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Up-regulation of CHAF1A, a poor prognostic factor, facilitates cell proliferation of colon cancer

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ABSTRACT

Deregulation of chromatin assembly factor 1, p150 subunit A (CHAF1A) has recently been reported to be involved in the development of some cancer types. In this study, we identified that the frequency of positive CHAF1A staining in primary tumor mucosa (45.8%, 93 of 203 samples) was significantly elevated compared to that in paired normal mucosa (18.7%, 38 of 203 samples). The increased expression was strongly associated with cancer stage, tumor invasion, and histological grade. The five-year survival rate of patients with CHAF1A-positive tumors was remarkably lower than that of patients with CHAF1A-negative tumors. Colon cancer cells with CHAF1A knockdown exhibited decreased cell growth index, reduction in colony formation ability, elevated cell apoptosis rate as well as impaired colon tumorigenicity in nude mice. Hence, CHAF1A upregulation functions as a poor prognostic indicator of colon cancer, potentially contributing to its progression by mediating cancer cell proliferation.

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1. Introduction

Colon cancer is one of the most commonly diagnosed cancers and the fourth leading cause of cancer mortality worldwide. Despite improvements in surveillance and therapeutic approaches, there are approximately 1 million cases of colon cancer reported annually, with over 600,000 deaths per year [1]. Changes in dietary pattern and life style have increased colon cancer incidence rates, which have been historically low in China but have rapidly increased over the last 20 years [2,3]. Surgery cannot always protect against tumor recurrence, the major factor in cancer treatment failure. Furthermore, the existence of varied genetic and epigenetic backgrounds makes it difficult to implement personalized treatment regimens [4]. Therefore, the identification of novel diagnostic and prognostic biomarkers and therapeutic targets of colon tumors is of crucial importance for optimizing the choice and efficiency of individualized therapies.

Chromatin assembly factor 1 (CAF-1), a nuclear heterotrimeric complex comprised of subunits p150, p60, and p48, is well-known as the only histone chaperone protein that recruits histones H3 and H4 to newly synthesized DNA both in vitro and in vivo [5–7]. Previous reports have proposed that CAF-1, specifically the p150 subunit (CHAF1A), is the key gene responsible for bridging cell cycle progression and activating DNA damage checkpoints, and CAF-1/CHAF1A malfunction leads to sustained cell proliferation, resistance to cell death, increased genomic instability, and eventually a more aggressive cancer phenotype [8,9]. Recently, CHAF1A has been reported to be involved in the development of several cancer types [10–15]. It is worth noting that CHAF1A regulates histone H3 lysine 9 trimethylation of several key target genes (including wingless/integrated, Kirsten rat sarcoma, protein kinase B, and dehydrogenase/reductase member 2), and this epigenetic modification promotes neuroblastoma aggression [13]. Hence, CHAF1A could be inferred as being a pleiotropic mediator of cancer evolution. Nevertheless, the mechanism underlying the involvement of CHAF1A in colon cancer progression remains quite unknown.

In the current study, we evaluated the expression of CHAF1A in tissue microarrays (TMA) of paired normal and cancerous colon tissue samples by immunohistochemistry (IHC). We further validated the findings by real-time quantitative polymerase chain reaction (qPCR) and western blot analysis by using fresh patient-derived samples. We determined the clinical value of CHAF1A expression by the Kaplan–Meier survival and Cox proportional hazard models. Lastly, we evaluated whether CHAF1A has effects on colon cancer

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cell biological characteristics by using RNA interference (RNAi) approaches in vitro and in vivo.

2. Methods

2.1. Human tissue samples and cell lines

For TMA construction, tissue samples from 203 patients with colon cancer who underwent tumor resection between 2001 and 2003 by the same surgical team were archived. 86 men and 117 women, with a median age of 68 years at the time of surgery (range: 22–95 years of age), were enrolled. The median follow-up time was 61 months post-operatively (range: 9–89 months). The samples were comprised of formalin-fixed, paraffin-embedded primary neoplasms, matched non-tumor tissues (10 cm distance from primary neoplasms), and 66 paired lymph node metastasized specimens. For qPCR and western-blot analysis, tissue samples were gathered from 40 additional patients who had recently undergone colectomy. These 40 pairs of fresh tissue were subpackaged, immediately frozen in liquid nitrogen, and subsequently stored at −80 °C. None of the patients had been treated with chemotherapy or radiotherapy prior to surgery, and patients with stage II, III, or IV colon cancer received standard adjuvant chemotherapy. The study was approved by the Ethics Committee of Shanghai Jiaotong University affiliated with the First People's Hospital. Informed consent, according to the Declaration of Helsinki, was obtained from each patient.

Healthy human colon mucosa cell line, NCM460, was obtained from INCELL (San Antonio, USA). The human colon cancer cell lines DLD-1, HCT116, RKO, SW620, and LoVo cells were purchased from Type Culture Collection of the Chinese Academy of Science (Shanghai, China). Cells were maintained in DMEM medium, supplemented with 1% penicillin–streptomycin (Gibco, USA) and 10% FBS (Gibco, USA), at 37 °C in 5% CO_2.

2.2. Tissue microarray construction and immunohistochemistry

TMA construction and IHC staining were performed as previously described [16]. Briefly, slides were dewaxed, rehydrated, and then epitope retrieved. After blocking the enzymatic activity of endogenous peroxidase, slides were immunolabeled using a primary rabbit monoclonal antibody against human CHAF1A (1:200; Epitomics Inc., USA). The slides were then incubated with an anti-rabbit secondary antibody and 3,3′- diaminobenzidine (Dako EnVision Detection System, Denmark). Finally, each slide was counterstained with hematoxylin.

IHC staining was scored independently in a blinded fashion and in the absence of clinicopathological information by 2 pathologists. The scoring standard applied has been previously described [16].

2.3. RNA extraction and real-time quantitative polymerase chain reaction

Total RNA was extracted from frozen tissue samples or cultured cells using AllPrep DNA/RNA Mini Kit (Qiagen, Germany). After RNA intensity and purity were checked, first strand cDNA was reverse transcribed from 3-μg of RNA using the Access Reverse Transcriptase (RT)-PCR System (Promega, USA). A 1-μl aliquot of cDNA was used as the template for real-time PCR with GoTaq qPCR Master Mix (Promega, USA) by employing the 7900 system (Applied Biosystems Inc., USA). The following specific primers were used: *CHAF1A*, sense 5′-agggaaggtgcctatggtg-3′ and antisense 5′-cagggacgaatggctgagta-3′ (160 base pairs); *GAPDH*, sense 5′-tgacttcaacagcgacaccca-3′ and antisense 5′-caccctgttgctgtagccaaa-3′ (121 base pairs). GAPDH was used as the internal control. Each reaction was run in triplicate. The relative *CHAF1A* mRNA expression was calculated using the $2^{-\Delta\Delta Ct}$ comparative method.

2.4. Western blot analysis

Total protein was isolated from tissue samples or cultured cells using RIPA lysis buffer with inhibitor cocktail and then quantified by the BCA assay kit (Thermo Fisher, USA). Each 30-μg aliquot of total protein was loaded onto a 10% SDS–PAGE gel and then transferred to 0.2-μm PVDF membranes (Merck Millipore, Germany). The membranes were blocked in 5% (w/v) non-fat milk and probed with primary rabbit antibody against human CHAF1A protein (1:1000; Epitomics Inc., USA) or GAPDH (1:2000; Cell Signaling Technology, USA). Following incubation with a horseradish peroxidase-conjugated secondary antibody (1:5000; Santa Cruz Biotechnology, USA), the blots were visualized by electrochemiluminescence (Merck Millipore, Germany) and graphed. GAPDH was used as the loading control. Each sample was loaded in triplicate.

2.5. CHAF1A knockdown plasmid construction and cell transfection

For small hairpin RNA (shRNA)-mediated CHAF1A (NM_005483.2) knockdown, the following RNAi target sequence was used: 5′-ccgactcaattcctgtgtaaa-3′. The non-target shRNA sequence, 5′-ttctccgaacgtgtcacgt-3′, was employed as a negative control (available at Qiagen Company website). The specific double-stranded oligonucleotides were chemosynthesized, annealed, and then inserted into a shRNA expression vector, pGCSIL-GFP (in collaboration with Shanghai GeneChem Company, China). The CHAF1A-shRNA and negative control shRNA plasmids were transfected into RKO cells by using Lipofectamine 2000 (Life Technologies, USA). Stably transfected cell clones were selected in 2 μg/ml puromycin-containing medium (Sigma–Aldrich, USA) and enriched by the limited dilution method. CHAF1A clone expressions were confirmed by qPCR and western blot analysis.

2.6. Cell proliferation and colony formation assays

The effect of CHAF1A knockdown on colon cancer cell proliferation was measured with the Cellomics system (Thermo Scientific ArrayScan, USA). In brief, log-phase cells treated with CHAF1A-shRNA or non-target–shRNA were seeded onto 96-well plates (2×10^3 cells/well) in replicates of five. The cell number counting assay was carried out at 24, 48, 72, 96, and 120 h post seeding. A cell growth curve was generated by using the obtained growth data, which were expressed as mean ± standard deviation.

To evaluate colony formation ability, 800 treated log-phase cells were suspended and then plated on to 6-well plates. Cells were cultured in an incubator for 14 days. Following fixation, the cells were stained by Giemsa solution. Colonies were then counted and photographed. The tests were independently performed in triplicates.

2.7. Flow cytometry cell cycle and apoptosis assays

Cell cycle and apoptosis analyses were conducted by using the FACSCalibur flow cytometer (BD Biosciences, USA). Transfected cells were synchronized by serum starvation for 12–14 h. For cell cycle analysis, collected cells were stained with 50 μg/ml propidium iodide (Sigma–Aldrich, USA) staining solution containing 100 μg/ml RNase, following which the cells were fixed in ice-cold 70% ethanol at 4 °C for at least 2 h. For the apoptosis assay, harvested cells were washed with binding buffer, resuspended in staining buffer, and then incubated with Annexin V-APC (eBioscience, USA) according to the manufacturer's instructions.

Triplicate samples containing at least 1×10^5 cells each were subjected to flow cytometry.

2.8. Tumorigenicity in nude mice

Male BALB/c nude mice were obtained (Shanghai Slac Laboratory Animal Co. Ltd, China) and bred under specific pathogen-free conditions. Six-week-old mice were randomly assigned to 2 groups ($n = 6$) and were subcutaneously implanted with 5×10^6 cells into their flanks. Tumor size was measured with a caliper every 5 days from day 1 to 21 post-implantation. All mice were killed 21 days after inoculation and their tumors were weighed. The tumor volumes were calculated using the following formula: volume = width^2 × length × 0.5. All animal studies were performed in compliance with guidelines established by the Shanghai Medical Experimental Animal Care Commission, Chinese Academy of Science.

2.9. Statistical analysis

The differences between the 2 groups were compared with the *t* test, χ^2 test or Fisher's exact test, as appropriate. Survival curves were calculated by Kaplan–Meier method with the log-rank test. To investigate independent risk factors, Cox proportional hazard regression models were used and expressed as hazard ratios with 95% confidence interval. Significant factors estimated by the univariate Cox analysis were put to the multivariate Cox proportional hazard model with the forward conditional method. All statistical analyses were set to a significance level of 0.05 and performed using the SPSS 20.0 statistical software (SPSS Inc., USA).

3. Results

3.1. CHAF1A expression is significantly upregulated in human colon cancer

To explore the effect of CHAF1A expression on colon cancer progression, we determined CHAF1A expression in 203 primary colon cancer tissue samples and matched normal tissue samples as well as in 66 lymph node metastasized samples. Based on IHC staining of TMAs, CHAF1A predominantly stained positive in nuclei of primary and lymph node-infiltrated tumor cells (Fig. 1A–b and A–c), whereas CHAF1A staining was minimally detectable in paired normal epithelia (Fig. 1A–a). The frequency of CHAF1A positive

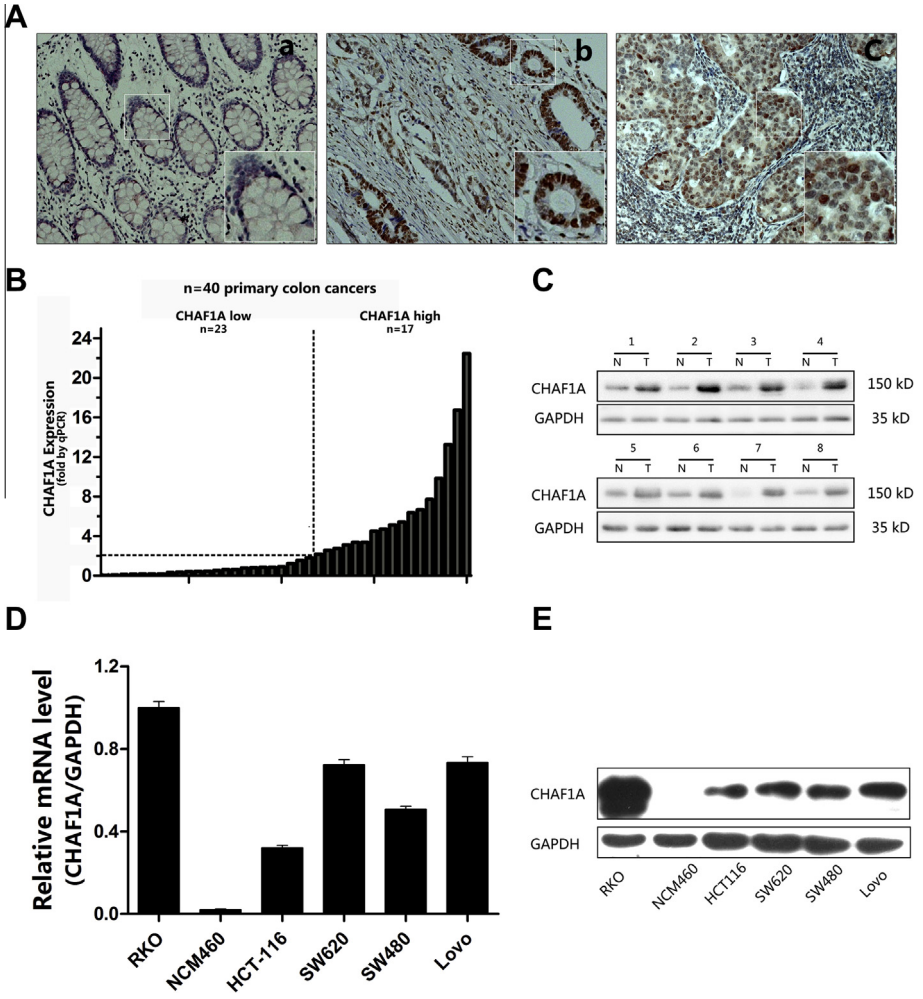


Fig. 1. CHAF1A expression is upregulated in human colon cancer tissues and cell lines. (A) Immunohistochemical staining of chromatin assembly factor 1, p150 subunit A (CHAF1A) in normal and cancerous mucosa samples as well as lymph node metastasized tissue samples. Representative illustrations are shown: (a), (b), and (c): CHAF1A staining in paired normal and cancerous mucosa samples and corresponding lymph node metastasized tissue samples, 200×. (B) Real-time quantitative polymerase chain reaction analysis of CHAF1A expression in human colon cancer tissues. (C) Western blot analysis of CHAF1A expression in 8 representative cases. The dashed line represents a 2-fold change normalized to paired normal tissue. 'N' represents normal tissue. 'T' represents tumor tissue. (D) Real-time quantitative polymerase chain reaction and (E) western blot analysis of CHAF1A expression in human colon cell lines. Error bars represent standard deviation; $n = 3$. NCM460: normal colon cell line; RKO, HCT-116, SW620, SW480, and LoVo: colon cancer cell lines.

staining in primary tumor mucosa (45.8%, 93 of 203 samples) was significantly higher (Table 1, $P < 0.001$) than that observed in paired normal mucosa (18.7%, 38 of 203 samples), while positive staining levels in primary cancers was similar to that in lymph node-metastasized cancers (48.5%, 32 of 66 samples). To confirm the presence of abnormal CHAF1A expression, total RNA and protein extracted from additional 40 fresh paired cancerous and normal mucosal tissue samples, were analyzed by qPCR and western blotting, respectively (Fig. 1B and C). In agreement with the IHC results, compared to normal mucosa samples, *CHAF1A* mRNA levels in cancerous tissue samples were significantly upregulated in 17 of 40 cases (42.5%, fold change $\geqslant 2$; Fig. 1B). The result was confirmed by western blot analysis and the representative data is shown in Fig. 1C. Finally, CHAF1A mRNA and protein expression were found to be significantly higher in colon cancer cell lines than in normal colon epithelial cells (NCM460) as depicted in Fig. 1D and E, which was identical to the findings obtained from clinical samples. These data indicate that CHAF1A expression is commonly elevated in human colon cancer tissues.

3.2. Correlation between CHAF1A overexpression and colon cancer clinicopathologic characteristics

We next investigated the correlation between upregulated CHAF1A expression and clinicopathological features of 203 colon cancer cases. As summarized in Table 2, elevated CHAF1A expression was significantly associated with cancer stage (AJCC, $P = 0.022$), tumor invasion (T stage, $P = 0.001$), and histologic grade (differentiation, $P = 0.037$), while no relationship was found between CHAF1 expression and age, gender, tumor location, regional lymph node metastasis, distant metastasis, or vessel invasion status. These findings indicated that upregulated CHAF1A expression may be an early event in cancer development and could play a critical role in colon cancer progression.

3.3. CHAF1A overexpression is an independent prognostic indicator of poor colon cancer survival

To evaluate the clinical value of CHAF1A overexpression in colon cancer, the results of TMA IHC staining and relevant clinical parameters were used for Kaplan–Meier (Fig. 2) and Cox proportional hazard analyses (Table 3). The 5-year overall survival (OS) and disease-free survival (DFS) rates of patients with CHAF1A-positive tumors were remarkably lower than those of patients with CHAF1A-negative tumors (OS: 56.1% vs. 77.0%, $P = 0.002$; DFS: 58.1% vs. 73.5%, $P = 0.013$, Fig. 2). Additionally, univariate analysis of OS indicated that CHAF1A expression level, tumor invasion, lymph node metastasis, distant metastasis, cancer stage, histological differentiation, and vascular invasion were prognostic factors. These significant factors were selected for the multivariate Cox proportional hazard analysis. Multivariate analysis showed that CHAF1A expression level was an independent prognostic indicator for the OS of patients with colon cancer (hazard ratio = 1.905, $P = 0.011$, 95% confidence interval: 1.159–3.131, Table 3). Therefore, CHAF1A overexpression represents a powerful prognostic indicator, independent of other acknowledged factors, of poor colon cancer survival.

Table 1
Expression of *CHAF1A* in colonic normal tissues, cancerous tissues and lymph node metastasis tissues.

Tissue sample	*n*	Expression of *CHAF1A*		*P* value
		Negative (*n*, %)	Positive (*n*, %)	
Normal tissue	203	165 (81.3)	38 (18.7)	<0.001*
Cancerous tissue	203	110 (54.2)	93 (45.8)	
LNM tissue	66	32 (48.5)	34 (51.5)	

Abbreviations: LNM, lymph node metastasis.
* $P < 0.05$ indicates a significant difference among the variables.

Table 2
CHAF1A expression and clinicopathologic characteristics.

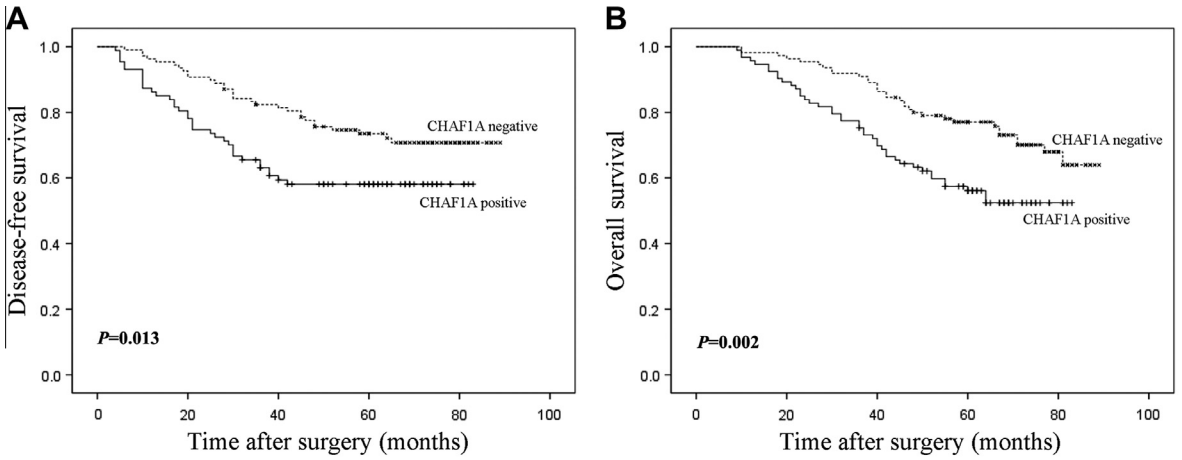
	CHAF1A expression		*P* value
	Negative ($n = 110$, %)	Positive ($n = 93$, %)	
Age			
<65	39 (35.5)	42 (45.2)	0.159
⩾65	71 (64.5)	51 (54.8)	
Gender			
Male	47 (42.7)	39 (41.9)	0.909
Female	63 (57.3)	54 (58.1)	
Location			
Right	44 (40.0)	40 (43.0)	0.664
Others	66 (60.0)	53 (57.0)	
AJCC stage			
I + II	65 (59.1)	40 (43.0)	**0.022***
III + IV	45 (40.9)	53 (57.0)	
T stage			
T1 + T2	25 (22.7)	5 (5.4)	**0.001***
T3 + T4	85 (77.3)	88 (94.6)	
N stage			
N0	65 (59.1)	43 (46.2)	0.067
N1 + N2	45 (40.9)	50 (53.8)	
M stage			
M0	104 (94.5)	81 (87.1)	0.063
M1	6 (5.5)	12 (12.9)	
Differentiation			
Well + moderate	99 (90.0)	74 (79.6)	**0.037***
Poor	11 (10.0)	19 (20.4)	
Vascular invasion			
Yes	103 (93.6)	86 (92.5)	0.745
No	7 (6.4)	7 (7.5)	

* $P < 0.05$ indicates a significant difference among the variables.

3.4. Knockdown of CHAF1A inhibits colon cancer cell growth both in vitro and in vivo

To determine the biological effect of upregulated CHAF1A expression on colon cancer development, a series of functional studies combined with a loss-of-function approach were employed to assess the role of CHAF1A in colon cancer cell growth. RKO cells were transfected with CHAF1A-shRNA or scrambled shRNA lentivirus and stably transfected cells were established. The efficacy of CHAF1A knockdown was confirmed by western blot (Fig. 3A). Based on the Cellomics cell number counting assay, compared to cell growth of control cells, cells with CHAF1A knockdown exhibited significantly decreased cell growth with prolonged culture time ($P < 0.05$, Fig. 3B). CHAF1A knockdown consistently induced a reduction in colony formation ability ($P < 0.05$, Fig. 3C). As illustrated in Fig. 3D, compared to negative control cells, cells lacking CHAF1A accumulated increased S phase DNA content (S phase DNA content: $54.74 \pm 0.68\%$ vs. $45.28 \pm 0.36\%$; $P < 0.05$). Furthermore, the apoptosis rate was dramatically elevated from 5.91% in mock cells to 33.68% in CHAF1A knockdown cells. Collectively, these data suggest that CHAF1A knockdown suppresses colon cancer cell proliferation and induces cell apoptosis.

To elucidate the role of CHAF1A in colon tumorigenicity in vivo, CHAF1A-shRNA–treated RKO cells were subcutaneously implanted into nude mice. The growth index of CHAF1A-shRNA–treated tumors distinctly dropped in comparison to that of control tumors (Fig. 4). Specifically, the average tumor size and weight of CHAF1A knockdown tumors were dramatically lower than those of the control group: tumor size at day 21 was $0.48 \pm 0.14\ cm^3$ vs. $2.01 \pm 0.68\ cm^3$, respectively, while tumor weight was 0.47 ± 0.04 g vs. 2.18 ± 0.07 g, respectively. Taken together, the



		Time (months)							
		0	12	24	36	48	60	72	84
Negative	No. at Risk	110	104	98	86	76	55	39	6
	No. of events	0	4	6	9	7	2	2	0
Positive	No. at Risk	93	75	65	51	43	31	12	0
	No. of events	0	12	10	10	4	0	0	0

		Time (months)							
		0	12	24	36	48	60	72	84
Negative	No. at Risk	110	108	105	100	86	62	42	6
	No. of events	0	2	3	5	12	3	5	2
Positive	No. at Risk	93	89	78	69	57	37	12	0
	No. of events	0	4	11	8	11	6	2	0

Fig. 2. Kaplan–Meier curves and life tables based on CHAF1A expression level. (A) Disease-free survival curves and (B) overall survival curves of 203 primary colon tumors. The survival rate of CHAF1A-positive group ($n = 93$) is significantly lower than that of the CHAF1A-negative group ($n = 110$; log-rank test; $P = 0.013$ and $P = 0.002$ respectively). The relevant life tables are presented beneath the curves.

Table 3
Association between clinical characteristics and overall survival by Cox regression model analysis.

	Overall survival			
	Univariate		Multivariate	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
CHAF1A expression				
Negative	–		–	
Positive	2.016 (1.267, 3.208)	0.003*	1.905 (1.159, 3.131)	0.011*
Age				
<65	–			
⩾65	0.967 (0.608,1.539)	0.888		
Gender				
Male	–			
Female	1.347 (0.838, 2.164)	0.219		
Location				
Right	–			
Others	1.019 (0.639, 1.625)	0.937		
T stage				
T1 + T2	–		–	
T3 + T4	3.772 (1.376, 10.341)	0.010*	3.382 (1.230, 9.302)	0.018*
N stage				
N0	–		–	
N1 + N2	6.195 (3.571, 10.747)	<0.001*	2.494 (0.549, 11.321)	0.236
M stage				
M0	–		–	
M1	14.741 (8.148, 26.668)	<0.001*	5.828 (2.836, 11.975)	<0.001*
AJCC				
I-II	–		–	
III-IV	6.804 (3.830,12.088)	<0.001*	5.830 (3.264, 10.415)	0.003*
Differentiation				
Well	–		–	
Moderate	2.324 (1.318, 4.097)	0.004*	1.854 (1.042, 3.297)	0.036*
Poor	7.413 (4.067, 13.513)	<0.001*	3.221 (1.536, 6.755)	0.002*
Vascular invasion				
No	–		–	
Yes	4.730 (2.573, 8.694)	<0.001*	1.064 (0.518, 2.186)	0.865

HR = Hazard ratio, CI = confidence interval.
* $P < 0.05$ indicates that the 95% CI of HR was not including 1.

aforementioned findings indicated that elevated CHAF1A expression contributes to hyperproliferation of colon cancer cells both in vitro and in vivo.

4. Discussion

CHAF1A, located on chromosome 19p13.3, serves as the main functional subunit of the CAF-1 protein, participating in DNA replication, regulation of gene expression, and DNA mismatch repair [8,9,17–19]. Recently, there has been an increase in reports indicating that CHAF1A expression pattern is related to breast cancer pathogenesis, prostate squamous cell carcinoma, glioma, and neuroblastoma [10,11,13–15]. Additionally, CHAF1A deregulation strongly correlates with genomic instability in the recessively inherited Bloom syndrome, resulting in high predisposition to leukemia, lymphoma, and other solid carcinomas [20]. Therefore, the oncogenic contribution of CHAF1A needs to be continuously explored. In this study, we discovered for the first time that CHAF1A expression is elevated in both human colon cancer tissue samples and cell lines, and this deregulation is significantly associated with poor clinical outcome, independent of age, cancer stage, and tumor histology.

These clinical findings suggest that CHAF1A might play a critical role in colon cancer development. The 938-amino acid protein CHAF1A binds via its C-terminal to the p60 subunit of CAF-1 to activate nucleosome assembly [7,21]. CHAF1A binds via its N-terminal residues to the proliferating cell nuclear antigen and heterochromatin protein 1 to stimulate the function of DNA polymerases [22,23]. The KER domain, a region rich in lysine, glutamine, and arginine residues, the ED domain, a region rich in asparagine and glutamic acid residues, and the two internal regions of CHAF1A, serve as binding sites for acetylated histones H3 and H4 [5,24]. These biochemical docking sites confer CHAF1A with a pivotal regulatory role in cell cycle progression from middle-to-late S phase [25], while CHAF1A inhibition blocks DNA synthesis, induces DNA damage, and activates the S phase checkpoint [26]. Based

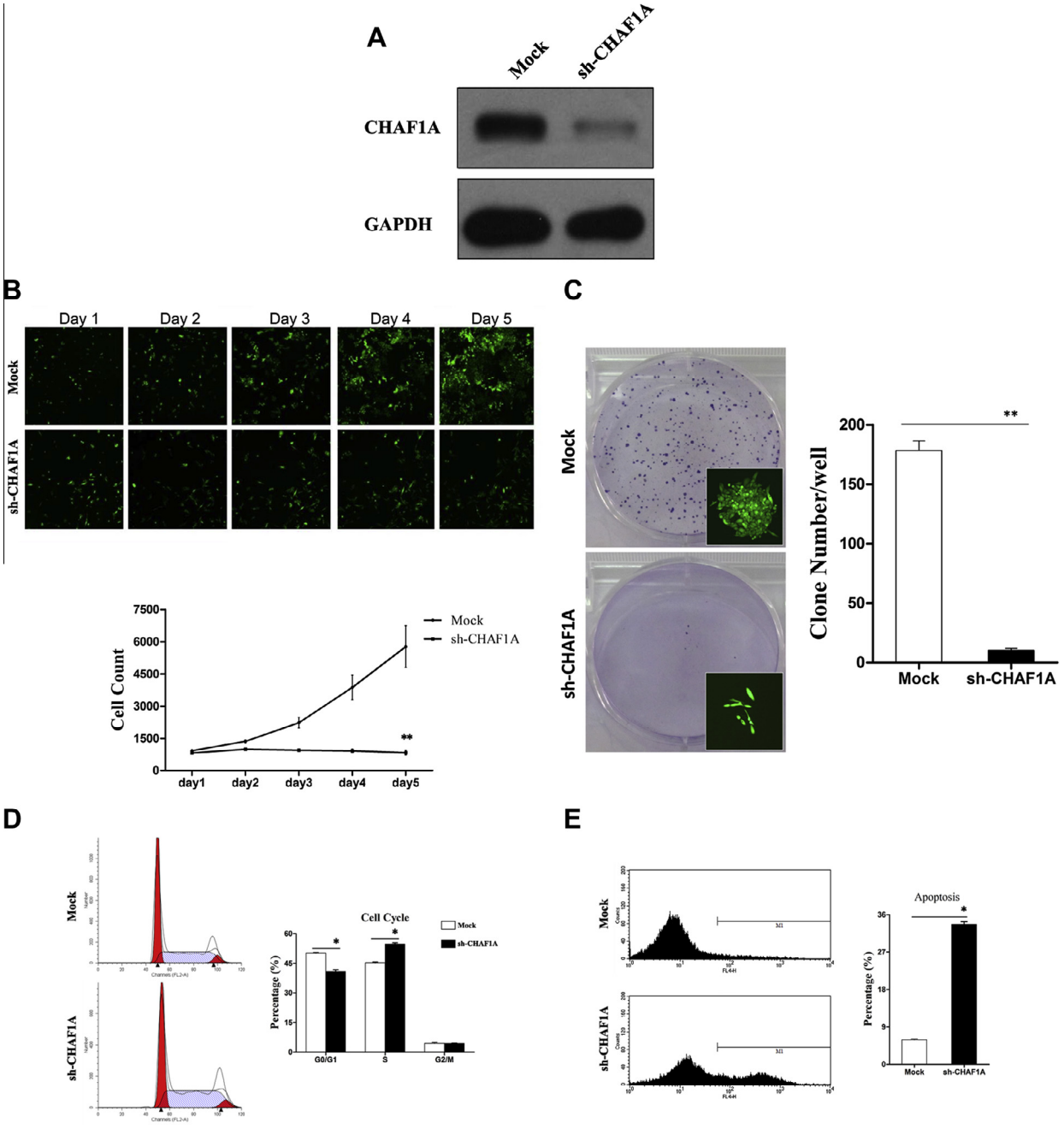


Fig. 3. CHAF1A knockdown inhibits colon cancer cell growth and induces apoptosis. (A) CHAF1A knockdown clones and mock clones of RKO cell line were identified and confirmed by western blot analysis. Effect of CHAF1A knockdown on cell growth were evaluated by (B) cell counting assay and (C) colony formation assay ($n = 3$; $^{**}P < 0.01$). Error bars represent standard deviation. Flow cytometry analysis of (D) cell cycle and (E) apoptosis ($n = 3$; $^{*}P < 0.05$). Representative illustrations are shown. Error bars represent standard deviation.

on the functional experiments shown here, CHAF1A knockdown in RKO cells led to low cell growth rate in vitro and reduced tumorigenicity in nude mice, marked especially by S-phase arrest, which is consistent with the role of CHAF1A in cell cycle progression. Once CHAF1A expression is inhibited, the S phase checkpoint machinery is activated, specifically checkpoint kinase 1 and p53, successively [21,26]. Activated checkpoint kinase 1 interferes with the activity cyclin-dependent kinases, thereby leading to slow S-phase progression [27]. Activated p53 is well known for inducing cell cycle arrest and cell death, which would explain the increased cell apoptosis index in CHAF1A-shRNA RKO cells observed in the current study. Moreover, a previous study demonstrated that CHAF1A acts as an epigenetic silencing factor by modulating the histone H3 lysine 3 trimethylation epigenetic modification in pluripotent embryonic cells [28,29]. CHAF1A also regulates DNA methylation by forming a complex with methyl-CpG binding protein [9]. These findings suggest that CHAF1A might have an epigenetic role in cancer biology. Hence, it is plausible that CHAF1A functions as a pleiotropic modulator of colon cancer progression, although the molecular mechanisms need to be further elucidated.

In summary, the present study identified that the expression of CHAF1A mRNA and protein was upregulated in colon tumor mucosa samples and cell lines in comparison to that in normal tissues. CHAF1A overexpression was strongly associated with poor survival in large colon cancer patient cohorts. Therefore, elevated CHAF1A may be a powerful indicator for colon cancer prognosis.

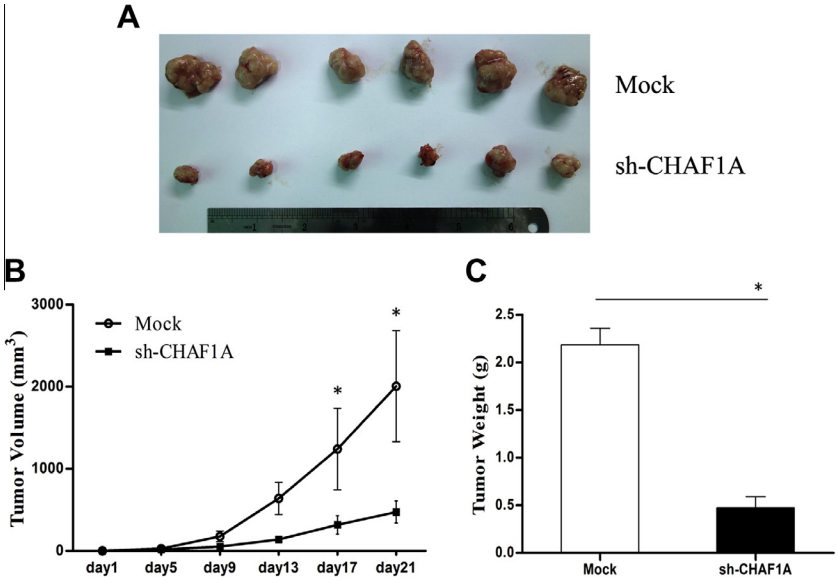


Fig. 4. Downregulation of CHAF1A expression inhibits tumorigenicity in nude mice. (A) Stable CHAF1A knockdown clones and mock clones of RKO cells were subcutaneously implanted into the flank of nude mice ($n = 6$). At day 21, the mice were killed. (B) The tumor volumes were calculated in each group every 5 days from day 1 to day 21. Error bars represent standard deviation; $^*P < 0.01$. (C) The tumor weights were measured at day 21. Data is expressed as mean ± standard deviation; $^*P < 0.01$.

Furthermore, knocking down CHAF1A inhibited cell growth, induced cell apoptosis, and impaired tumorigenicity. It is, thus, reasonable to speculate that CHAF1A plays an important role malignant transformation of colon cancer and serves as a potential target for cancer prevention and treatment. Further mechanistic studies may advance our knowledge of the role of CHAF1A in cancer development.

Competing interests

No competing interests were disclosed.

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