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Characterization of the oncogenic function of centromere protein F in hepatocellular carcinoma

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ABSTRACT

Centromere protein F (CENPF) is an essential nuclear protein associated with the centromere-kinetochore complex and plays a critical role in chromosome segregation during mitosis. Up-regulation of CENPF expression has previously been detected in several solid tumors. In this study, we aim to study the expression and functional role of CENPF in hepatocellular carcinoma (HCC). We found CENPF was frequently overexpressed in HCC as compared with non-tumor tissue. Up-regulated CENPF expression in HCC was positively correlated with serum AFP, venous invasion, advanced differentiation stage and a shorter overall survival. Cox regression analysis found that overexpression of CENPF was an independent prognosis factor in HCC. Functional studies found that silencing CENPF could decrease the ability of the cells to proliferate, form colonies and induce tumor formation in nude mice. Silencing CENPF also resulted in the cell cycle arrest at G2/M checkpoint by down-regulating cell cycle proteins cdc2 and cyclin B1. Our data suggest that CENPF is frequently overexpressed in HCC and plays a critical role in driving HCC tumorigenesis.

1. Introduction

Hepatocellular carcinoma (HCC) is the fifth most common and the third most deadly cancer in the world [1]. Although progress has been made in recent decades to improve detection and treatment of HCC, the 5-year survival rate for the disease remains poor due to late presentation and the high incidence of recurrence and metastasis [2,3]. Thus, there is a need to identify better markers to detect the disease at an earlier stage, as well as to develop new therapeutic regimens that can better target HCC. Amplification of the long arm of chromosome 1 (1q) is one of the most frequent genetic alterations in HCC [4,5]. Several candidate oncogenes at 1q have been reported to be associated with the development and progression of HCC, including CHD1L [6,7], JTB and SHC1 [5]. In the present study, another candidate oncogene, centromere protein F (*CENPF*) at 1q41, was characterized.

CENPF is a member of the centromere protein family of kinetochore proteins [8]. When bound together with nuclear proteins like CENP-E, cytoplasmic dynein, MAD1, MAD2, Bub1 and BubR1, CENPF acts as a subunit of the protein complex which is responsible for kinetochore assembly, microtubule attachment, microtubule dynamics and spindle checkpoint signaling during mitosis [9]. *CENPF* is expressed in a cell cycle-dependent manner. At S phase, only low levels of CENPF can be detected in cell nucleus. At early G2 phase, CENPF level increases, and at late G2 phase, a dynamic spatial and temporal distribution of CENPF appears at the nuclear envelope [10]. Following nuclear envelope breakdown, CENPF becomes soluble in the mitotic cytoplasm while a subset remains bound at the outer kinetochore region until metaphase–anaphase transition [11]. During telophase, CENPF accumulates to the intraceullar bridge between the daughter cells [10], and then rapidly proteolyzed by farnesylation and ubiquitylation at the end of mitosis [12].

Abbreviations: CENPF, centromere protein F; HCC, hepatocellular carcinoma; CHD1L, chromodomain helicase DNA binding protein 1-like; JTB, jumping translocation breakpoint; SHC1, Src homology 2 domain containing transforming protein 1; MAD1, mitotic arrest deficient-like 1; MAD2, mitotic arrest deficient-like 2; BUB1, mitotic checkpoint serine/threonine kinase; TMA, tissue microarray; IHC, immunohistochemistry; DAB, Diaminobenzidine; qRT-PCR, quantitative real time polymerase chain reaction; siRNAs, small interfering RNAs; HE, hematoxylin and eosin.

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Overexpression of CENPF has previously been reported to be associated with non-Hodgkin's lymphoma [13], pancreatic ductal carcinoma [14], Wilm's tumor [15], neuroblastoma [16], breast cancer [17], colorectal gastrointestinal stromal tumors [18] and nasopharyngeal carcinoma [19]. CENPF overexpression has been suggested to serve as a poor prognostic factor in breast cancer and colorectal gastrointestinal stromal tumors [17,18]. Recently, CENPF was also found to be frequently amplified in HCC [20]. However, the oncogenic role of CENPF and its clinicopathological significance in HCC has not been explored to date. In the present study, immunohistochemistry was applied to investigate the expression pattern of CENPF in a large cohort of HCC specimens, as well as the clinical significances of CENPF overexpression in HCC. Oncogenic function of CENPF was characterized by various functional assays.

2. Materials and methods

2.1. Clinical samples and cell lines

Forty-one pairs of freshly frozen tissue samples containing HCC and adjacent non-tumor counterparts were obtained from the Department of Hepatobiliary Tumor, Sun Yat-Sen University Cancer Center (Guangzhou, China). Patient's consent and approval from the Institute's Research Ethics Committee was obtained before clinical material collection for research purposes. HCC cell lines QGY7703, BEL7402, SMMC7721, QSG7701, HepG2, PLC8024, Huh7, H2P and H2M, and immortalized normal human liver cell lines MIHA and LO2 have been described in previous studies [7,21,22].

2.2. Tissue microarray (TMA) construction and immunohistochemistry (IHC)

A total of 142 pairs of paraffin-embedded HCC samples were used for construction of the TMA. Samples were histologically and clinical diagnosed between 2003 and 2010 at the Sun Yat-Sen University Cancer Center. TMA blocks construction and IHC were performed as previously described [23]. The degree of immunostaining was assessed and scored independently by two investigators, blinded from the clinical parameters, according to both intensity and extent of staining. Only cells stained in the nucleus were taken into account. The extent of immunopositive cells was categorized as follow: 0 (0%), 1 (1–25%), 2 (26–50%), 3 (51–75%) or 4 (76–100%). The staining intensity was categorized by relative intensity as follow: 0 (no staining), 1 (weak staining), 2 (moderate staining) and 3 (strong staining). An overall score taking into account both parameters were then given: low CENPF expression with sum of both scores (extent and intensity) <3 or high CENPF expression with sum of both scores (extent and intensity) $\geqslant 3$.

2.3. RNA extraction and quantitative real-time PCR (qRT-PCR)

Total RNA was extracted from cultured cells or frozen tissues using TRIZOL Reagent (Invitrogen, Carlsbad, CA). Reverse

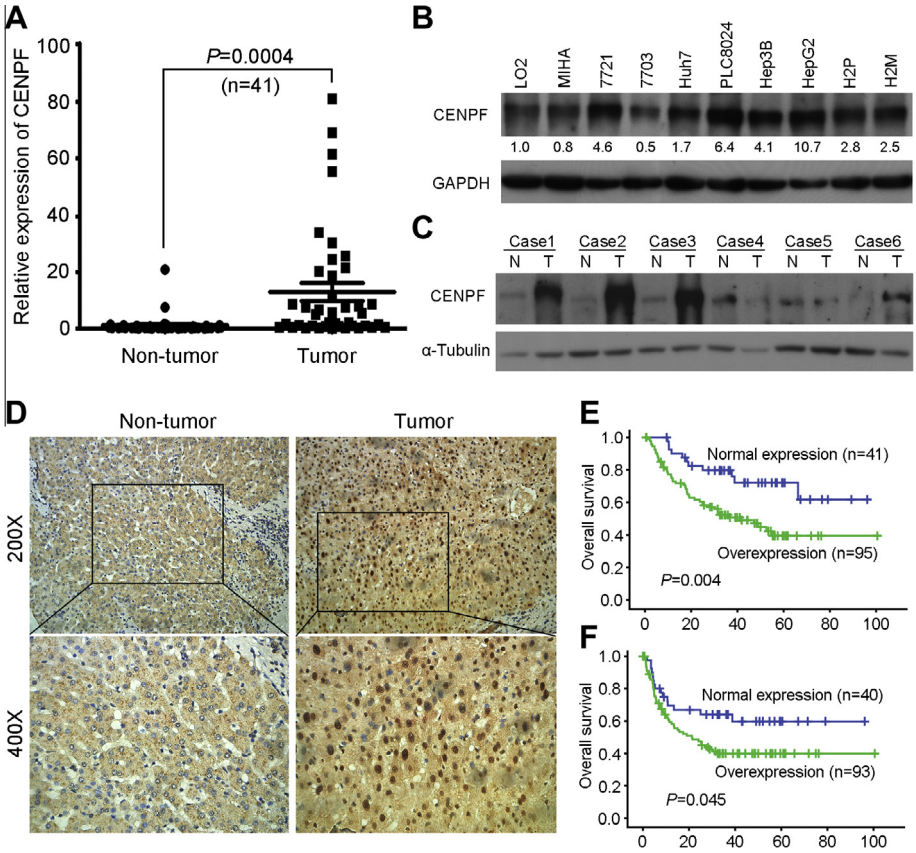

Fig. 1. Overexpression CENPF was determined in HCC and serve as an independent prognosis factor for cancer patients. (A) mRNA expression of CENPF in 41 matched non-tumor and primary HCC cases as detected by qRT-PCR. Average relative expression in tumor tissues (12.63 ± 3.10) was markedly higher than as compared with adjacent non-tumor tissues (1.00 ± 0.51; $P = 0.0004$). 18S was amplified as an internal control. (B) Protein expression of CENPF in a panel of liver cell lines as detected by Western blot. LO2 and MIHA are immortalized, normal liver cell lines. 7721, 7703, Huh7, PLC8024, Hep3B, HepG2, H2P and H2M are HCC cell lines. Numeric number represents the intensities of the bands of CENPF related to GAPDH. (C) Protein expression of CENPF in 6 pairs of non-tumor and HCC clinical samples as detected by Western blot. CENPF was up-regulated in 4 of the 6 samples examined. α-Tubulin was used as a loading control. (D) Representative IHC images of CENPF staining on the TMA. Only nuclear CENPF staining was regarded as positive signals. (E) Probability of survival of all patients with HCC: low CENPF expression, $n = 41$; high CENPF expression, $n = 95$ ($P = 0.004$). (F) Recurrence-free survival analysis of CENPF expression in tumor tissues ($P = 0.045$).