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FOXM1 promotes proliferation in human hepatocellular carcinoma cells by transcriptional activation of CCNB1

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ABSTRACT

The transcription factor Forkhead box protein M1 (FOXM1) plays critical roles in cancer development and progression, including human hepatocellular carcinoma (HCC). However, the regulatory role and underlying mechanisms of FOXM1 is still limited. Here, we found that the high level expression of FOXM1 and CCNB1 is closely associated with poor prognosis in HCC patients. And FOXM1 and CCNB1 were overexpressed concomitantly in liver tumor tissues. Knockdown of FOXM1 significantly inhibited the expression levels of CCNB1 in HCC cell lines at both the mRNA and protein levels. Mechanistic studies revealed that FOXM1 binds directly to the promoter region of CCNB1 and regulates the expression levels of the CCNB1 gene in the transcriptional level. Furthermore, the loss of functional and rescue experiments showed that CCNB1 is essential for FOXM1-driven proliferation in HCC cells. In the present study, our results partially explained the dysregulated expression of FOXM1 play an important role in proliferation of human hepatocellular carcinoma cells via transcriptional activation of CCNB1 expression. And it also highlights a FOXM1/CCNB1 axis could be a potential target for the treatment of HCCs.

1. Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignant diseases and the third leading cause of cancer death globally [1]. Despite the advances in diagnosis and treatment, HCC remains one of the most lethal cancers with sever poor prognosis in patients. Surgical resection and chemotherapy have only limited effect for patients at advanced stages due to frequent relapse and metastasis. Therefore, it is emergent to identify novel therapeutic targets and develop new strategies for the treatment of HCC [2]. Although the substantial functional studies on the oncogenes and tumor suppressor genes have greatly contributed to the advance of knowledge on the molecular mechanism of initiation and progression of HCC, the detailed mechanism of HCC development remains far from fully understood, and few biomarkers for the prognosis of patients are available in the clinic so far [3].

FOXM1 is a member of the Fox transcription factor family that they share homology within the winged-helix/Forkhead DNA-binding domain, involved in the regulation of organism development, cell proliferation, and differentiation [4]. FOXM1 is known to be a master regulator in both G1–S and G2–M phases of the cell-cycle and mitotic-spindle integrity [5–7]. FOXM1-null mouse embryos were neonatal lethal as a result of the development of polyploid hepatocytes and cardiomyocytes, highlighting the critical role of FOXM1 in mitotic division [8]. The recent study in FOMX1-knockout MEF cells revealed that FOXM1 regulates expression of a large number of G2/M-specific genes, such as Plk1, CCNB2, Nek2, CENPF and et al., indicating that it plays an essential role in maintenance of chromosomal segregation and genomic stability [8,9]. As

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a transcriptional factor, FOXM1 binds directly to the consensus (5′-TAAACA-3′) or non-consensus sequences in the genome to transcriptionally upregulate the downstream genes [9,10]. Recently, a growing body of evidence has demonstrated that FOXM1 is implicated in the DNA-damage response pathway. It can transcriptionally regulate the expression levels of a series of DNA damage repair associated genes, like BRCA1/2, RAD51, XRCC1, BRIP1 and NBE1 [11–14]. All these evidence support the view the FOXM1 plays a pleiotropic role in multiple signaling pathways. More importantly, it has been noted that FOXM1 is abundantly expressed in a wide range of human cancers, including human hepatocellular carcinoma [15,16]. To deeply understand the underlying mechanisms of FOXM1 in the malignant behaviors of cancers will help us to further explore the potentially therapeutic strategy in controlling malignant diseases [17].

In the present study, we sought to determine the prognostic value of FOXM1 and its downstream gene(s) in HCC patients and to deeply explore its underlying molecular mechanisms in the pathogenesis of HCC. Our study partially explained the dysregulated expression of FOXM1 plays an important role in proliferation of human hepatocellular carcinoma via transcriptional activation of CCNB1 expression and provided evidence to support that the FOXM1-CCNB1 axis could be a potential target for the treatment of HCC patients.

2. Materials and methods

2.1. Antibodies for Western blotting

The detailed information of primary antibodies was as the following: FOXM1 Rabbit mAb (clone D12D5) (cat. no. 5436), CCNB1 Rabbit mAb (clone D5C10) (cat. no. 12231) are from Cell Signaling Technology. β-actin (cat. no. A5316) are from Sigma-Aldrich.

2.2. Statistical analysis

Statistical validation of data was conducted using the SPSS

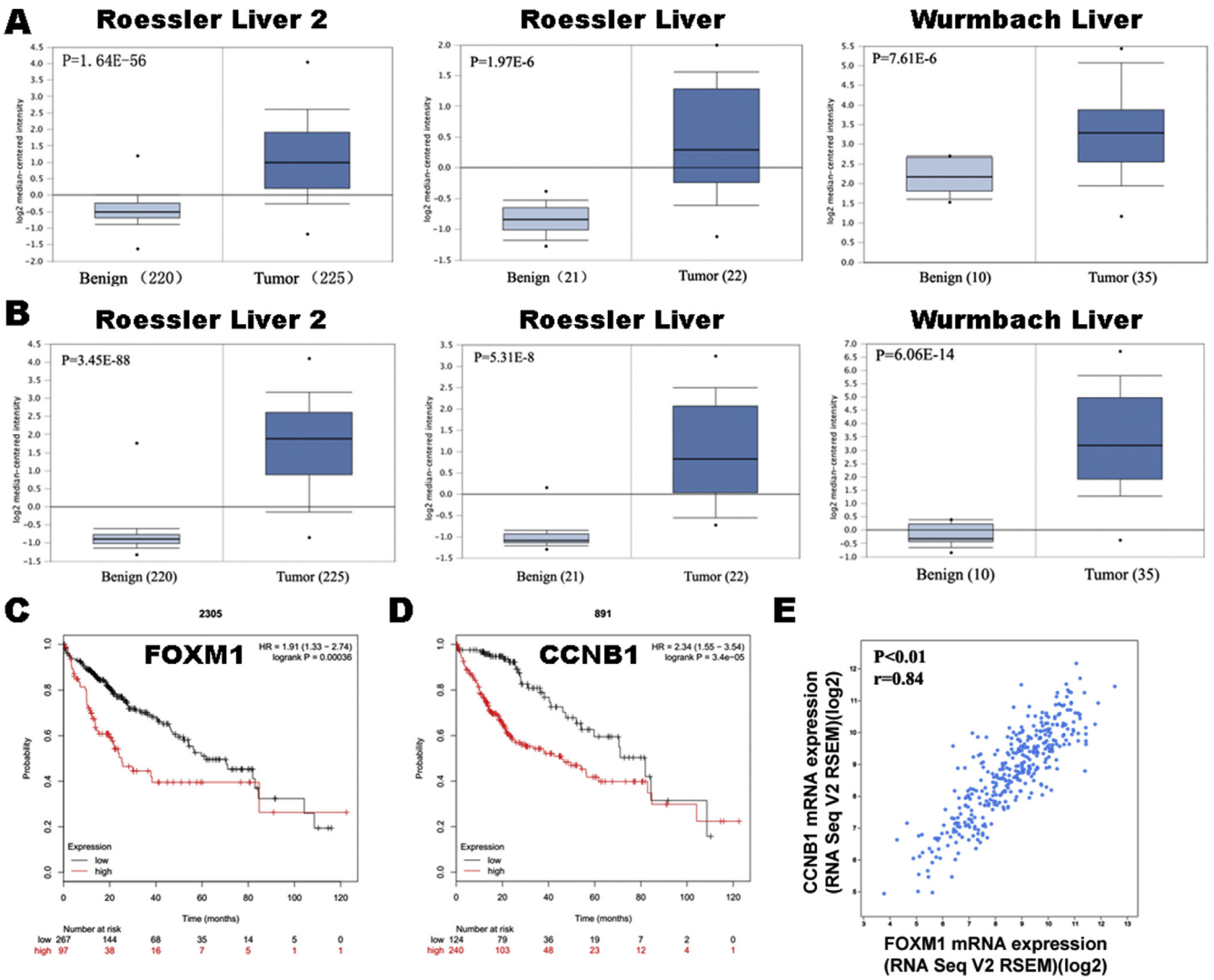

Fig. 1. The expression pattern and prognostic value of FOXM1 and CCNB1 in HCC.
(A) Analysis of Oncomine dataset to determine the expression pattern of FOXM1 in 3 independent cohorts.
(B) Analysis of Oncomine dataset to determine the expression pattern of CCNB1 in 3 independent cohorts which were similar to Fig. 1A.
(C) Kaplan–Meier analysis of the relevance between FOXM1 level and overall survival in human hepatocellular cancers.
(D) Kaplan–Meier analysis of the relevance between CCNB1 level and overall survival in human hepatocellular cancers.
(E) Analysis of Cbioportal dataset to determine the correlation between the mRNA expression levels of FOXM1 and CCNB1 among human hepatocellular cancers in TCGA.

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