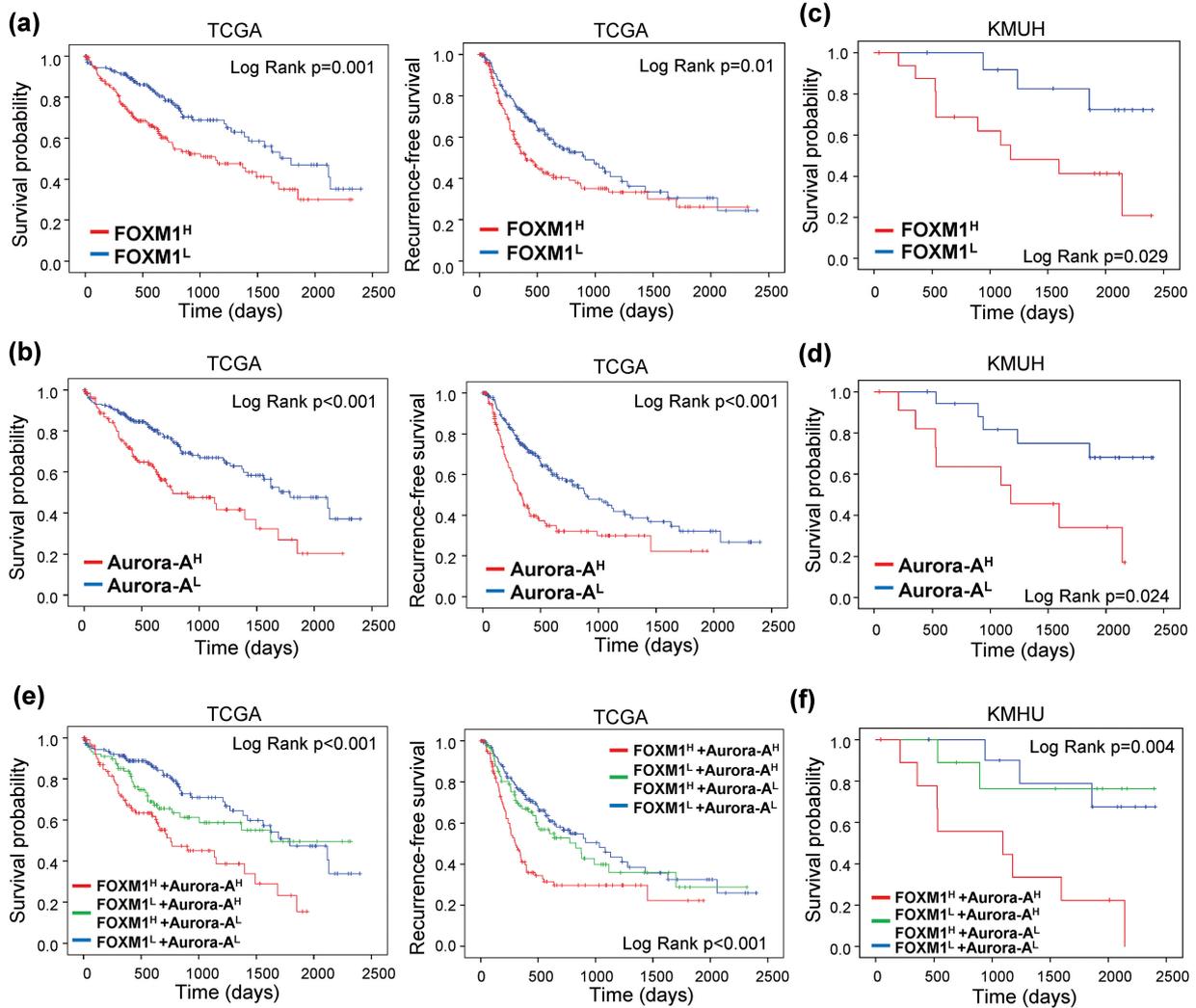


Fig. 2. Overexpression of FOXM1 and Aurora-A are associated with poor prognosis in HCC. KaplanMeier curves of OS (Left) and RFS (Right) were estimated with different FOXM1 and Aurora-A expression status in the TCGA ($n = 341$) (a–b), and OS in KMHU ($n = 30$) datasets (c–d). KaplanMeier curves for OS (Left) and RFS (Right) among three subgroups according to FOXM1 and Aurora-A expression status in the TCGA ($n = 341$) (e), and OS in KMHU ($n = 30$) datasets (f).

341 HCC and 44 normal tissues were included in the TCGA dataset. Eight FOXM1-related Gene Ontology (GO) biological process gene sets were significantly enriched in malignant HCC tissues (Table 2). Six of eight FOXM1-related gene sets were associated with cell cycle and DNA damage regulation. Knockdown of FOXM1 by siRNA decreased the expression of cell cycle-regulating proteins (Supplementary Fig. 1). This result showed that FOXM1 is a critical cell cycle regulator. We found that Aurora-A (AURKA), a mitotic serine/threonine kinase, was involved in five FOXM1-related gene sets and showed significantly high enrichment in HCC than normal tissues. Overexpression of

Aurora-A has been reported in a variety of solid tumors, including breast [16], colon [15], lung [17], and pancreas cancer [18]. The deregulation of Aurora-A may result in chromosomal instability and thus be related to tumorigenesis. Furthermore, Aurora-A has been identified as a target of FOXM1 in melanoma [19] and breast cancer [20]. Thus, we speculate that the level of FOXM1 may be increased in parallel with Aurora-A expression. We found that the relative expression of both the FOXM1 and Aurora-A mRNAs were significantly higher in HCC tissues than in adjacent liver tissues from the TCGA RNA-seq dataset. Consistently, the results of RT-qPCR indicated that the expression of

Table 3
The correlation between clinical variables and FOXM1 and Aurora-A expression in HCC patients obtained from the TCGA dataset

Clinical variables	F ^L + A ^L	F ^L + A ^H /F ^H + A ^L	F ^H + A ^H	p-value ^a
	N = 145 (42.5%)	N = 90 (26.4%)	N = 106 (31.1%)	
Age				0.113
< 60	56 (37.1%)	40 (26.5%)	55 (36.4%)	
≥ 60	89 (46.8%)	50 (26.3%)	51 (26.8%)	
Gender				0.707
Male	101 (43.7%)	58 (25.1%)	72 (31.2%)	
Female	44 (40.0%)	32 (29.1%)	34 (30.9%)	
TNM stage				
T1-2	117 (46.2%)	66 (26.1%)	70 (27.7%)	0.024
T3-4	27 (31.4%)	23 (26.7%)	36 (41.9%)	
N0	88 (38.1%)	67 (29.0%)	76 (32.9%)	0.886
N1	2 (50.0%)	1 (25.0%)	1 (25.0%)	
M0	99 (40.4%)	60 (24.5%)	86 (35.1%)	0.438
M1	2 (66.7%)	1 (33.3%)	0 (0.0%)	
Stage				0.031
1-2	110 (46.4%)	62 (26.2%)	65 (27.4%)	
3-4	26 (31.7%)	22 (26.8%)	34 (41.5%)	
Grade				< 0.001
1-2	110 (51.6%)	53 (24.9%)	50 (23.5%)	
3-4	33 (26.8%)	36 (29.3%)	54 (43.9%)	
Child pugh				0.027
A	90 (44.8%)	55 (27.4%)	56 (27.9%)	
B	7 (35.0%)	11 (55.0%)	2 (10.0%)	
Vascular invasion				0.594
No	88 (46.1%)	56 (29.3%)	47 (24.6%)	
Yes	44 (44.0%)	26 (26.0%)	30 (30.0%)	
Etiology				0.003
HBV	18 (37.5%)	18 (37.5%)	12 (25.0%)	
HCV	8 (40.0%)	10 (50.0%)	2 (10.0%)	
HBV + HCV	26 (32.1%)	16 (19.8%)	39 (48.1%)	

^aThe data were analyzed using Pearson's chi-square test. F^H, high FOXM1; F^L, low FOXM1; A^H, high Aurora-A; A^L, low Aurora-A.

FOXM1 and Aurora-A mRNAs were highly expressed in HCC tissues in the KMUH dataset (Fig. 1a). It was showed that 50.7% (173/341) and 37.8% (129/341) of patients with high FOXM1 and Aurora-A expression tissues from the TCGA dataset, respectively. The proportion of high FOXM1 and Aurora-A expression tissues in the KMUH dataset was 57.5% (23/40) and 35.0% (14/40), respectively. High Aurora-A expression was observed more frequently detected in high FOXM1 than in low FOXM1 tissues (61.3% vs. 13.7%, TCGA; 52.2% vs. 11.8%, KMUH) (Fig. 1b). We also found a positive linear correlation between FOXM1 and Aurora-A expression in HCC tissues ($r = 0.7961$, $P < 0.0001$) (Fig. 1c). Overall, these results indicate that both FOXM1 and Aurora-A were parallel overexpressed in HCC.

3.2. Coordinated overexpression of FOXM1 and Aurora-A are associated with poor prognosis of HCC patients

To investigate the prognostic values of FOXM1 and

Aurora-A expression in HCC, we separated the HCC patients into low- and high-expression groups. Patients with high FOXM1 expression (FOXM1^H) or high Aurora-A expression (Aurora-A^H) had significantly shorter OS and RFS than that with low FOXM1 expression (FOXM1^L) or low Aurora-A expression (Aurora-A^L) in TCGA datasets (Fig. 2a and b). In the KMUH cohort, patients with FOXM1^H or Aurora-A^H also exhibited poor OS (Fig. 2c and d). We further classified the patients into three groups according to the FOXM1 and Aurora-A expression: both high-expression (F^H + A^H), both low-expression (F^L + A^L), and intermediate (F^H + A^L/F^L + A^H) groups. We found that the five-year OS rates for the TCGA dataset were 23% in the F^H + A^H group, 50% in the F^H + A^L/F^L + A^H group, and 47% in the F^L + A^L group (log-rank $p < 0.001$) (Fig. 2e). Five-year OS rates for the three groups in KMUH dataset were 22%, 76%, and 79%, respectively (log-rank $p = 0.004$) (Fig. 2f). Five-year RFS was 22% in F^H + A^H group, compared with 29% and 32% in F^H + A^L/F^L + A^H and F^L + A^L group, respectively

Table 4
Univariate and multivariate Cox regression analysis of variables associated with overall survival and recurrence-free survival in HCC from the TCGA dataset

Variables	Univariate		Multivariate	
	HR (95% CI)	<i>p</i> value ^a	HR (95% CI)	<i>p</i> -value
<i>Overall survival</i>				
Age (≥ 60 vs. < 60)	1.168 (0.812–1.680)	0.404		
Gender (Male vs. Female)	1.343 (0.931–1.937)	0.115		
T (T3-T4 vs. T1-T2)	2.624 (1.823–3.778)	< 0.001	1.182 (0.158–8.841)	0.871
N (N1 vs. N0)	1.821 (0.446–7.440)	0.404		
M (M1 vs. M0)	3.648 (1.146–11.609)	0.028	2.946 (0.875–9.925)	0.081
Stage (3-4 vs. 1-2)	2.515 (1.714–3.692)	< 0.001	2.307 (0.311–17.125)	0.414
Grade (3-4 vs. 1-2)	1.096 (0.756–1.589)	0.629		
Child pugh (B vs. A)	1.854 (0.878–3.915)	0.105		
Vascular invasion (Yes vs. No)	1.398 (0.909–2.149)	0.127		
FOXM1 (High vs. Low)	1.858 (1.287–2.682)	0.001		
Aurora-A (High vs. Low)	2.042 (1.423–2.932)	< 0.001		
FOXM1/Aurora-A				
F ^H + A ^L /F ^L + A ^H vs. F ^L + A ^L	1.405 (0.880–2.244)	0.154		
F ^H + A ^H vs. F ^L + A ^L	2.497 (1.634–3.816)	< 0.001	3.065 (1.771–5.307)	< 0.001
<i>Recurrence-free survival</i>				
Age (≥ 60 vs. < 60)	1.027 (0.752–1.404)	0.865		
Gender (Male vs. Female)	1.017 (0.729–1.419)	0.920		
T (T3-T4 vs. T1-T2)	2.381 (1.713–3.309)	< 0.001	0.354 (0.046–2.727)	0.319
N (N1 vs. N0)	1.395 (0.344–5.661)	0.641		
M (M1 vs. M0)	2.379 (0.582–9.732)	0.228		
Stage (3-4 vs. 1-2)	2.369 (1.681–3.337)	< 0.001	4.772 (0.642–35.476)	0.127
Grade (3-4 vs. 1-2)	1.111 (0.805–1.533)	0.523		
Child pugh (B vs. A)	1.265 (0.634–2.524)	0.504		
Vascular invasion (Yes vs. No)	1.910 (1.332–2.738)	< 0.001	1.595 (1.075–2.367)	0.020
FOXM1 (High vs. Low)	1.502 (1.099–2.054)	0.011		
Aurora-A (High vs. Low)	1.961 (1.434–2.683)	< 0.001		
FOXM1/Aurora-A				
F ^L + A ^H /F ^H + A ^L vs. F ^L + A ^L	1.206 (0.808–1.800)	0.359		
F ^H + A ^H vs. F ^L + A ^L	2.111 (1.469–3.032)	< 0.001	1.749 (1.126–2.717)	0.013

^a*p*-value given in bold was used to adjust for Multivariate analysis. F^H, high FOXM1; F^L, low FOXM1; A^H, high Aurora-A; A^L, low Aurora-A.

(log-rank $p < 0.001$) (Fig. 2e). From the results, patients with both FOXM1 and Aurora-A high-expression had significantly shorter overall survival and earlier recurrence.

Moreover, F^H + A^H expression was positively correlated with larger tumor size ($p = 0.024$), advanced stage ($p = 0.031$), higher grade ($p < 0.001$), and double-positive for HBV and HCV ($p = 0.003$). However, patients with F^H + A^H expression had high proportion of Child pugh A class ($p = 0.027$) (Table 3).

We further confirm the association of FOXM1 and Aurora-A expression with OS and RFS by Cox regression analysis. Univariate Cox regression analysis demonstrated that T classification, M classification, stage, and FOXM1 and/or Aurora-A expression were all significantly associated with OS. T classification, stage, vascular invasion, and FOXM1 and/or Aurora-A expression were correlated with RFS. Subsequently, the significant characteristics factors from the univariate analyses were included for multivariate Cox regression

analysis. The results showed that F^H + A^H expression was the most significant independent predictor for OS (HR: 3.065, $p < 0.001$) and was for RFS (HR: 1.749, $p = 0.013$), and vascular invasion was also an independent prognostic factor for RFS (Table 4).

3.3. Co-expression of FOXM1 and Aurora-A predicts the prognosis of sorafenib-treated patients in HCC

In a subsequent analysis, we found that the F^H + A^H expression in 29 sorafenib-treated patients (TCGA cohort) was associated with worse survival in HCC. The five-year OS rates was significantly worse in FOXM1^H patients compared with FOXM1^L patients (15% vs. 67%, $p = 0.03$) (Fig. 3a). A significant difference in five-year OS rates was also seen in Aurora-A^H between Aurora-A^L patients (0% vs. 40%, $p = 0.029$) (Fig. 3a). The five-year OS rates was zero for F^H + A^H patients, compared with 28% and 67% in F^H + A^L/F^L + A^H and

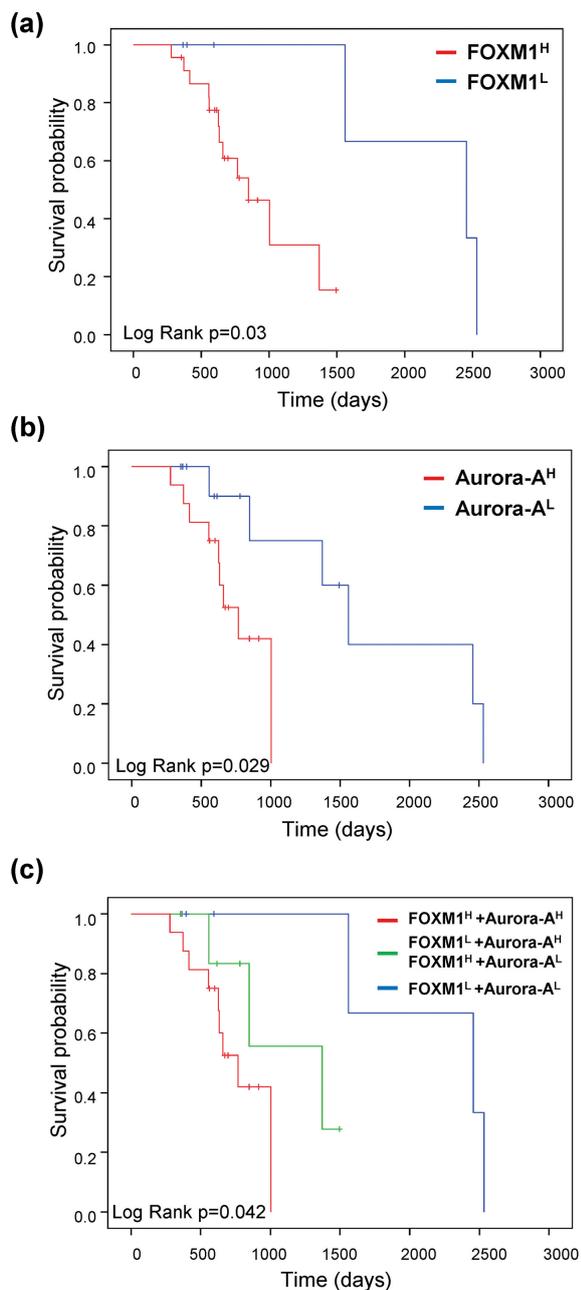


Fig. 3. FOXM1 and Aurora-A expression predict the prognosis of sorafenib-treated patients in HCC. KaplanMeier curves of overall survival probability were estimated with different FOXM1 (a), Aurora-A (b), and FOXM1/Aurora-A (c) expression status in sorafenib-treated patients obtained from the TCGA dataset ($n = 29$).

$F^L + A^L$ patients, respectively ($p = 0.042$) (Fig. 3c). These results suggest that FOXM1 and Aurora-A expression are key factors associated with a poor prognosis in sorafenib-treated HCC patients, and they may be effective in predicting the efficacy of sorafenib.

4. Discussion

Valid and precise biomarkers for identifying patients who have a poor prognosis are urgently needed to optimize their individual therapy. The development of powerful target-therapeutic drugs for HCC is also critical for this aggressive cancer. FOXM1 is a critical regulator in the G1/S and G2/M transitions of the cell cycle through regulating downstream targets such as centromere protein (CENP) F, cyclin B1, kinesin family member (KIF), p27^{Kip1} and p21^{cip1} in HCC cells [21,22]. Our GSEA data also showed that FOXM1-related gene sets were mostly associated with cell cycle regulation in HCC (Table 2). We suppose that the abnormal expression of FOXM1 may contribute to deregulating cell cycle and induce cell proliferation in HCC. Our finding revealed that FOXM1 overexpression is positively correlated with a vital cell cycle regulator, Aurora-A, in human HCC (Fig. 1c). The proportion of FOXM1^H and Aurora-A^H tissues was as many as 50% and 35% in both TCGA and KMUH cohort, respectively. It is noteworthy that FOXM1^H and Aurora-A^H expression were more associated with the Asian race than the White race (data not shown). Moreover, it was demonstrated that FOXM1 could predict the prognosis of HCC in two Asian cohorts, and Aurora-A was a critical hub gene with prognostic value using the bioinformatics analysis [7,23,24]. From the literature review, we hypothesized that the expression of FOXM1 and Aurora-A could be prognostic and predictive biomarkers for HCC.

In this study, we determined the relationship of FOXM1 and Aurora-A expression and analyzed the significance of their expression on the prognosis of HCC. There was 31.1% (106/341) of patients with $F^H + A^H$ expression and they were associated with larger tumor size, advanced stage, higher-grade tumors, and poorer outcome (Table 3 and Fig. 2). A meta-analysis demonstrated that FOXM1^H expression could predict worse prognosis of HCC with advanced vascular invasion, differentiation, and TNM stage [25]. These results suggested that FOXM1 and Aurora-A could cooperatively play essential roles in inducing tumor proliferation and progression.

$F^H + A^H$ expression was positively correlated with double-positive for HBV and HCV (Table 3). It seems that virus infection could be a key factor in affecting the expression of FOXM1 or Aurora-A. It is consistent with a previous report that HBx can upregulate FOXM1 expression to promote tumor metastasis and poor prognosis [26], and HCV can increase the expression Aurora-A in HCC tissues [27]. However, there are

still no reports showing synergetic effects of coexisting HBV and HCV to FOXM1 or Aurora-A expression.

In univariate Cox regression analysis, $F^H + A^H$ expression (HR: 2.497, $p < 0.001$) was the most significant factor associated with OS than FOXM1 (HR: 1.858, $p = 0.001$) or Aurora-A (HR: 2.042, $p < 0.001$) alone. $F^H + A^H$ expression (HR: 2.111, $p < 0.001$) enhanced the prognostic value for RFS than FOXM1 alone (HR: 1.502, $p = 0.011$). Although some studies have identified FOXM1 and Aurora-A differentially expressed, respectively, in HCC, their combinational predictive value has not been specifically investigated. So far, this is the first study to propose that the co-expression of FOXM1 and Aurora-A can be a new prognostic biomarker for HCC patients.

Unlike other malignancies, such as breast [28] and lung cancer [29], which have reliable biomarkers for treatment decisions in clinical practice, there is no biomarker for selecting HCC patients who could benefit most from sorafenib. Previous studies showed that FOXM1 and Aurora-A are related to TKI resistance in lung cancer [11,30]. The level of FOXM1 was correlated with sorafenib resistance in HCC cells. High FOXM1 expression was found in sorafenib-resistant cells, whereas low levels were observed in the comparatively sorafenib-sensitive cells [31,32]. Furthermore, Aurora-A has been suggested to be a critical therapeutic target combine with sorafenib to improve the HCC patient's drug resistance and prolong survival [33]. According to the above experimental results, we suppose that different levels of FOXM1 and Aurora-A react differently when exposed to sorafenib. In our pilot analysis of HCC specimens prove that the $F^H + A^H$ expression in sorafenib-treated patients (TCGA cohort) was associated with worse survival in HCC (Fig. 3), suggesting that the FOXM and Aurora-A expression could affect the therapeutic efficiency of sorafenib. Thus, FOXM1 and Aurora-A could be a reliable biomarker to predict drug response and prognosis of HCC patients. It is also reasonable to propose that adding FOXM1 or Aurora-A inhibitor to sorafenib may enhance the cytotoxic effects of sorafenib in HCC.

In conclusion, we found that the expression of FOXM1 was positively associated with the levels of Aurora-A in HCC. $F^H + A^H$ expression was associated with poor prognosis and was the most significant independent prognostic factor for OS and RFS. Moreover, $F^H + A^H$ expression was a key factor associated with a poor prognosis of sorafenib-treated HCC patients, and it was effective in predicting sorafenib response, suggesting that the co-expression of FOXM1 and Aurora-A

could be a reliable biomarker to predict sorafenib response and prognosis of HCC patients. Both FOXM1 and Aurora-A are potential therapeutic targets for HCC.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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