

Original Research Article

Centrosomal protein55 enhances melanoma cell proliferation, invasion and migration through PI3K/AKT signaling

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Abstract

Purpose: To investigate the role of centrosomal protein55 (CEP55) in melanoma.

Methods: The CEP55 expression in human melanoma cells and normal human melanocytes were determined by quantitative reverse transcription-polymerase chain reaction (qRT-PCR) and western blot. Cell proliferation was evaluated by colony formation and CCK8 assays, while cell cycle was assessed by flow cytometry. Transwell assay was used to investigate cell migration and invasion.

Results: The CEP55 protein expression was higher in A-2058 and A375 melanoma cells than in normal HEMn-LP cells. Overexpression of CEP55 increased melanoma cell viability and the number of melanoma cell colonies ($p < 0.01$), while CEP55 silencing reduced melanoma cell viability and proliferation. Overexpression of CEP55 promoted cell cycle ($p < 0.01$) via decreased p21 expression, but increased CyclinD1 and CDK1 expression. Knockdown of CEP55 enhanced p21 expression and reduced CyclinD1 and CDK1 expression to induce cell cycle arrest. Knockdown of CEP55 suppressed melanoma cell migration and invasion by downregulating p-AKT and p-PI3K.

Conclusion: Centrosomal protein55 functions as an oncogene in melanoma by activating PI3K/AKT signaling. Therefore, CEP55 might be a promising target for melanoma diagnosis and treatment.

Keywords: Centrosomal protein55, Melanoma, Proliferation, Migration, Invasion, Cycle, PI3K/AKT

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INTRODUCTION

Melanoma is a deadly form of skin cancer that results from the malignant transformation of melanocytes in the epidermis [1]. Although melanoma has the lowest incidence among skin cancers, it is the most malignant and aggressive skin cancer and accounts for 75 % of skin cancer-related deaths [2]. The 5-year survival

rate for patients with metastatic melanoma is < 10 % [3]. Therefore, further research on a promising drug target for the treatment of melanoma is needed.

Centrosomal protein55 (CEP55) is a midbody- and centrosome-associated protein that facilitates abscission and cytokinesis [4]. It is dysregulated in various tumors and functions as

an oncogene by promoting tumorigenesis [5]. The oncogenic function of CEP55 in melanoma cells remains unclear.

The PI3K/AKT signaling regulates cell proliferation, metastasis, and multidrug resistance of distinct cancers [6]. Its signaling is activated in human melanoma [7], and inhibition of PI3K/AKT showed clinical benefits for the treatment of melanoma [8]. Centrosomal protein55 was shown to enhance the stability of AKT during embryonic growth and development [4]. In addition, CEP55 interacts with the catalytic subunit of PI3K leading to activation of AKT during tumorigenesis of various cancers [5]. Therefore, CEP55/PI3K/AKT may be involved in melanoma development. The effects of CEP55 on cell proliferation, the cell cycle, and metastasis of melanoma cells were investigated in this study.

EXPERIMENTAL

Bioinformatics analysis

Gene Expression Profiling Interactive Analysis (GEPIA, <http://gepia.cancer-pku.cn/index.html>) was used to assess expression of CEP55 in melanoma tissues (n = 461) and normal tissues (n = 558).

Cell culture

A-2058 and A375 melanoma cells and HEMn-LP human normal melanocytes were purchased from the Chinese Academy of Sciences (Beijing, China). Cells were cultured in Dulbecco's modified Eagle's medium with 10 % fetal bovine serum (Thermo Fisher Scientific, Waltham, MA, USA) in a 37 °C incubator with 5 % CO₂.

Quantitative reverse transcription PCR (qRT-PCR)

Cells were lysed and RNAs were extracted using a TRIzol kit (Invitrogen, Carlsbad, CA, USA). The RNAs were reverse transcribed into cDNAs, and cDNAs were used for qRT-PCR analysis using the PreTaq II kit (Takara, Dalian, Liaoning, China). Relative expression of CEP55 was normalized to GAPDH and calculated using the 2^{-ΔΔC_q} method. Primers are shown in Table 1.

Table 1: qRT-PCR primers

Gene	Forward (5'-3')	Reverse (5'-3')
CEP55	TGAAGAGAAAGACGTATTGAAACAA	GCAGTTTGGAGCCACAGTCT
GAPDH	TCAACGACCACTTTGTCAAGCAGAGT	GCTGGTGGTCCAGGGGTCTTACT

Cell proliferation assay

A375 cells were seeded into a 96-well plate and transfected with shRNA targeting CEP55 (shCEP55) or negative control shRNA (shNC) (Invitrogen) for 48 h. pcDNA3.1-CEP55 (pc-CEP55) or pcDNA3.1 vector (NC) was also transfected into A375 cells. Then, the cells were incubated with 10 μL of CCK8 solution (Beyotime, Beijing, China) for an additional 2 h. Absorbance at 450 nm were measured using a microplate reader (Thermo Fisher Scientific). For the colony formation assay, A375 cells were seeded into 6-well plates, transfected with pcDNA or shRNA, cultured for 10 days, and then fixed in methanol. The cells were stained with crystal violet and examined using a microscope (Olympus, Tokyo, Japan).

Flow cytometry and transwell assays

A375 cells were harvested and resuspended in the binding buffer in the BD Cycletest™ Plus DNA Reagent Kit (BD Biosciences, San Jose, CA, USA), stained with propidium iodide, and analyzed using a FACS flow cytometer (Life Technologies, Darmstadt, Germany). For the transwell assay, A375 cells in serum-free medium were placed in the upper Matrigel-coated Transwell insert chamber (Corning Incorporated, Corning, NY, USA), and the lower chamber contained medium with 15 % fetal bovine serum. The cells that invaded the lower chamber were stained with crystal violet and examined using a microscope (Olympus). For the cell migration analysis, A375 cells were placed into the upper chamber without Matrigel coating, and cells that migrated into the lower chamber were stained with crystal violet and examined using a microscope (Olympus).

Western blot assay

A375 cells were lysed in RIPA buffer (Beyotime), and proteins were separated by 10 % SDS-PAGE and transferred onto nitrocellulose membranes. The membranes were blocked in 5 % bovine serum albumin and then probed with the following primary antibodies overnight at 4 °C: anti-CEP55 and anti-β-actin (1:2000); anti-CDK1 and anti-CyclinD1 (1:2500); anti-p21, anti-p-AKT, and anti-AKT (1:3000); and anti-p-PI3K and anti-PI3K (1:3500).

The membranes were then washed and incubated with horseradish peroxidase-conjugated secondary antibody (1:4000). Immunoreactivities were visualized using enhanced chemiluminescence (Sigma-Aldrich, St. Louis, MO, USA). All the antibodies were acquired from Abcam (Cambridge, MA, USA). β -actin was used as the control.

Statistical analysis

All data with at least three replicates were expressed as mean \pm SEM and analyzed by student's t test or one-way analysis of variance in SPSS software. P -values < 0.05 were considered statistically significant.

RESULTS

Upregulation of CEP55 in melanoma

Analysis of data from the cancer genome atlas (TCGA) showed that CEP55 expression was elevated in melanoma tissues compared to normal tissues (Figure 1 A). In addition, CEP55 was upregulated in melanoma cells when compared to HEMn-LP cells (Figure 1 B and C) suggesting that CEP55 may be involved in the development of melanoma.

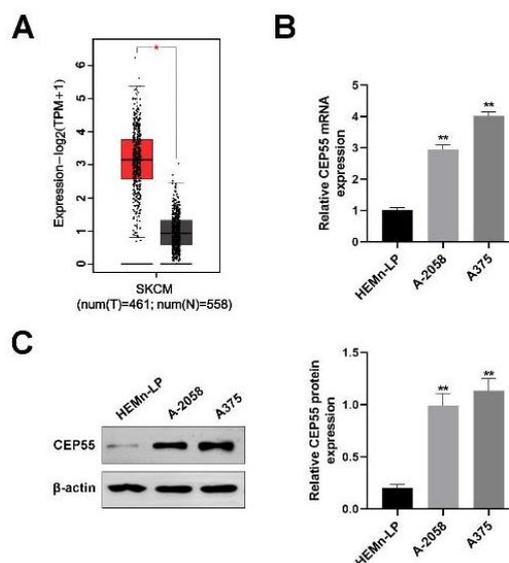


Figure 1: Upregulation of CEP55 in melanoma. (A) TCGA analysis showed that CEP55 is elevated in melanoma tissues ($n = 461$) when compared to normal tissues ($n = 558$). SKCM: Skin cutaneous melanoma, T: tumor tissues, N: normal tissues. (B) qRT-PCR showed that CEP55 mRNA is elevated in A375 and A-2058 melanoma cells when compared to HEMn-LP cells. (C) Western blot showed that CEP55 protein is elevated in A375 and A-2058 melanoma cells when compared to HEMn-LP cells. All experiments were repeated at least three independent times. $**p < 0.01$

CEP55 enhanced melanoma cell proliferation

A375 cells were transfected with shRNA or pcDNA to decrease or increase CEP55 protein expression, respectively (Figure 2 A). A375 cell viability increased upon transfection with pc-CEP55 and decreased upon transfection with shCEP55 (Figure 2 B). Transfection with pc-CEP55 promoted A375 cell proliferation and transfection with shCEP55 reduced the number of A375 colonies (Figure 2 C and D) demonstrating the antiproliferative effect of CEP55 silencing on melanoma cells.

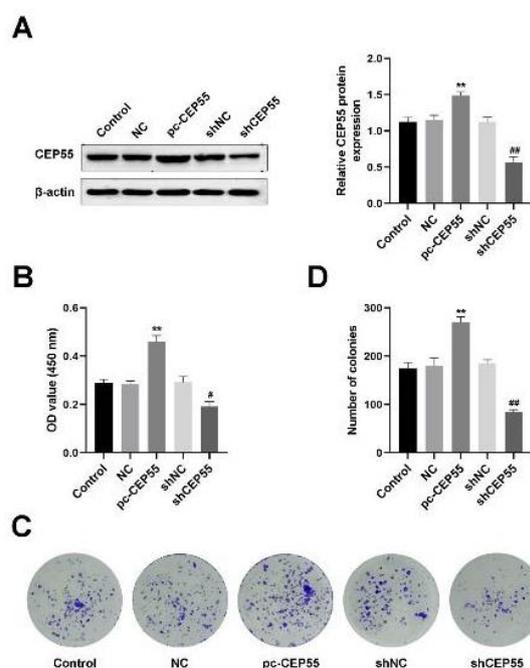


Figure 2: CEP55 promoted melanoma cell proliferation. (A) Western blot showed that CEP55 protein expression in A375 cells decreased or increased upon transfection with shRNA or pcDNA, respectively. (B) The CCK8 assay showed that transfection with pc-CEP55 increased A375 cell viability and that transfection with shCEP55 decreased A375 cell viability. (C) The colony formation assay showed that transfection with pc-CEP55 promoted A375 cell proliferation and transfection with shCEP55 suppressed A375 cell proliferation. (D) Transfection with pc-CEP55 increased the number of A375 colonies and transfection with shCEP55 reduced the number of A375 colonies. All experiments were repeated at least three independent times. $**p < 0.01$ vs NC. $##p < 0.01$ vs shNC

CEP55 promoted the melanoma cell cycle

Overexpression of CEP55 increased the frequencies of G2/M and S phases and knockdown of CEP55 increased the frequency of the G1 phase in A375 cells (Figure 3 A). In addition, overexpression of CEP55 reduced p21 protein expression and enhanced CyclinD1 and

CDK1 protein expression in A357 cells (Figure 3 B). Silencing of CEP55 upregulated p21 expression and downregulated CyclinD1 and CDK1 expression in A357 cells (Figure 3 B) indicating that CEP55 silencing induced cell cycle arrest in melanoma.

CEP55 promoted metastasis of melanoma cells

Overexpression of CEP55 enhanced migration (Figure 4A) and invasion (Figure 4B) of A357 cells. Knockdown of CEP55 inhibited A357 cell migration (Figure 4A) and invasion (Figure 4B) indicating that a CEP55 deficiency has an anti-invasive effect on melanoma cells.

CEP55 enhanced activation of PI3K/AKT in melanoma cells

The CEP55 did not affect PI3K and AKT protein expression; however, overexpression of CEP55 increased p-AKT and p-PI3K expression in A357 cells (Figure 5). Knockdown of CEP55 decreased p-AKT and p-PI3K expression in A357 cells (Figure 5) demonstrating that CEP55 silencing inhibited PI3K/AKT signaling in melanoma cells.

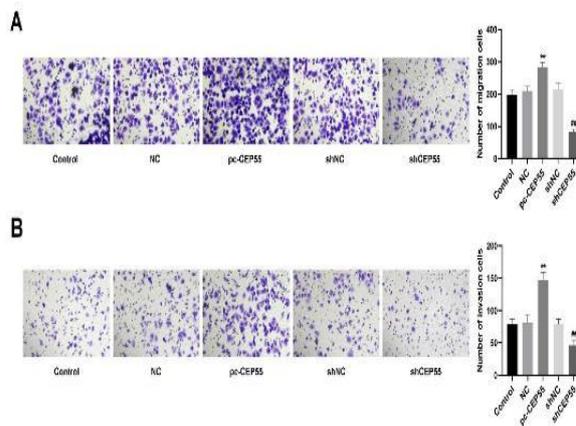


Figure 4: CEP55 enhanced metastasis of melanoma cells. (A) The transwell assay showed that overexpression of CEP55 enhanced A357 cell migration and silencing of CEP55 suppressed cell migration. (B) The transwell assay showed that overexpression of CEP55 enhanced invasion of A357 cells and silencing of CEP55 suppressed cell invasion. All experiments were repeated at least three independent times. ** $p < 0.01$ vs. NC. ## $p < 0.01$ vs. shNC

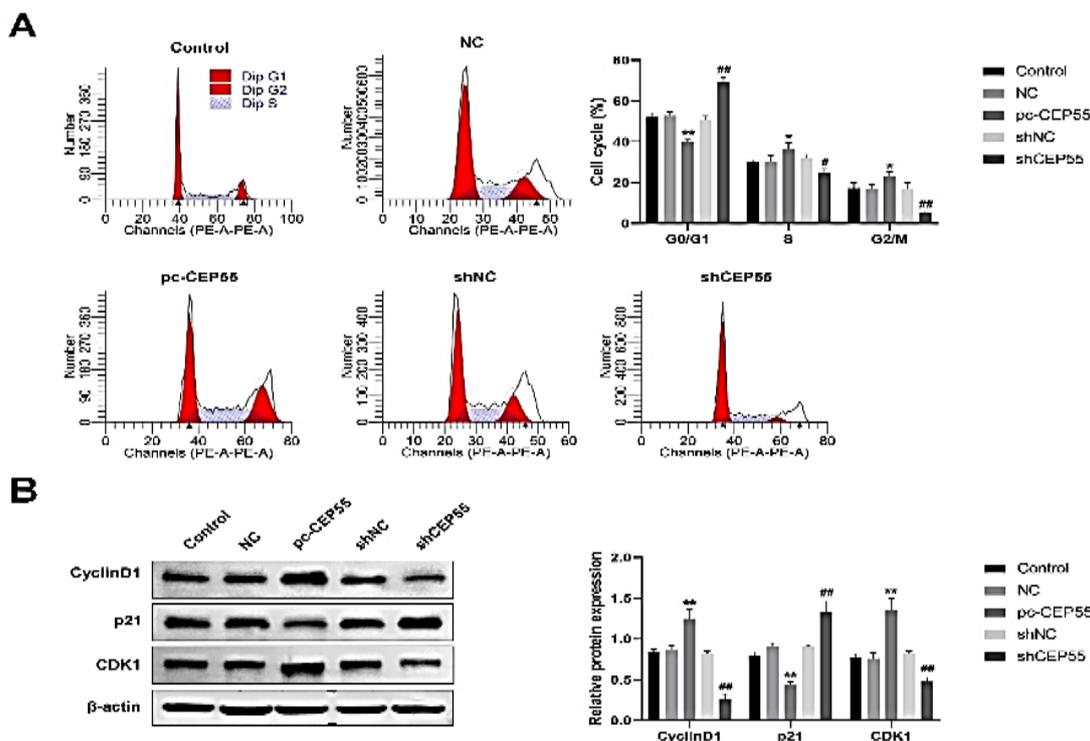


Figure 3: CEP55 promoted the melanoma cell cycle. (A) Flow cytometry showed that overexpression of CEP55 increased the frequencies of the G2/M and S phases and knockdown of CEP55 increased the frequency of the G1 phase in A357 cells. (B) Western blot showed that overexpression of CEP55 reduced p21 protein expression and enhanced CyclinD1 and CDK1 protein expression in A357 cells. Silencing of CEP55 upregulated p21 expression and downregulated CyclinD1 and CDK1 expression in A357 cells. All experiments were repeated at least three independent times. * $p < 0.05$, ** $p < 0.01$ vs. NC. # $p < 0.05$, ## $p < 0.01$ vs. shNC

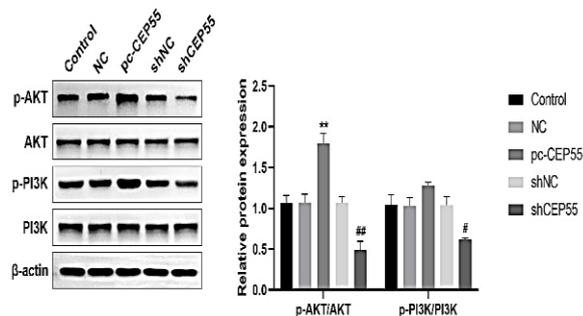


Figure 5: CEP55 activated PI3K/AKT in melanoma cells. Overexpression of CEP55 increased p-AKT and p-PI3K expression in A375 cells and silencing of CEP55 reduced p-AKT and p-PI3K expression in A375 cells. All experiments were repeated at least three independent times. ** $p < 0.01$ vs. NC. # $p < 0.05$, ## $p < 0.01$ vs. shNC

DISCUSSION

The CEP55 is abnormally upregulated in various tumors, associated with tumor metastasis, aggressiveness, and the tumor stage, and can function as a prognostic biomarker or therapeutic target for tumorigenesis [5]. This study found that CEP55 is elevated in melanoma and contributes to melanoma cell proliferation and metastasis.

A previous study showed that high CEP55 expression predicted poor prognosis in patients with colorectal cancer [9]. Although CEP55 is upregulated in melanoma tissues and cells, the relationships between CEP55 expression and the clinicopathological parameters of melanoma patients must be evaluated to determine the diagnostic and prognostic values of CEP55 for melanoma. Knockdown of CEP55 reduced melanoma cell proliferation, invasion, and migration confirming the oncogenic role of CEP55 in melanoma. The epithelial-mesenchymal transition is essential for metastasis and intravasation of tumor cells into the blood during melanoma development [10]. The CEP55 was shown to downregulate the epithelial marker E-cadherin and upregulate the mesenchymal biomarkers ZFE-box binding homeobox 1 and N-cadherin to induce the epithelial-mesenchymal transition in renal cell carcinoma [11]. Therefore, silencing of CEP55 may reduce the epithelial-mesenchymal transition and suppress metastasis of melanoma cells.

Ultraviolet radiation, the most important cause of melanoma occurrences, induces dysregulation of the cell cycle in melanoma [12]. Cell cycle regulators, such as CyclinD1, CDK1, and p21, are frequently dysregulated in melanoma [13]. In addition, CEP55 suppressed p21 activity during

the cell cycle of glioma cells [14], and silencing of CEP55 reduced CyclinD1 expression to induce cell cycle arrest in gastric carcinoma [15]. In this study, knockdown of CEP55 enhanced p21 expression and reduced CDK1 and CyclinD1 expression to inhibit cell cycle progression in melanoma cells.

The PI3K/AKT signaling is important for melanoma cell migration, invasion, epithelial-to-mesenchymal transition, and proliferation [16]. CEP55 interacts directly with the p110 catalytic subunit of PI3K to enhance activation of AKT during tumorigenesis [17]. Blocking PI3K/AKT signaling attenuated the CEP55-induced epithelial-mesenchymal transition and metastasis of esophageal squamous cell carcinoma cells [18]. In this study, CEP55 knockdown attenuated PI3K and AKT phosphorylation indicating that CEP55 may contribute to melanoma cell metastasis and proliferation by activating PI3K/AKT signaling.

CONCLUSION

Centrosomal protein55 is upregulated in melanoma cells, and its knockdown reduces melanoma cell viability, induces cell cycle arrest, and inhibits melanoma cell migration and invasion by inactivating PI3K/AKT pathway. Therefore, CEP55 might be a promising target for melanoma diagnosis and treatment. However, the effect of CEP55 on melanoma tumor growth should be further investigated.

DECLARATIONS

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Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Huaikang Hua designed and carried out the study, supervised the data collection, and analyzed and interpreted the data. Huifang Yang prepared the manuscript for publication and reviewed the draft of the manuscript. All authors read and approved the manuscript.

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