Original Article

Overexpression of CENPU promotes cancer growth and metastasis and is associated with poor survival in patients with nasopharyngeal carcinoma

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Background: Centromere protein U (CENPU) is key for mitosis in the carcinogenesis of cancers. However, the roles of CENPU have not been inspected in nasopharyngeal carcinoma (NPC). Thus, we aimed to explore the functions and mechanisms of CENPU in NPC.

Methods: Expression of CENPU was evaluated by real-time quantitative polymerase chain reaction, western blotting and immunohistochemistry. The biological functions of CENPU were evaluated *in vitro* and *in vivo*. Gene chip analysis, ingenuity pathway analysis, and coimmunoprecipitation experiments were used to explore the mechanisms of CENPU.

Results: CENPU was highly expressed in NPC. High expression of CENPU was associated with advanced tumor, node and metastasis (TNM) stage and poor overall survival. Cox regression analysis demonstrated that CENPU expression was an independent prognostic factor in NPC. Knockdown of CENPU inhibited proliferation and migration *in vitro* and *in vivo*. Knockdown of CENPU upregulated dual specificity phosphatase 6 (DUSP6) expression. The expression of CNEPU was inversely correlated with the expression of DUSP6 in NPC tissues. Mechanistic studies confirmed that CENPU increased the activation of the ERK1/2 and p38 signaling pathways by suppressing the expression of DUSP6.

Conclusions: CENPU acts as an oncogene in NPC by interacting with DUSP6, and may represent a promising prognostic biomarker for patients with NPC.

Keywords: Nasopharyngeal carcinoma (NPC); centromere protein U (CENPU); growth; metastasis; prognosis

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Introduction

Nasopharyngeal carcinoma (NPC) is characterized by a unique geographic distribution and is particularly prevalent in southeast China (1). Over the past decade, with the advancement of tumor staging, intensity-modulated radiation therapy, and therapeutic strategies, the overall survival (OS) of NPC has remarkably improved (2). However, 5% to 15% of NPC patients will experience local recurrence, and 15% to 30% will develop distant metastasis after treatment (3), the exact underlying mechanisms of recurrence and metastasis remain elusive and warrant further research.

Genetic alterations are essential events in the

pathogenesis of NPC (4). Frequent chromosomal deletions of specific genes on chromosomes usually results in the disruption of normal biological behavior, leading to the development and progression of NPC (5). Mitosis is a key process of chromosome behavior that could be easily influenced by multiple factors, including centromere protein (CENP) (6,7). Members of CENP family, like CENPA, CENPE, CENPK, CENPM and CENPU, have been reported to be associated with the tumorigenesis and are potential prognostic factors in human tumors (8-13). Signal pathways, such as ERK pathway, PI3K/AKT/NF-κB pathway, Wnt/beta-catenin pathway, etc. are involved in the tumorigenesis (14-16). However, little has been reported about the roles of CENP in NPC.

CENPU is a centromere component crucial for mitosis and has a vital role in orchestrating kinetochore-microtubule attachment (17-19). Studies have reported that CENPU is implicated in the growth and metastasis in cancers (8,10,20-24), such as triple-negative breast cancer (TNBC), lung adenocarcinoma, prostate cancer, cervical cancer and liver cancer. CENPU usually acts as cancer promoting gene, and overexpression of CENPU is associated with poor survival, suggesting CENPU is a promising target for therapy. However, the roles and mechanisms of CENPU have not been inspected in NPC.

Thus, the aim of this study was to explore the clinical significance and functions of CENPU, and the underlying molecular mechanisms of CENPU in the pathogenesis of NPC. We present this article in accordance with the ARRIVE and MDAR reporting checklists (available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-2395/rc).

Highlight box

Key findings

- Centromere protein U (CENPU) is upregulated in nasopharyngeal carcinoma (NPC) patients and is associated with poorer survival. CENPU promotes growth and metastasis by activating the ERK1/2 and p38 pathways in NPC.

What is known and what is new?

- CENPU promotes growth and metastasis in NPC.
- CENPU promotes the development of NPC by suppressing DUSP6 expression and activating the ERK1/2 and p38 pathways.

What is the implication, and what should change now?

- CENPU acts as an oncogene in NPC and may represent a promising prognostic biomarker for patients with NPC.

Methods

Cell lines

NPC cells, including CNE-1, CNE-2, SUNE-1 and 5-8F, were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium (HyClone, New York, USA) supplemented with 10% fetal bovine serum (FBS) (Gibco, New York, USA). The immortalized normal nasopharyngeal epithelial cell line (NP69) was cultured in Keratinocyte-SFM medium supplemented with epidermal growth factor (Invitrogen). CNE-1 and CNE-2 cell lines were obtained from Wuhan Institute of Cell Biology, China Center for Type Culture Collection. SUNE-1 and 5-8F cell lines was obtained from Sun Yat-sen University Cancer Center. NP69 cell lines were kindly provided by Prof. George S. W. Tsao of University of Hong Kong. All cells were maintained at 37 ℃ under 5% CO_2. A protocol was prepared before the study without registration.

Patients issues

Normal nasopharyngeal epithelial tissues, NPC tissues and the samples used for tissue microarray (TMA) were all obtained from Fujian Medical University, Fujian Cancer Hospital. OS was calculated from the day of diagnosis until death from any cause. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013) and was approved by the Ethical Review Committee of Fujian Medical University, Fujian Cancer Hospital (approval No. SQ2020-038). Informed consent was obtained from all the patients.

Short hairpin RNA (shRNA) and lentiviruses

ShRNA targeting CENPU (shCENPU-1 and shCENPU-2) and a scrambled control shRNA (shCtrl) were synthesized by Shanghai Genechem (Shanghai, China). NPC cells were infected with shRNA-encoding lentiviruses. After 72 hours of transfection, NPC cells were further screened with puromycin for 48 hours.

Real-time quantitative polymerase chain reaction (RT-qPCR)

Total RNA was extracted using TRIzol (Invitrogen). To measure CENPU mRNA expression, complementary DNA (cDNA) was synthesized using a Transcriptor cDNA

Synth Kit (Roche, Mannheim, Germany). RT-qPCR was performed using FastStart Universal SYBR Green Mast (Roche). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used to normalize the expression of mRNA. The fold changes (FCs) were calculated using the relative quantification method ($2^{-\Delta\Delta Ct}$). All reactions were performed in triplicate.

Cell growth and clone formation assay

Celigo (Nexcelom), a cell count instrument, was used to detect cell growth. A total of 2,000 cells/well were seeded. From the second day after laying, the Celigo reading board was tested once a day for 5 days continuously. The number of cells with green fluorescence in each scanning orifice plate was accurately calculated. For colony formation assays, a total of 1,000 cells/well were seeded onto 6-well plates. Following 2-week incubation, cell colonies were washed, fixed with 100% methanol and stained with crystal violet staining solution for 15 min. The number of more than 50 cell clones was counted under microscope.

Apoptosis experiment

NPC cells were collected and washed with cold phosphate buffered saline (PBS) and resuspended in 1× binding buffer. Two hundred µL of the solution (5×10^5 cells) was transferred to a new tube. Next, 10 µL of PE Annexin V and 5 µL 7-AAD (PE Annexin V Apoptosis Detection Kit I, BD Pharmingen™, BD Biosciences, Franklin Lakes, USA) were added into the tube. The cells were incubated for 15min at room temperature in the dark. Finally, cells were analyzed by Beckman-Coulter system (Beckman Coulter, Inc., Bera, USA).

Migration and invasion assays

For migration and invasion assays, CNE-2 cells in serum-free medium were added to the upper chamber with an 8.0 µm pore size (Corning, kennebunk, ME, USA) without or with Matrigel (BD Biosciences), and 20% FBS was added to the lower chamber. After 24 hours (migration assays) or 48 hours (invasion assays), cells in the lower chamber were fixed, stained, and photographed. Representative images of the gap distance were captured at 0 and 24 hours using an inverted microscope.

Western blotting analyses and immunohistochemistry (IHC)

Western blotting was performed using standard protocols. The primary antibodies used in our study are provided in Table S1. The CENPU scores were determined by multiplying the intensity score and percentage score to determine a semiquantitative H-score. The mean H-score from all patients was used as the cutoff value to define CENPU positivity, which was described in our previous article (25).

Xenograft growth and lung metastasis model

For the xenograft growth model, 4-week-old female nude BALB/c mice were purchased from Shanghai SLAC Laboratory Animal Center (Shanghai, China). CNE-2 cells were subcutaneously injected into nude mice at a concentration of 2×10^7 cells in each group (n=5). Tumor volume and the average of total fluorescence expression were measured once a day and compared 11 days after injection. For the lung metastasis model, CNE-2 cells were intravenously injected through the tail vein at a concentration of 1.0×10^6 cells in each group (n=10). Fluorescent imaging and total radiant efficiency were taken and compared once a week. The number of metastatic nodes in the lung was sampled and quantified after the mice were sacrificed by CO_2 asphyxiation after 47 days of injection. Animal experiments were performed under a project license (No. 2021-0322) granted by the Ethical Review Committee of Fujian Medical University, Fujian Cancer Hospital, and in compliance with institutional guidelines for the care and use of animals.

GeneChip and ingenuity pathway analysis (IPA)

GeneChip PrimeView human was used to identify differentially expressed genes (DEGs) between the knockdown groups (shCENPU) and normal control groups (shCtrl) by Shanghai Genechem. Genes with P value ≤0.05 and FC >2.0 were considered significant. IPA was used to perform classical pathway analysis, biological downstream effect analysis, disease and functional analysis, regulatory effect analysis and interaction network analysis according to the DEGs.

Coimmunoprecipitation (co-IP) experiments

Co-IP experiments were conducted using a Pierce™ Co-

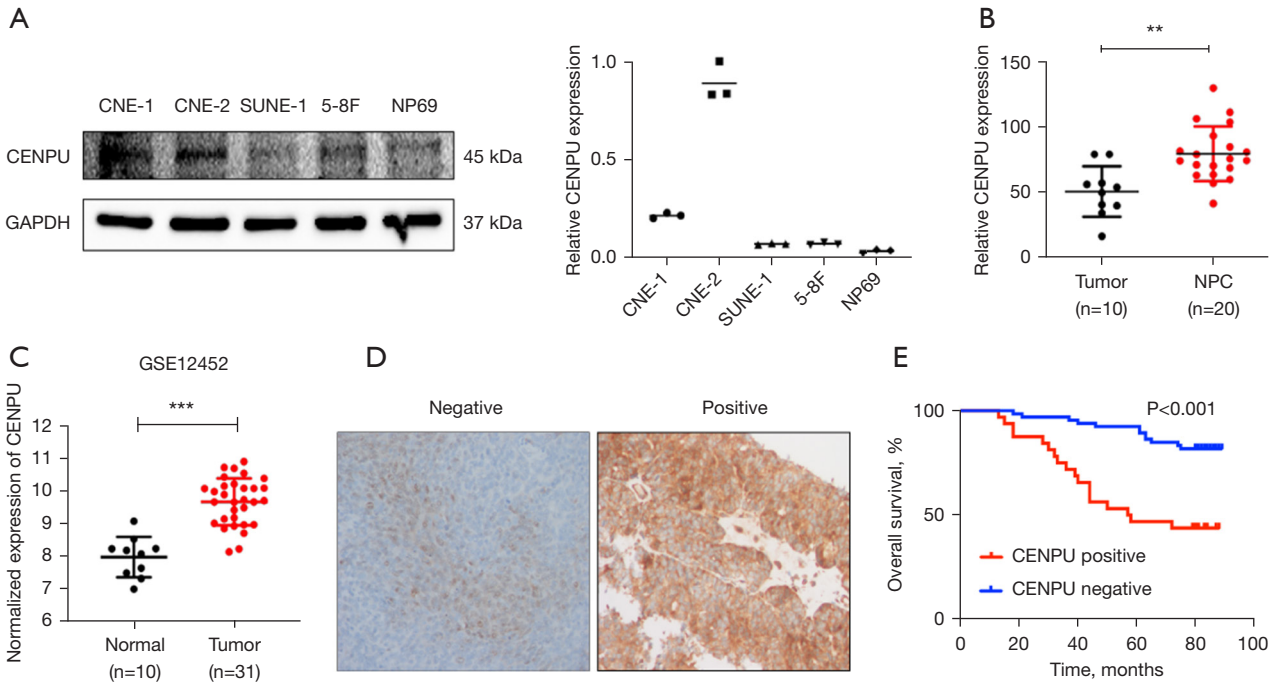

Figure 1 CENPU expression in NPC cells, tissue and its clinical significance. (A) The protein and mRNA expression of CENPU in NPC cells and NP69 cells. (B) CENPU mRNA expression in normal nasopharyngeal tissues and NPC tissues. (C) CENPU expression in the Gene Expression Omnibus database (GSE12452). (D) Representative images of CENPU expression by immunohistochemistry (magnification: ×200). (E) Overall survival analysis of CENPU expression. **, P<0.01; ***, P<0.001. CENPU, centromere protein U; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; NPC, nasopharyngeal carcinoma.

IP kit (Thermo Scientific, Rockford, USA) according to the manufacturer's protocol. Briefly, cell lysates were incubated with antibodies against CENPU (Abcam, Waltham, USA) or dual specificity phosphatase 6 (DUSP6) (Abcam) at 4 ℃ overnight. After incubation, the antigen/antibody complex was combined with magnetic beads for 1 hour at room temperature. Then, magnetic beads were washed twice using immunoprecipitation lysis and washed once using pure water. Then, the protein was eluted using elution buffer. IgG (Santa Cruz Biotechnology, Dallas, USA) was used as a negative control. Finally, the samples were resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and analyzed by western blotting.

Statistical analysis

All statistical analyses were performed using SPSS version 24.0 (SPSS, Chicago, USA) and GraphPad Prism 8 (GraphPad Prism, San Diego, USA). The relationship between CENPU expression and characteristics was analyzed using the χ^2 test. The *t*-test and one-way analysis of variance (ANOVA) were used to compare the intergroup differences. Nonparametric test was used to identify the significant differences for nonparametric data. The Kaplan-Meier method was used to estimate the survival probability, and the differences were evaluated with the log-rank test. The Cox proportional hazards regression model was used to estimate the hazard ratio of CENPU. The correlation between CNEPU and DUSP6 mRNA expression was evaluated by Spearman's correlation analysis. A P value less than 0.05 was considered statistically significant.

Results

CENPU is overexpressed in NPC and is associated with poor survival

Western blotting and RT-qPCR showed that the protein and mRNA of CENPU were both upregulated in NPC cells, especially in CNE-2 cells (*Figure 1A*). Normal nasopharyngeal epithelial tissues and NPC tissues further

Table 1 Characteristics of 98 patients with nasopharyngeal carcinoma grouped by levels of CENPU expression

Variables	Overall	CENPU expression		P
		Low (n=66)	High (n=32)	
Gender				0.27
Male	74	52	22	
Female	24	14	10	
Age				0.43
≤50 years	36	26	10	
>50 years	62	40	22	
Histology				0.40
NKDC	3	2	1	
NKUC	95	64	31	
T stage				>0.99
T1–2	50	35	15	
T3–4	48	31	17	
N stage				0.19
N0–1	30	23	7	
N2–3	68	43	25	
Clinical stage				0.03*
I–II	14	13	1	
III–IV	84	53	31	

*, P<0.05. CENPU, centromere protein U; NKDC, nonkeratinizing differentiated carcinoma; NKUC, nonkeratinizing undifferentiated carcinoma.

Table 2 Cox proportional hazards regression model analysis for overall survival in 98 patients with nasopharyngeal carcinoma

Variables	Multivariable	
	HR (95% CI)	P
Gender (female *vs.* male)	1.533 (0.658–3.573)	0.32
Age (≤50 *vs.* >50 years)	1.825 (0.9765–4.352)	0.17
T stage (T1–2 *vs.* T3–4)	1.491 (0.650–3.422)	0.34
N stage (N0–1 *vs.* N2–3)	1.114 (0.513–2.419)	0.47
Clinical stage (I–II *vs.* III–IV)	3.843 (0.410–36.013)	0.23
CENPU expression (positive *vs.* negative)	4.379 (2.063–9.292)	<0.001

HR, hazard ratio; CI, confidence interval; CENPU, centromere protein U.

confirmed that CENPU was significantly upregulated in NPC tissues (*Figure 1B*), which was further demonstrated using the Gene Expression Omnibus database (GSE12452) (*Figure 1C*).

To examine the relationship between CENPU expression and patients' prognosis, TMA of 98 NPC specimens and 33 normal nasopharyngeal epithelial samples were performed (*Figure 1D*). The results showed that CENPU was upregulated in 32.65% (32/98) of NPC patients, while CENPU was almost lost in normal nasopharynx epithelia, with 12.12% (4/33) positive rates. Expression of CENPU was closely associated with tumor, node and metastasis (TNM) stage, while no significance was found between CENPU expression and gender, age, histology, T stage and N stage (*Table 1*). Kaplan-Meier analysis indicated that patients with high expression of CENPU had worse OS than those with low expression of CENPU (59.21 *vs.* 82.27 months, P<0.001) (*Figure 1E*). Cox regression model analysis demonstrated that CENPU expression was independent prognostic factor in NPC (hazard ratio =4.379, 95% confidence interval: 2.063–9.292, P<0.001) (*Table 2*). Taken together, these data reveal that CENPU is upregulated in NPC patients and high expression of CENPU is associated with poorer survival.

Knockdown of CENPU in NPC cells suppresses growth in vitro and in vivo

As CENPU were highly upregulated in CNE-2 cells, CNE-2 cells were selected for further biological study. CNE2 cells were transfected with control vector (shCtrl) and lentiviral vector that downregulated CENPU expression (shCENPU-1 and shCENPU-2) (*Figure 2A*). By performing Celigo and colony-forming assays, we found that downregulation of CENPU remarkably attenuated CEN2 growth and colony formation (*Figure 2B*,*2C*). Meanwhile, the apoptosis assay indicated that knockdown of CENPU expression dramatically promoted the death of CNE-2 cells (*Figure 2D*). Moreover, subcutaneous xenograft tumor growth *in vivo* further confirmed that CENPU showed a negative effect on tumor proliferation (*Figure 2E*).

Knockdown of CENPU in NPC cells suppresses metastasis in vitro and in vivo

Transwell assays, Matrigel invasion assays (*Figure 3A*) and wound healing assays (*Figure 3B*) showed that

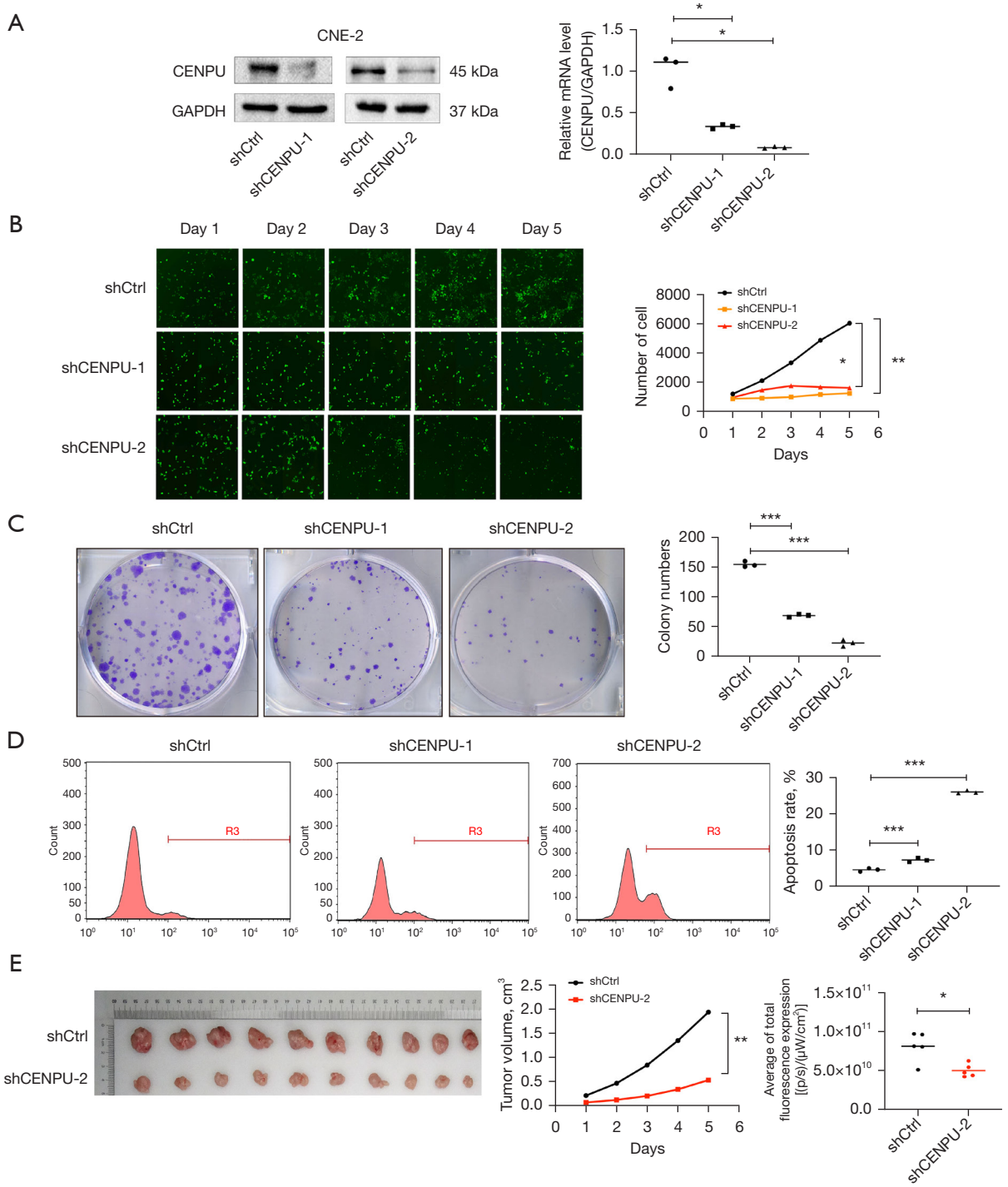

Figure 2 Effects of CENPU on NPC cell proliferation and apoptosis. (A) CEN-2 cells that transfected with sh-CNEPU-1 and sh-CENPU-2 lentiviral vector (shCENPU-1 and shCENPU-2) or control vector (shCtrl), and transfection efficiency was verified by western blotting and real-time quantitative polymerase chain reaction. (B) Cell growth assays (NPC cells with green fluorescent protein) (magnification: 100×). (C) Clone formation assays (0.1% crystal violet staining) (magnification: 100×). (D) The apoptosis rate was analyzed by flow cytometry. (E) Representative images of tumors (n=5) transfected with shCENPU-2 (left column). Quantitative analysis of xenografted tumor volumes (middle column) and fluorescence expression (right column). *, P<0.05; **, P<0.01; ***, P<0.001. CENPU, centromere protein U; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; sh, short hairpin; NPC, nasopharyngeal carcinoma.

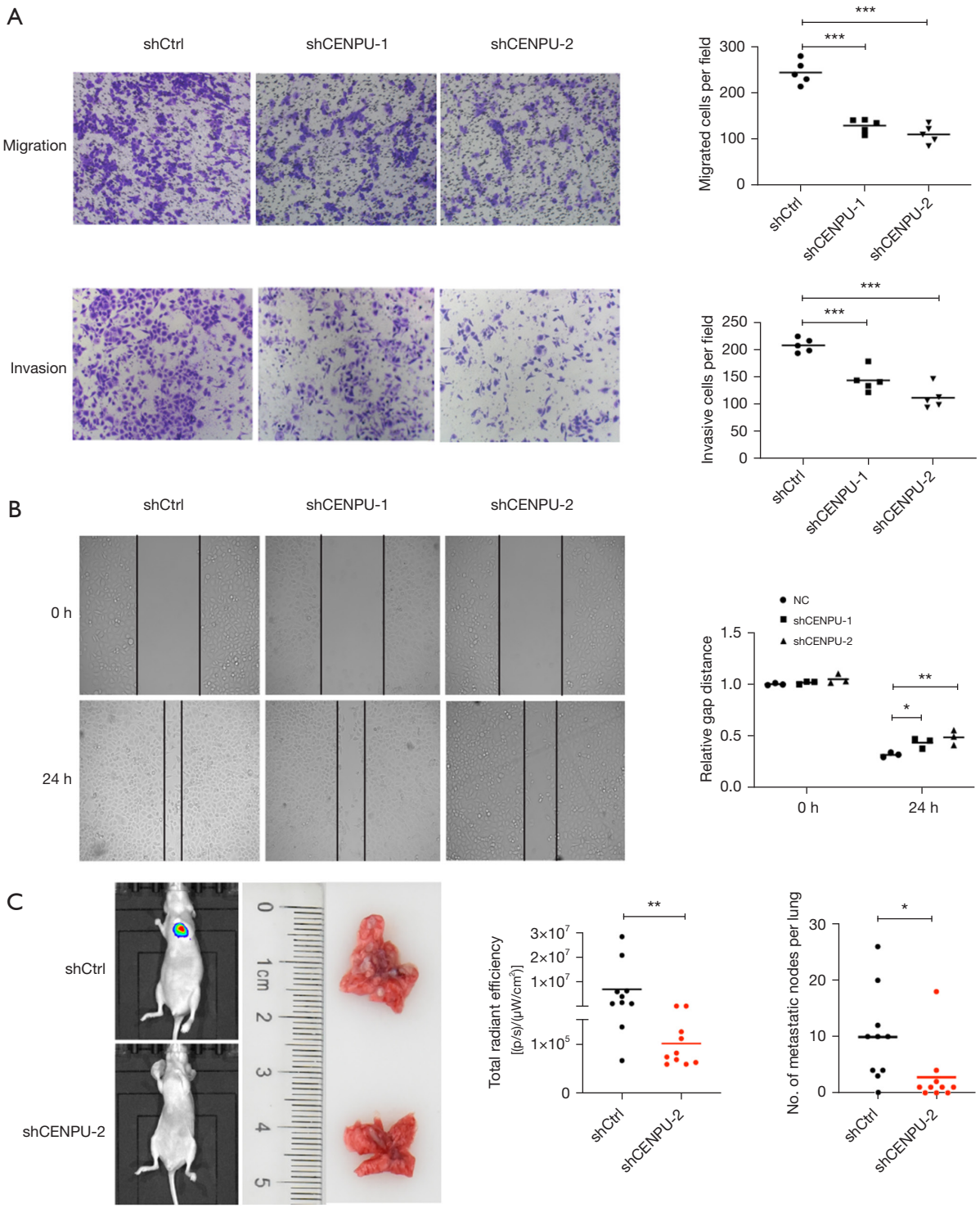

Figure 3 Effects of CNEPU on CNE-2 cell migration and invasion *in vitro* and *in vivo*. (A) Migration and invasion assays (0.1% crystal violet staining) (original magnification: ×100). (B) Cell wound healing assays (magnification: ×100). (C) Representative images of nude mice and metastatic nodes of the lung in nude mice (n=5) (left column). Quantitative analysis of radiant efficiency (middle column) and metastatic nodes of the lung in xenografted tumors (right column). *, P<0.05; **, P<0.01; ***, P<0.001. sh, short hairpin; CENPU, centromere protein U; NC, negative control.

downregulation of CENPU significantly attenuated the metastatic mobility of NPC cells. To determine the effect of CENPU function *in vivo*, we injected cancer cells into the tail vein of nude mice to observe the rate of nodule formation in the lungs. Compared with controls, lower radiant efficiency and smaller lung metastatic nodules were found in the lungs of mouse xenografts injected with shCENPU-2 (*Figure 3C*).

Gene chip analysis and IPA

To explore the molecular mechanisms by which CENPU contributes to the development of NPC, we conducted a microarray analysis comparing the DEGs in the shCENPU group and the shCtrl group (Table S1). Genes with P value ≤0.05 and FC >2.0 were considered significant and were chosen for further analysis. Hierarchical clustering showed that the similarity of the data pattern within the group was high, and the similarity of the data pattern between the groups was low (*Figure 4A*). Gene chip found that 172 genes were upregulated and 397 genes were downregulated, as shown in the volcano plot (*Figure 4B*). The classical signaling pathway analysis suggested that p38/MAPK and ERK1/2 were strongly activated when CENPU was knocked down (*Figure 4C*).

Moreover, disease and function analysis revealed that DEGs were significantly enriched in cancer and functions, including cellular movement, cell death and survival, cellular development, cell growth, and proliferation (Figure S1A). The network of DEGs involved in the movement of tumor cell lines suggested that DUSP6 had a negative effect on the movement of NPC cell lines (Figure S1B). A heatmap of diseases and functions affected by DEGs showed that knockdown of CENPU suppressed cellular movement (Figure S1C), which was closely consistent with our study.

The upstream regulatory network suggested that lipopolysaccharides were strongly inhibited when CENPU was downregulated (Figure S2A). The regulatory network of interactions between DEGs, regulators and functions indicated that CENPU not only regulated the proliferation and migration of cell lines but also participated in the activity of lymphocytes, monocytes and phagocytes (Figure S2B).

Knockdown of CENPU inhibits the p38 and ERK1/2 pathways by interacting with DUSP6

Western blotting analysis showed that knockdown of CENPU reduced the phosphorylation of p38, ERK1/2 and MAPK (*Figure 5A*). To study the downstream genes that led to the carcinogenesis of NPC, 30 candidate downstream genes that enriched in cyclins and cell cycle regulation, integrin signaling, ERK/MAPK signaling and p38 MAPK signaling were chosen and tested (*Figure 5B*). Then, six candidate genes were further tested by western blotting (*Figure 5C*). Our data suggested that knockdown of CENPU upregulated the expression of tumor suppressor DUSP6. Using our own samples and the database GSE12452, we found that DUSP6 was downregulated in NPC tissues (*Figure 5D,5E*). And the expression of CNEPU was inversely correlated with DUSP6 (*Figure 5F*). Co-IP and reciprocal western blotting analysis further revealed that CENPU was coimmunoprecipitated with DUSP6 and, conversely, that DUSP6 was coimmunoprecipitated with CENPU in CNE-2 cells (*Figure 5G*). Taken together, these findings suggest that CENPU promotes the development of NPC by negatively regulating DUSP6 expression.

Discussion

CENPU is a key member of the CENP family, which is associated with centromere mitosis and chromosome movement. In our study, we found that the expression of CENPU was significantly increased in NPC. Overexpression of CENPU promotes growth and metastasis and was associated with worse prognosis. Preliminary mechanistic study suggested that CENPU suppressed the ERK1/2 and p38 pathways by interacting with DUSP6, ultimately leading to the inhibition of NPC progression.

Recently, studies have shown that CENPU acts as an oncogene in human cancers (8,10,20-23,26,27). In TNBC, CENPU promotes angiogenesis through activation of the COX-2-p-ERK-HIF-1alpha-VEGFA pathway (8). In hepatocellular cancer, high expression of CENPU is related with worse OS and recurrence-free survival (10). In non-small cell lung cancer, CENPU facilitates growth and metastasis via the Wnt/beta-catenin pathway (22) and PI3K/AKT signaling (23). Consistent with the above findings, CENPU was a tumor promoter in NPC. We found that high expression of CENPU was significantly associated with advanced tumor stage and inferior OS in NPC. And like TNBC, activation of the ERK signaling pathway plays an important role in CENPU knockdown NPC cells. The ERK1/2 signaling pathway is frequently activated in cancers (26). In NPC, activation of ERK1/2 pathway facilitates cancer cell growth and metastasis (27,28),

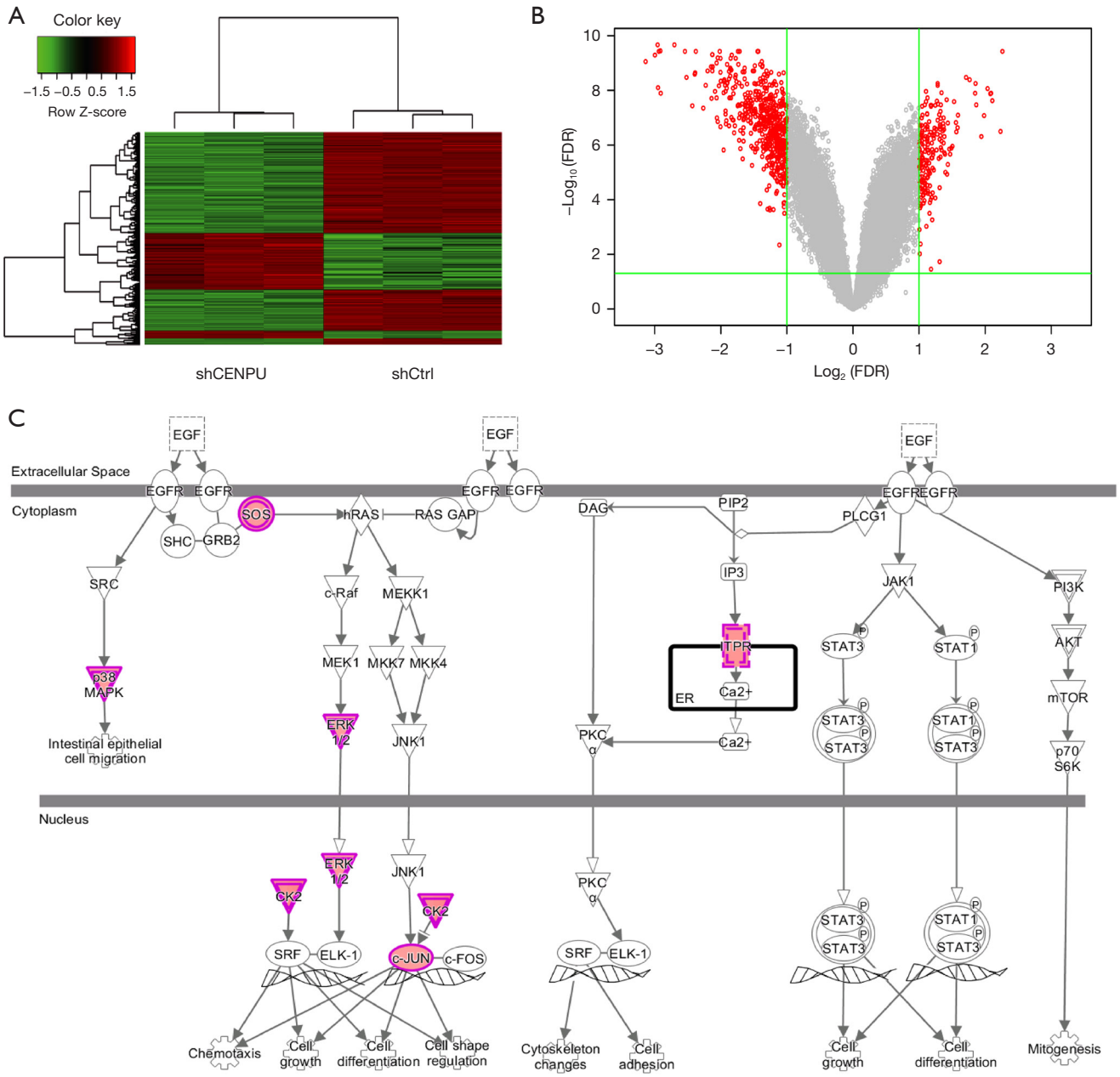

Figure 4 Gene chip analysis and ingenuity pathway analysis. (A) Hierarchical clustering of the DEGs in the CENPU knockdown group (shCENPU) and control group (shCtrl). (B) The volcano plot of the DEGs. (C) Classic signal pathways based on DEGs according to ingenuity pathway analysis. The red dots refer to the differentially expressed genes with P value ≤0.05 and fold change >2.0 between shCENPU group and shCtrl group. The grey dots refer to other genes with no significant difference between those two groups. sh, short hairpin; FDR, false discovery rate; DEGs, differentially expressed genes; CENPU, centromere protein U.

and overexpression of p-ERK was associated with advanced clinical stage and worse outcome in patients with NPC (29). Collectively, CENPU leads to the carcinogenesis in NPC, and acts as a predictive biomarker for NPC patients.

DUSP6 belongs to the dual specificity protein phosphatase subfamily, which negatively regulates members of the MAPK superfamily in cancers (30-35). Wong and colleagues reported that DUSP6 suppresses metastasis of

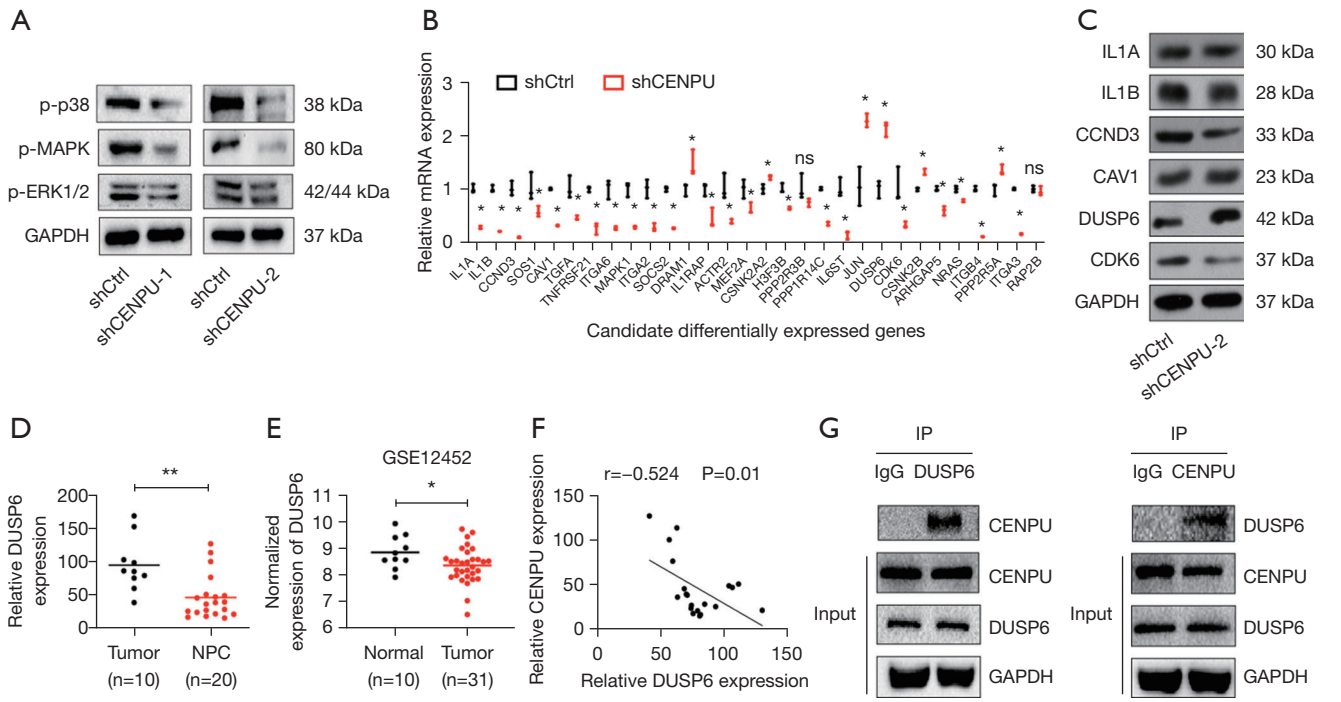

Figure 5 Effects of CENPU on signaling pathways. (A) Expression of p-p38, p-MAPK and p-ERK1/2 by western blotting. (B) Candidate differentially genes. (C) Six candidate downstream genes. (D) Expression of DUSP6 in normal nasopharyngeal mucosa and NPC samples. (E) Expression of DUSP6 in database (GSE12452). (F) Correlations between CENPU and DUSP6 in NPC samples. (G) Immunoblot analysis. *, P<0.05; **, P<0.01. GAPDH, glyceraldehyde-3-phosphate dehydrogenase; sh, short hairpin; CENPU, centromere protein U; ns, not significant; DUSP6, dual specificity phosphatase 6; NPC, nasopharyngeal carcinoma; IP, immunoprecipitation.

NPC cells by inhibiting ERK1/2 pathway (36). However, the relationship between DUSP6 and CENPU remains unknown. Of note, DUSP6 has been considered to have a tumor suppressive effect in NPC and acts as a negative feedback regulator for ERK/MAPK in NPC (37), which is consistent with the results of gene chip in our study. Thus, we hypothesized that CENPU may contribute to tumor progression through downregulation of DUSP6, leading to inactivation of the MAPK signaling pathway in NPC cells. Fortunately, co-IP confirmed our hypothesis that CENPU interacts with DUSP6. Abnormal ubiquitin modification is related to carcinogenesis in NPC (38,39), and CENPU has been reported to play a role in the occurrence and development of liver cancer and TNBC via ubiquitination (8,10). Thus, a better understanding of the roles of CENPU in NPC requires deeper research.

The study had some limitations. First, the concrete mechanism between CENPU and DUSP6 is unclear. Second, the upstream regulatory network of CENPU in NPC remains unknown.

Conclusions

In summary, CENPU is upregulated in NPC patients and is associated with poorer survival. CENPU promotes growth and metastasis by suppressing DUSP6 expression and activating the ERK1/2 and p38 pathways in NPC. CENPU could be a promising prognostic biomarker for patients with NPC.

Acknowledgments

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Footnote

Reporting Checklist: The authors have completed the

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Data Sharing Statement: Available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-2395/dss

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-2395/coif). The authors have no conflicts of interest to declare.

Ethics Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This study was approved by the Ethical Review Committee of Fujian Medical University, Fujian Cancer Hospital (approval No. SQ2020-038). Written informed consent was obtained from the patients for their anonymized information to be published in this article. Animal experiments were performed under a project license (No. 2021-0322) granted by the Ethical Review Committee of Fujian Medical University, Fujian Cancer Hospital and in compliance with institutional guidelines for the care and use of animals.

References

1. Rotolo F, Pignon JP, Bourhis J, et al. Surrogate End Points for Overall Survival in Loco-Regionally Advanced Nasopharyngeal Carcinoma: An Individual Patient Data Meta-analysis. J Natl Cancer Inst 2017;109:djw239.
2. Chen YP, Chan ATC, Le QT, et al. Nasopharyngeal carcinoma. Lancet 2019;394:64-80.
3. Lee AW, Ma BB, Ng WT, et al. Management of Nasopharyngeal Carcinoma: Current Practice and Future Perspective. J Clin Oncol 2015;33:3356-64.
4. Bruce JP, Yip K, Bratman SV, et al. Nasopharyngeal Cancer: Molecular Landscape. J Clin Oncol 2015;33:3346-55.
5. Lo KW, To KF, Huang DP. Focus on nasopharyngeal carcinoma. Cancer Cell 2004;5:423-8.
6. Wordeman L, Mitchison TJ. Identification and partial characterization of mitotic centromere-associated kinesin, a kinesin-related protein that associates with centromeres during mitosis. J Cell Biol 1995;128:95-104.
7. Samejima K, Platani M, Wolny M, et al. The Inner Centromere Protein (INCENP) Coil Is a Single α-Helix (SAH) Domain That Binds Directly to Microtubules and Is Important for Chromosome Passenger Complex (CPC) Localization and Function in Mitosis. J Biol Chem 2015;290:21460-72.
8. Pan T, Zhou D, Shi Z, et al. Centromere protein U (CENPU) enhances angiogenesis in triple-negative breast cancer by inhibiting ubiquitin-proteasomal degradation of COX-2. Cancer Lett 2020;482:102-11.
9. Chen X, Shao Y, Li Y, et al. The cell cycle gene centromere protein K (CENPK) contributes to the malignant progression and prognosis of prostate cancer. Transl Cancer Res 2022;11:1099-111.
10. Liu Y, Yao Y, Liao B, et al. A positive feedback loop of CENPU/E2F6/E2F1 facilitates proliferation and metastasis via ubiquitination of E2F6 in hepatocellular carcinoma. Int J Biol Sci 2022;18:4071-87.
11. Zhang X, Yang Z, Hu Q, et al. Centromere protein U is highly expressed in colorectal cancer and associated with a poor long-term prognosis. Nan Fang Yi Ke Da Xue Xue Bao 2022;42:1198-204.
12. Yuan Y, Deng X, Wang S, et al. Comprehensive pan-cancer analysis identifies centromere-associated protein E as a novel prognostic and immunological biomarker in human tumors. Biochim Biophys Acta Gen Subj 2023;1867:130346.
13. Liu Y, Yu W, Ren P, et al. Upregulation of centromere protein M promotes tumorigenesis: A potential predictive target for cancer in humans. Mol Med Rep 2020;22:3922-34.
14. Hao X, Qiu Y, Cao L, et al. Over-Expression of Centromere Protein U Participates in the Malignant

Neoplastic Progression of Breast Cancer. Front Oncol 2021;11:615427.
15. Chen H, Wu F, Xu H, et al. Centromere protein F promotes progression of hepatocellular carcinoma through ERK and cell cycle-associated pathways. Cancer Gene Ther 2022;29:1033-42.
16. Yu J, Wang K, Yang S, et al. The inhibition of centromere protein K causes anticancer effects in breast carcinoma via effects on the FAK/PI3K/AKT/mTOR pathway. Toxicol Appl Pharmacol 2022;454:116232.
17. Hua S, Wang Z, Jiang K, et al. CENP-U cooperates with Hec1 to orchestrate kinetochore-microtubule attachment. J Biol Chem 2011;286:1627-38.
18. Singh P, Pesenti ME, Maffini S, et al. BUB1 and CENP-U, Primed by CDK1, Are the Main PLK1 Kinetochore Receptors in Mitosis. Mol Cell 2021;81:67-87.e9.
19. Eskat A, Deng W, Hofmeister A, et al. Step-wise assembly, maturation and dynamic behavior of the human CENP-P/O/R/Q/U kinetochore sub-complex. PLoS One 2012;7:e44717.
20. Wang X, Chen D, Gao J, et al. Centromere protein U expression promotes non-small-cell lung cancer cell proliferation through FOXM1 and predicts poor survival. Cancer Manag Res 2018;10:6971-84.
21. Li H, Zhang H, Wang Y. Centromere protein U facilitates metastasis of ovarian cancer cells by targeting high mobility group box 2 expression. Am J Cancer Res 2018;8:835-51.
22. Zhang Q, Li YD, Zhang SX, et al. Centromere protein U promotes cell proliferation, migration and invasion involving Wnt/β-catenin signaling pathway in non-small cell lung cancer. Eur Rev Med Pharmacol Sci 2018;22:7768-77.
23. Li J, Wang ZG, Pang LB, et al. Reduced CENPU expression inhibits lung adenocarcinoma cell proliferation and migration through PI3K/AKT signaling. Biosci Biotechnol Biochem 2019;83:1077-84.
24. Zhang X, Wang M, Zhang Y, et al. Knockdown of CENPU inhibits cervical cancer cell migration and stemness through the FOXM1/Wnt/β-catenin pathway. Tissue Cell 2023;81:102009.
25. Lin C, Chen X, Li M, et al. Programmed Death-Ligand 1 Expression Predicts Tyrosine Kinase Inhibitor Response and Better Prognosis in a Cohort of Patients With Epidermal Growth Factor Receptor Mutation-Positive Lung Adenocarcinoma. Clin Lung Cancer 2015;16:e25-35.
26. Roberts PJ, Der CJ. Targeting the Raf-MEK-ERK mitogen-activated protein kinase cascade for the treatment of cancer. Oncogene 2007;26:3291-310.
27. Lin C, Zong J, Lin W, et al. EBV-miR-BART8-3p induces epithelial-mesenchymal transition and promotes metastasis of nasopharyngeal carcinoma cells through activating NF-κB and Erk1/2 pathways. J Exp Clin Cancer Res 2018;37:283. Erratum in: J Exp Clin Cancer Res 2019;38:34.
28. Wu M, Li X, Li X, et al. Signaling Transduction Network Mediated by Tumor Suppressor/Susceptibility Genes in NPC. Curr Genomics 2009;10:216-22.
29. Wang SS, Guan ZZ, Xiang YQ, et al. Significance of EGFR and p-ERK expression in nasopharyngeal carcinoma. Zhonghua Zhong Liu Za Zhi 2006;28:28-31.
30. Fan MJ, Liang SM, He PJ, et al. Dusp6 inhibits epithelial-mesenchymal transition in endometrial adenocarcinoma via ERK signaling pathway. Radiol Oncol 2019;53:307-15.
31. Ramkissoon A, Chaney KE, Milewski D, et al. Targeted Inhibition of the Dual Specificity Phosphatases DUSP1 and DUSP6 Suppress MPNST Growth via JNK. Clin Cancer Res 2019;25:4117-27.
32. Gao Y, Li H, Han Q, et al. Overexpression of DUSP6 enhances chemotherapy-resistance of ovarian epithelial cancer by regulating the ERK signaling pathway. J Cancer 2020;11:3151-64.
33. Ingram K, Samson SC, Zewdu R, et al. NKX2-1 controls lung cancer progression by inducing DUSP6 to dampen ERK activity. Oncogene 2022;41:293-300.
34. Wang H, Liu D, Sun Y, et al. Upregulation of DUSP6 impairs infectious bronchitis virus replication by negatively regulating ERK pathway and promoting apoptosis. Vet Res 2021;52:7.
35. Zhang B, Yuan P, Xu G, et al. DUSP6 expression is associated with osteoporosis through the regulation of osteoclast differentiation via ERK2/Smad2 signaling. Cell Death Dis 2021;12:825.
36. Wong VC, Chen H, Ko JM, et al. Tumor suppressor dual-specificity phosphatase 6 (DUSP6) impairs cell invasion and epithelial-mesenchymal transition (EMT)-associated phenotype. Int J Cancer 2012;130:83-95.
37. Tulalamba W, Janvilisri T. Nasopharyngeal carcinoma signaling pathway: an update on molecular biomarkers. Int J Cell Biol 2012;2012:594681.
38. Yarza R, Bover M, Agulló-Ortuño MT, et al. Current

approach and novel perspectives in nasopharyngeal carcinoma: the role of targeting proteasome dysregulation as a molecular landmark in nasopharyngeal cancer. J Exp Clin Cancer Res 2021;40:202.

39. Lin C, Li M, Lin N, et al. RNF38 suppress growth and metastasis via ubiquitination of ACTN4 in nasopharyngeal carcinoma. BMC Cancer 2022;22:549.

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