

CEP55 contributes to human gastric carcinoma by regulating cell proliferation

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Abstract Centrosomal protein 55 (CEP55) is the latest found member in the centrosomal relative protein family, which participates in cell-cycle regulation. CEP55 exists in many kinds of normal tissues and tumour cells such as hepatocellular carcinoma, and is important in carcinogenesis. However, the role of CEP55 in the pathogenesis of gastric cancer (GC) remains unclear. The mRNA levels of CEP55 in GC tissues and GC cell lines were examined by quantitative real-time PCR, and the protein expression of CEP55 in GC tissues was detected by Western blot and immunohistochemistry. The role of CEP55 in regulating the proliferation of GC cell lines was investigated both in vitro and in vivo. CEP55 was strongly upregulated in human GC, indicating that CEP55 contributed to carcinogenesis and progression of GC. Ectopic overexpression of CEP55 enhanced the cell proliferation, colony formation, and tumorigenicity of GC cells, whereas CEP55 knock-down inhibited these effects. We discovered that cell transformation induced by CEP55 was mediated by the AKT signaling pathway. Overexpression of CEP55 enhanced the phosphorylation of AKT and inhibited the activity of p21 WAF1/Cip1. In addition, cellular proliferation was suppressed as a result of cell cycle arrest at the G2/M phase in CEP55-knockdown cells. CEP55 expression was elevated in GC compared with normal control tissues. Credible evidence

showed that CEP55 can be a potential therapeutic target in GC.

Keywords Gastric cancer · CEP55 · Proliferation · PI3K/AKT/p21 · Cell cycle

Introduction

Gastric cancer (GC) is one of the most common malignant tumours worldwide. GC-related morbidity and mortality have gradually decreased in recent years, but approximately 989,600 new cases and 738,000 people died of GC in 2008 [1]. Significant progress has been made in surgical techniques, adjuvant therapy, radiochemotherapy, molecular targeted therapy and early detection for GC prognosis. Nevertheless, 5-year survival rate remains at $\leq 25\%$, especially in eastern Asian countries, including Korea, Japan and China [2–4]. The main virulence factors of GC including dietary factors, environmental exposures, chronic *Helicobacter pylori* infection and genetic susceptibility are gradually being determined. Oncogenes, tumour suppressor genes and many microRNAs have also been proven to be closely associated with GC, but the deep molecular mechanisms underlying its carcinogenesis, progression and aggressiveness are still under investigation [5]. Therefore, new clues to better understand GC proliferation and effective therapeutic strategies should be explored.

Centrosomal protein 55 (CEP55) is the latest found member in the centrosomal relative protein family, which participates in the regulation of the cell cycle. CEP55 is located in the centrosome in interphase cells and is recruited into the midbody during cytokinesis [6]. In addition, CEP55 is required for midbody structure and the completion of cytokinesis at the terminal stage [7]. CEP55 is known by several names, including FLJ10540, C10orf3 and URCC6. CEP55 is highly expression in certain human tumours [8–12] and

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various tumour cell lines [13]. However, information on the relationship between CEP55 and GC, and the molecular mechanisms underlying the participation of CEP55 in these malignant features, are lacking.

A major downstream target of phosphoinositide 3-kinase (PI3K) is the serine/threonine protein kinase AKT (protein kinase B, PKB) that regulates diverse processes, such as glucose homeostasis, transcription, apoptosis, cell motility, angiogenesis and proliferation [14–17]. AKT has been identified as an oncogene, and this kinase is frequently activated in malignant cells [18]. AKT promotes growth by regulating several components of the translational apparatus, including the target of p21 (also known as p21 WAF1/Cip1), which is the cyclin-dependent kinase inhibitor. As an inhibitor, p21 promotes cell cycle arrest in response to numerous stimuli [19].

Cell cycle is a complex and fine adjustment process, in which a large number of regulatory proteins are involved, including cyclins that obviously have cycle specificity [20, 21]. Previous studies have suggested that cyclins are closely related to the occurrence and development of tumour [22]. In hepatocellular carcinoma, CEP55 expression level is related with cyclins [8]. Fabbro et al. showed that CEP55-depleted cells induces cytokinesis failure, which results in aneuploidy and multiple spindle poles, and subsequently generates genomic instability, and facilitates the loss of tumour suppressor genes and activation of oncogenes. Furthermore, CEP55 overexpression also induces aneuploidy. Previous data suggest that CEP55 expression must be tightly regulated to ensure that the final stages of cell division occurs correctly [6].

In this study, we concluded that CEP55 was overexpressed in GC and ectopic expression of CEP55 promoted anchorage-independent growth in soft agar and induces tumourigenesis in nude mice. Moreover, we discovered that cell transformation induced by CEP55 was mediated by the AKT signalling pathway. Overexpression of CEP55 enhanced the phosphorylation of AKT and inhibited the activity of p21 WAF1/Cip1. In addition, cellular proliferation was suppressed as a result of cell cycle arrest at the G2/M phase in CEP55-knockdown cells. These findings identified the oncogenic accelerator function of CEP55 in GC, and provided new insights into the pathogenesis of GC which will aid the development of novel therapeutic strategies.

Materials and methods

Stomach tissue samples and GC cell lines cultures

Human GC tissues and adjacent normal tissues were obtained from 68 GC patients who underwent surgical resection at the Department of General Surgery, First Affiliated Hospital, Nanjing Medical University, China, with informed consent.

All patients were diagnosed pathologically according to the criteria of the American Joint Committee on Cancer. Histopathological diagnoses were performed by two professional pathologists independently. Tissue samples were flash frozen immediately after resection and stored in liquid nitrogen until RNA and protein extraction. The tissue specimens were collected using a protocol approved by the Nanjing Medical University Institutional Review Board.

The human GC cell lines AGS, MKN45, MKN28, SGC7901 and BGC823, and normal human gastric epithelial cells (GES-1) used in this study were purchased from the American Type Culture Collection (Manassas, VA, United States of America) and were grown in RPMI-1640 medium supplemented with 10 % foetal bovine serum at 37 °C in 5 % CO₂.

Quantitative real-time PCR

Total RNA was extracted from frozen tissues and cell lines using Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol and was reverse transcribed into cDNA using Primescript RT Reagent (Takara, Japan). The reaction system (20 µl) contained the corresponding cDNA. The CEP55 primers used in quantitative real-time PCR were as follows: forward primer of 5'-TTGGAACAACAGATGCAGGC-3' and reverse primer of 5'-GAGTGCAGCAGTGGGACTTT-3'. β-Actin, used as an internal control, was amplified with forward primer 5'-AGAGCCTCGCCTTTGCCGATCC-3' and reverse primer 5'-CTGGGCCTCGTCGCCACATA-3'. All procedures were performed in triplicate.

Immunohistochemical staining

The paraffin-embedded tissues were cut into 4-µm sections, and then incubated with rabbit anti-CEP55 antibody (Sigma, USA, dilution 1:200, HPA023430) at 4 °C overnight. After washing with PBS, the slices were incubated with horseradish peroxidase (HRP)-polymer-conjugated secondary antibody at 37 °C for 1 h. Subsequently, the slices were stained with the DAB solution for 3 min and the nuclei were counterstained with haematoxylin. Tumour slices were examined in a blinded manner. Three fields were selected for examination of the percentage of positive tumours and cell-staining intensity. Immunohistochemical staining was assessed according to the immunoreactive score (IRS) that evaluated the staining intensity and the proportion of positive cells. The staining intensity was graded as 0 (no staining), 1 (weak), 2 (moderate) and 3 (strong). The proportion of positive cells was scored as 0 (negative), 1 (<10 %), 2 (10–50 %) and 3 (>50 %). Both of the scores were multiplied and the IRS was determined: values ≥ 3 were defined as cytoplasmic expression positive, and values <3 were regarded as negative.

Construction of recombinant plasmids

For the construction of CEP55 recombinant plasmid, pcDNA3.1 (Invitrogen) was used. Based on the CEP55 nucleotide sequence from GeneBank, a pair of detection primers was designed. The sequence of the forward primer was 5'-EcoRI-AGAGAATTCATGTCTTCCAGAAGTACCAA-3', and sequence of the reverse primer was 5'-BamHI-AGAGGATCCCTACTTTGAACAGTATTCCA-3'. The GES-1 cDNA was used as template for PCR and subsequently inserted the PCR product into pcDNA3.1.

RNA interference

Small interference RNAs were chemically synthesised (GenePharma Co. Ltd., China). Synthesised DNA nucleotide fragment encoding short hairpin RNA (shRNA) for knock-down of endogenous CEP55 was inserted into pSUPER (OligoEngine, USA). The sequence of siRNA-312 was CCAGAAGUACCAAAGAUUUAdTdT (sense) and AAAUCUUUGGUACUUCUGGdTdT (antisense). Negative control (NC) siRNA synthesised by Shanghai GenePharma Co. was used as a control. The sequence of si-NC was as follows: UUCUCCGAACGUGUCACGUTT (sense) and ACGUGACACGUUCGGAGAATT (antisense).

The sequence of shRNA-312 was as follows: GATCCCCC CAGAAGTACCAAAGATTTTCAAGAGAAAATCTTTG GTACTTCTGGTTTTTGGAAA (sense) and AGCTTTTC CAAAAACCAGAAGTACCAAAGATTTTCTCTTGAA AAATCTTTGGTACTTCTGGGGG (antisense). The sequence of sh-NC was as follows: GATCCCC TTCTCCGA ACGTGTACGTTTCAAGAGAACGTGACACGTTTCG GAGAATTTTTGGAAA (sense) and AGCTTTTGGAAA AATTCTCCGAACGTGTACGTTCTCTTGAA ACGT GACACGTTTCGGAGAAGGG (antisense). The construct was verified by sequencing.

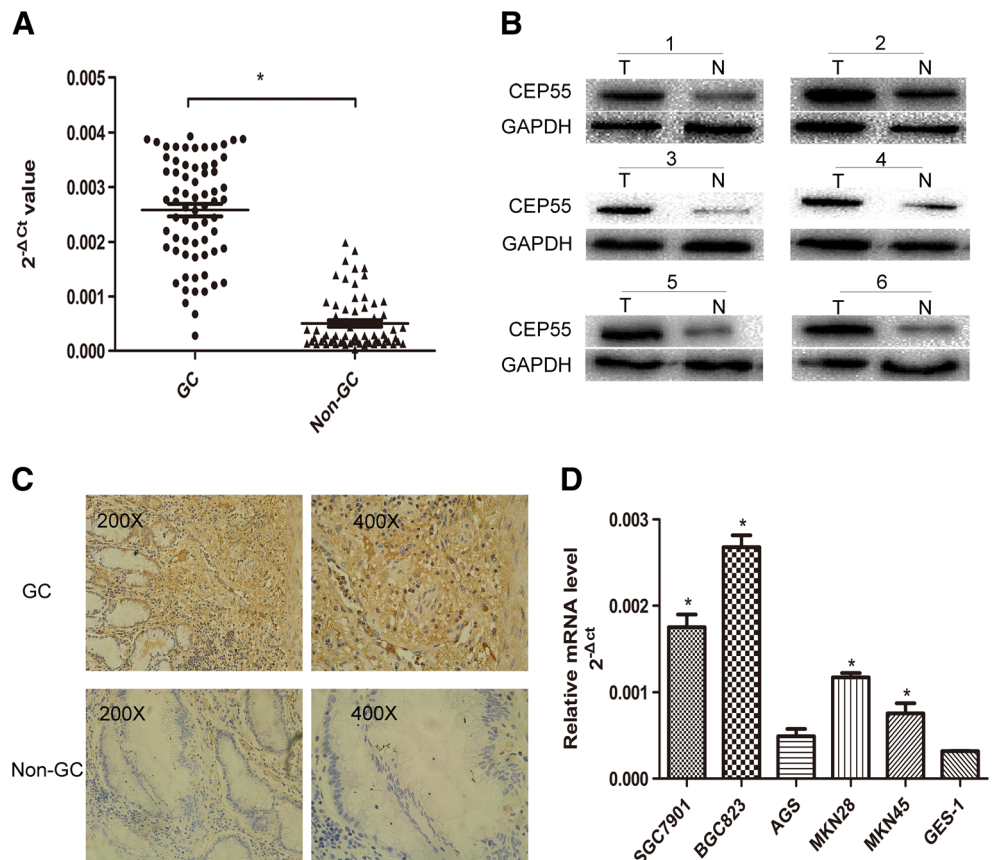
Cell transfection

All plasmids, siRNA and shRNA transfection were performed by Lipofectamine2000 (Invitrogen) according to the manufacturer's instructions.

Cell cycle assay

For cell cycle analysis, cells were trypsinised and rinsed twice with ice-cold PBS solution, and subsequently added to 75 % ice-cold ethanol while vortexing. Cells were incubated in -20 °C overnight. The fixed cells were washed with ice-cold PBS and incubated at 37 °C for 30 min in 0.5 ml PBS solution

Fig. 1 Upregulation of CEP55 in clinical specimens and GC-derived cell lines. **a** The expression of CEP55 mRNA in 68 paired GC specimens by real-time PCR (**P*<0.05). **b** Representative results of the upregulation of CEP55 protein in GC specimens by Western blot. **c** Representative results of the upregulation of CEP55 protein in GC specimens by immunohistochemistry. **d** CEP55 mRNA was upregulated in five GC cell lines and normal human gastric epithelial cells (**P*<0.05)



containing 20 mg/ml RNase A, 0.2 mM EDTA, 0.2 % Triton X-100 and 20 mg/ml of propidium iodide. The percentage of cells in G0/G1, S and G2/M phases was determined.

Western blot analysis

For Western blot analyses, protein was harvested from cells plated to 70 to 80 % confluence. A lysis buffer comprising 50 mM Tris–HCl (pH 7.4), 150 mM NaCl, 1 % Triton X-100, 0.1 % SDS and 1 mM EDTA was used. Protease inhibitors were added prior to use. The protein extracts were loaded, size-fractionated by SDS–polyacrylamide gel electrophoresis and transferred to PVDF membranes (Bio-Rad Laboratories). After blocking, the membranes were incubated with the specific first antibodies in dilution buffer at 4 °C overnight. The blotted membranes were incubated with HRP-conjugated anti-rabbit IgG (1:1,000) at room temperature for 2 h. Subsequently, the targeting protein expression level was detected by using an enhanced chemiluminescence (Millipore, Billerica, MA, USA) detection system following the manufacturer's instructions. Rabbit anti-AKT, rabbit anti-p-AKT, rabbit anti-p21 Waf1/Cip1, rabbit anti-cyclin A, rabbit anti-cyclin B1 and rabbit anti-cyclin D were purchased from Cell Signalling Technology (Beverly, MA, USA). Rabbit anti-CEP55 (AV46272) and rabbit anti-GAPDH antibodies were purchased from Sigma. GAPDH was used as the internal control.

Proliferation assay

Cells (2,000 cells/well) were seeded into 96-well plates in 100 μ l complete medium. The Cell Counting Kit-8 (Dojindo Labs) was used to measure cell viability according to the manufacturer's instructions. The plates were incubated for 5 days. The number of viable cells was assessed by measurement of the absorbance at 450 nm.

Soft agar colony formation assay

Both plasmid and shRNA transfection GC cells were resuspended in 0.5 ml 1 % low-melting-point agarose with complete culture medium, and layered on top of 0.5 ml 2 % low-melting agarose in 24-well plates (2,000 to 5,000 cells/well). The plates were incubated at 37 °C in a humidified atmosphere of 5 % CO₂ for 2 weeks. Colonies containing at least 50 cells were counted. All experiments were repeated three times.

Tumour xenograft in animals

Nude mice (BALB/c nude mice) were purchased from the Department of Laboratory Animal Centre of Nanjing Medical University. Cells with differential CEP55 expression were

subcutaneously injected into 4-week-old male nude mice. Bidimensional tumour measurements were obtained with vernier calipers every 4 days, and the mice were euthanised after about 3 weeks. The volume of the implanted tumour was calculated using the formula: Tumour volume=length \times width² \times 0.5. Care of experimental animals was in accordance with institutional animal care and use committee guidelines.

Statistical analysis

All quantitative data were expressed as mean \pm standard deviation. Statistical analyses were performed using Student's *t* test (two-tailed) using GraphPad Prism 5 software or comparisons among multiple groups were performed by one-way analysis of variance and the least significant

Table 1 Expression of CEP55 protein in human gastric cancer according to clinicopathological features of patients

Clinicopathological variables	CEP55 expression		<i>P</i> value
	T > N (<i>n</i> =57)	T < N (<i>n</i> =11)	
Age (year)			
<50	26	4	
\geq 50	31	7	0.572
Gender			
Male	31	6	
Female	26	5	0.992
Tumour size (cm)			
<3	21	8	
\geq 3	36	3	0.028*
Histological type			
Well	17	8	
Moderately and poorly	40	3	0.007*
Depth			
Localized in subserosa	19	5	
Beyond subserosa	38	6	0.441
Lymph node metastasis			
<i>n</i> ₀	15	6	
<i>n</i> _{1–3}	42	5	0.064
Lymphatic invasion			
Absent	21	4	
Present	36	7	0.0976
Stage			
I, II	18	9	
III, IV	39	2	0.002*

T > *N* CEP55 expression is strong in tumours and weak in normal tissues, which has statistical difference, *T* < *N* CEP55 expression is either lower or without significant difference in tumours compared with normal tissues

**P* < 0.05 statistically significant difference

difference *t* test. Categorical data were evaluated by the χ^2 test. The values of $P < 0.05$ are considered significant.

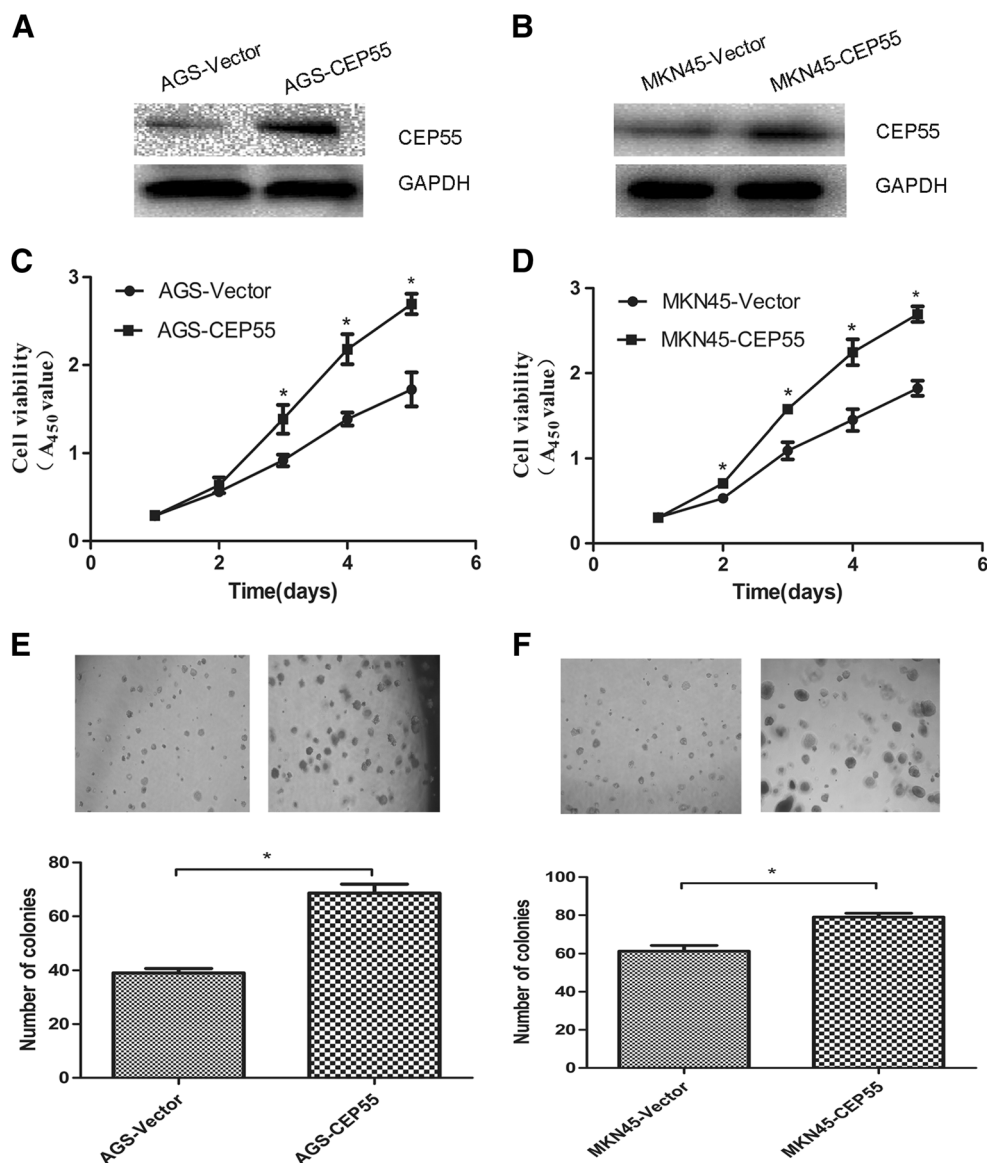
Results

Expression of CEP55 is amplified in most of the human GC tissue samples

RT-PCR detected the mRNA levels of CEP55 in 68 pairs of GC specimens, including GC tissues and adjacent normal tissues. CEP55 mRNA expression in GC was significantly upregulated (Fig. 1a). The CEP55 protein expression of six randomly matched GC samples was shown in Fig. 1b. We performed immunohistochemistry to evaluate CEP55 protein

expression in GC specimens and adjacent normal tissues in 68 matched samples. The adjacent non-tumour gastric cells in most of the cases showed weak but detectable CEP55 staining primarily in the perinuclear membrane, cytoplasm or nucleus. In contrast, strong positive immunoreactivity for CEP55 was found at the perinuclear membrane, cytoplasm or nucleus of tumour cells. There was a significantly lower level of CEP55 expression in adjacent non-tumour gastric cells from all clinical specimens examined, when these were compared with tumour cells from the same patients. In these specimens, most of non-GC tissues showed weak positive staining, whereas 57/68 (83.8 %) were strong positive staining in GC tissues (Fig. 1c). Furthermore, we assessed the expression of CEP55 in five GC cell lines and a normal human gastric epithelial cell line by RT-PCR. CEP55 mRNA was increased

Fig. 2 CEP55 overexpression contributes to proliferation and colony formation. **a** and **b** AGS and MKN45 cells transfected with pcDNA3.1-CEP55 stably enhanced CEP55 expression displayed by Western blot. **c** and **d** CEP55 overexpression contributes to proliferation of AGS and MKN45 cells ($*P < 0.05$). **e** and **f** CEP55 overexpression contributes to soft agar colony formation of AGS and MKN45 cells ($*P < 0.05$)



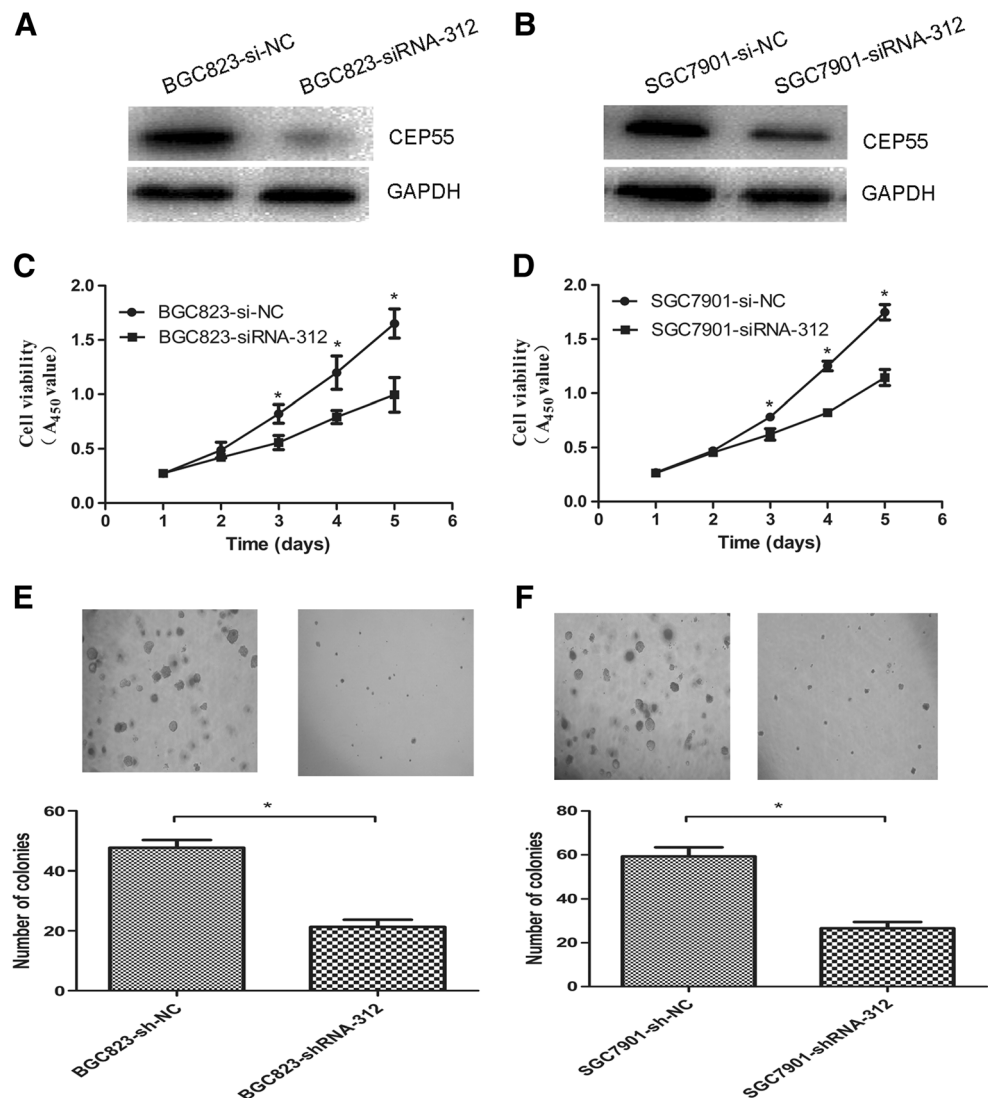
in all GC-derived cell lines (AGS, MKN45, MKN28, SGC7901 and BGC823) compared with GES-1 ($*P<0.05$; Fig. 1d). To investigate whether the increased expression of CEP55 was associated with various prognostic factors, such as age, gender, depth, lymph node metastasis and lymphatic invasion, we classified the patients into two groups on the basis of our immunohistochemical results for CEP55: weak expression and strong expression. As shown in Table 1, those patients with tumour size (≥ 3 cm) had a significantly higher expression of CEP55 compared with those patients with tumour size (< 3 cm; $*P<0.05$). In addition, in moderately and poorly differentiated type, CEP55 was dramatic higher expression than well-differentiated type. Furthermore, stages III and IV were significantly correlated with a strong CEP55 expression ($P<0.05$), whereas no significant difference was observed in terms of the patients' age, gender, depth, lymph node metastasis and lymphatic invasion. In summary, our data

showed that CEP55 was upregulated in GC, and that CEP55 functions was an oncogene accelerator in GC.

CEP55 overexpression promotes the cellular proliferation and colony formation in AGS and MKN45 cell lines

To obtain overexpression CEP55, the recombinant pcDNA3.1-CEP55 was transfected into AGS and MKN45 cells lines. CEP55 protein expression in pcDNA3.1-CEP55 cells was significantly higher than in cells transfected with empty vector (Fig. 2a and b). To investigate the proliferative effects in pcDNA3.1-CEP55 cells, cellular growth was monitored for 5 days. The pcDNA3.1-CEP55 infected AGS and MKN45 cells showed a significant enhancement in cellular growth compared with empty vector cells ($*P<0.05$; Fig. 2c and d). We used a soft agar assay for colony formation, which is the most stringent assay for detecting the anchorage-independent growth ability of

Fig. 3 CEP55 knockdown prevents proliferation and colony formation. **a** and **b** SGC7901 and BGC823 cells transfected with siRNA-312 stably depressed CEP55 expression displayed by Western blot. **c** and **d** CEP55 knockdown prevents proliferation of SGC7901 and BGC823 cells ($*P<0.05$). **e** and **f** CEP55 knockdown prevents soft agar colony formation of SGC7901 and BGC823 cells ($*P<0.05$)



cells. We observed enhanced formation of colonies in soft agar (Fig. 2e and f) seeded with AGS and MKN45 cells that were infected with pcDNA3.1-CEP55 compared with empty vector cells ($*P<0.05$). These results showed CEP55 contributed to tumour cell growth in vitro.

CEP55 knockdown prevents proliferation and colony formation in SGC7901 and BGC823 cell lines

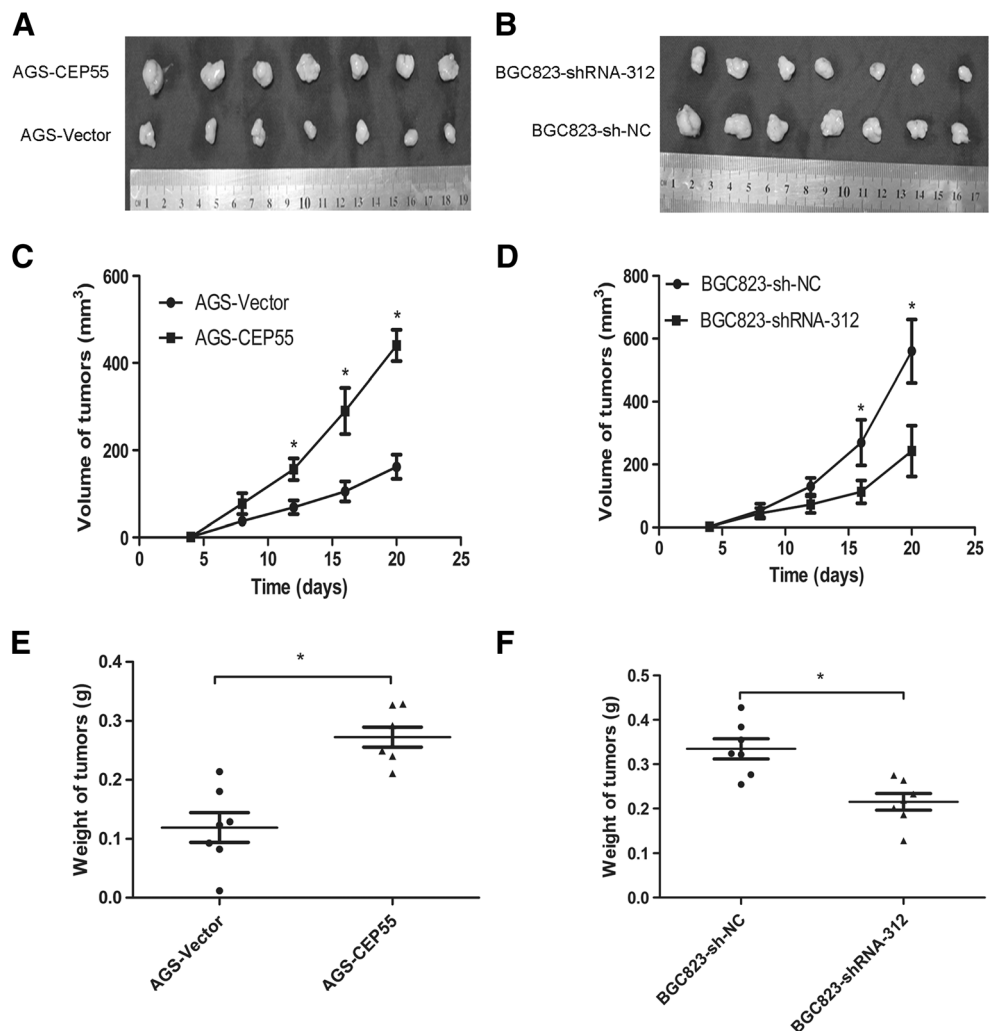
We used chemically synthesised siRNA-312 and shRNA-312 derived from recombinant pSUPER to knockdown endogenous CEP55, as well as si-NC and sh-NC, in SGC7901 and BGC7901 cell lines. Western blot analyses were performed to evaluate the efficiency of CEP55 knockdown in SGC7901 and BGC823 cells transfected with siRNA-312 48-h post-transfection. CEP55 protein expression in siRNA-312 transfected cells was significantly lower than that in si-NC transfected cells (Fig. 3a and b). The siRNA-312 transfected SGC7901 and BGC823 cells showed significant prevention of

cellular growth compared with si-NC transfected cells ($*P<0.05$; Fig. 3c and d). We also observed the prevented formation of colonies in soft agar that had been seeded with SGC7901 and BGC823 cells transfected with p-SUPER-shRNA-CEP55 compared with sh-NC transfected cells ($*P<0.05$; Fig. 3e and f).

Differential expression of CEP55 affects tumourigenesis and tumour burden

The effects of differential CEP55 expression on the tumourigenic potential of GC cells in vivo were also evaluated. AGS cells overexpressing CEP55 and BGC823 with downregulated CEP55 expression were injected subcutaneously into BALB/c nude mice. Tumour size was measured every 4 days after injection. After about 3 weeks, mice were sacrificed and photographed, and the tumours were removed and weighed. Comparison with the mice injected with AGS cells transfected with vector, the mice injected with AGS cells

Fig. 4 The effect of CEP55 expression on tumourigenesis in nude mice. **a** and **b** Tumours were excised 20 days after injection. **c** and **d** Tumour volume was measured every fourth day after injection ($*P<0.05$). **e** and **f** The average weight of tumours in each group was assessed ($*P<0.05$)



overexpressing CEP55 displayed larger tumours during the same period, and the average tumour volumes and weights

were significantly higher compared with those in the control group ($*P<0.05$; Fig. 5a, c and e). Compared with the mice

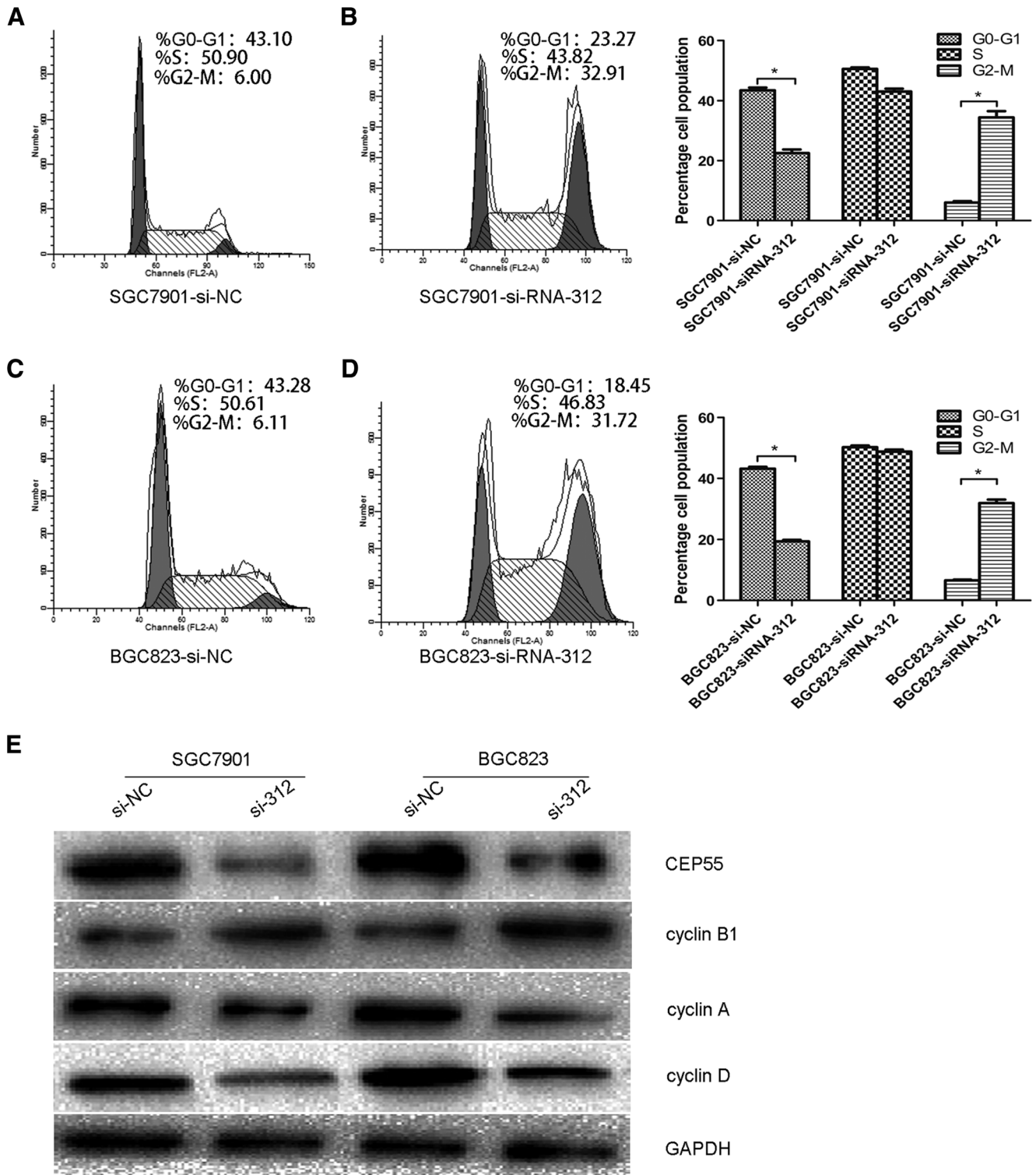


Fig. 5 Representative flow cytometric analysis showed the following. **a** and **b** The number of cells in the G2/M phase is significantly increased in siRNA-312 transfected SGC7901 cells compared with si-NC transfected cells ($*P<0.05$). **c** and **d** The number of cells in the G2/M phase is

significantly increased in siRNA-312 transfected BGC823 cells compared with si-NC transfected cells ($*P<0.05$). Knockdown of CEP55 upregulated cyclin B1 expression and downregulated cyclin A and cyclin D expressions in SGC7901 and BGC823 cells

injected with BGC823 cells transfected with p-SUPER-sh-NC, the mice injected with downregulated CEP55 cells showed an obviously decreased capacity for tumourigenesis ($*P < 0.05$; Fig. 5b, d and f). These results strongly suggested that CEP55 acted as an accelerator for tumour cell growth and tumourigenicity in vivo (Fig. 4).

CEP55-knockdown induces cell cycle arrest at G2/M phase in GC cells

Cell proliferation is closely related to the regulation of cell cycle progression. Previous studies have revealed that the inhibition of endogenous CEP55 against proliferation of hepatocellular carcinoma cells correlated with G2/M phase cell cycle arrest [8]. Many carcinogenic factors play a critical role in tumour progression and affect cell cycle progression. Thus, the siRNA-312 of CEP55 transfected cells were analysed by flow cytometry. Knockdown of CEP55 caused cell cycle arrest at the G2/M phase compared to negative cells in GC cells (Fig. 5a, b, c and d). The results indicated that CEP55 induces cell cycle arrest at G2/M phase in GC cells. The following data further proves that the protein expression of cyclin B1 was significantly higher in siRNA-312-treated cells than in the negative group, the protein expressions of cyclins A and D were decreased compared with the negative group (Fig. 5e). CEP55 played a critical role in GC cell proliferation and cell cycle progression.

CEP55 expression affects the PI3K/AKT/p21 signalling pathway and the expression of cyclin pathway-related proteins

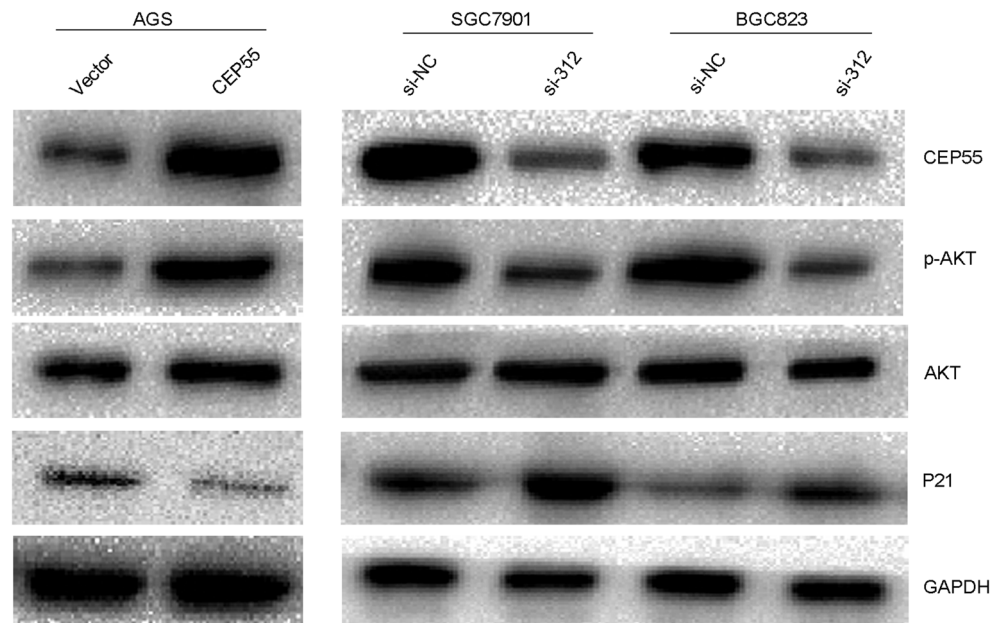
To determine the mechanism(s) by which CEP55 regulates tumour growth and progression, we examined potential

CEP55-regulated molecules. The protein expression data showed downregulation of p21 Waf1/Cip1 and upregulation of p-AKT in CEP55-overexpressed cell line AGS. Conversely, CEP55 silencing by siRNA-312 in the SGC7901 and BGC823 cell lines showed downregulation of p-AKT and upregulation of p21 Waf1/Cip1 (Fig. 6).

Discussion

Centrosome is a crucial organelle in vertebrate cells and plays key roles in cell cycle as the primary microtubule organising centre (MTOC) that comprises a pair of centrioles surrounded by pericentriolar material (PCM) [23]. PCM is the main area for nucleation of cytoplasmic microtubules and microtubules to form the meiotic and mitotic spindles. Members of the centrosome-associated protein family, such as CEP55, are included in PCM [6]. Many kinds of proteins located in the centrosome participate in the function of MTOC and abnormal levels of these proteins correlate with many kinds of diseases, including carcinoma [24]. In tumour cells, a number of apparent abnormalities of the centrosome are observed. Structural changes in centrosomes include the increasing in the number and volume of centrosome, the presence of supernumerary centrioles, accumulation of excess PCM and aberrant phosphorylation of centrosomal proteins [25]. CEP55 was overexpressed in hepatocarcinoma [8], colon carcinoma [10], oral cavity squamous cell carcinoma [11] and lung cancer [12]. Significantly, this study is the first to report that CEP55 was upregulated in the majority of GC specimens examined.

Fig. 6 Western blot analysis of AKT, p-AKT and p21 displayed the following. Upregulation of p-AKT and downregulation of p21 in CEP55-overexpressed AGS cells. Knockdown of CEP55 upregulated the p21 expression and downregulated the p-AKT expression in SGC7901 and BGC823 cells. AKT was present in CEP55-overexpressed AGS cells, and also in CEP55-silenced SGC7901 and BGC823 cells unchanged



PI3K is a major signalling component located downstream of many growth factor receptor tyrosine kinases, and is indispensable for the process of tumour occurrence [26]. Subsequent activation of the AKT downstream pathway by PI3K promotes cell proliferation and survival [27, 28]. Furthermore, AKT phosphorylation often appears in human lung cancer [29] and liver cancer [30], and has been recognised as a risk factor for early disease recurrence and poor prognosis [31]. As the downstream of AKT, activation of the PI3K/AKT pathway leads to a decrease in p21 levels [32–34]. The results of our study discovered that CEP55 enhances AKT phosphorylation and inhibited p21 in GC, which are consistent with the results of previous research on hepatocellular carcinoma [8].

We also demonstrated that ectopic CEP55 expression could regulate cell growth, and that knockdown CEP55 blocked the transition of G2/M phase of cell cycle progression in GC. Cell proliferation assays, colony formation assays and tumour xenografts in nude mice indicated a positive correlation between CEP55 expression and tumourigenesis of GC. Knockdown of CEP55 resulted in cell cycle arrest at G2/M phase and a decrease for S phase compared with negative si-NC transfected cells. Cyclin B1 is the major controlling cyclin in the G2 phase of the cell cycle [35], and cyclin A [36] and cyclin D [37] are the major controlling cyclins in the G1/S phase. Elevated protein expression of cyclin B1 and the inhibited expression of cyclin A and cyclin D were observed in CEP55 sh-RNA-312 transfected SGC7901 cells and BGC823 cells compared with negative transfected cells. Data strongly support the hypothesis that CEP55 is important in cell proliferation and alters cell cycle progression in the GC cell lines tested. The decreased expression of cyclin A and cyclin D may be mediated by the changed expression of p21 [38, 39], and further studies are necessary to identify the details underlying the changed expression of cyclins.

The expression of CEP55 increased in GC tissues and cell lines. Differential expression of CEP55 significantly influences the proliferation, colony formation and tumourigenesis of GC cells. Moreover, knockdown of CEP55 might be associated with the progression of GC cells by arrest of cell cycle progression at the G2/M phase. The feature of CEP55 tumour accelerator indicates that it is a novel therapeutic target for cancer therapy. Whether or not CEP55 could act as a therapeutic agent for GC patients should be investigated. Further research may focus on a specific mechanism through which CEP55 regulates the growth of GC and may confirm the potential effectiveness of CEP55 as a therapeutic target of GC in clinical practice.

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Conflicts of interest None.

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