Original Article

CircHIPK2 promotes proliferation of nasopharyngeal carcinoma by down-regulating *HIPK2*

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Background: Circular RNAs (circRNAs) are a new family of endogenous non-coding RNAs generated by a covalently closed loop, and a mounting body of data suggests they control gene expression. While the *circRNA*-homeodomain-interacting protein kinase-2 (*circHIPK2*) is generated from the homeodomain-interacting protein kinase 2 (*HIPK2*) gene, the function of *circHIPK2* in nasopharyngeal cancer (NPC) along with the responsible mechanisms are still unclear.

Methods: RNA-sequencing data was utilized to determine the differentially expressed circRNAs, and *circHIPK2* was established as a novel prospective circRNA. The expressions of circRNAs along with messenger RNAs (mRNAs) in NPC tissues and cells was assessed via quantitative real-time polymerase chain reaction (qRT-PCR), and the transfection of NPC cells with plasmids *in vitro* and *in vivo* was adopted to explore the effects of *circHIPK2* in NPC. Western blotting was adopted to assess the expressions of *HIPK2* and *β-catenin*, while Cell Counting Kit (CCK)-8 assay coupled with colony-forming assay were utilized to assess the biological functions. The expression of nuclear and cytoplasmic *HIPK2* was detected via nucleocytoplasmic separation assay.

Results: Herein, we established that *circHIPK2* was upregulated in NPC tissues. Over-expression of *circHIPK2* promoted cell proliferation *in vitro* and *in vivo*, and further studies revealed it inhibited the protein level of *HIPK2* in a post-transcriptional pattern, decreasing *β-catenin* expression and suppressing the proliferation of NPC.

Conclusions: Our findings demonstrated elevated *circHIPK2* facilitated the cell proliferation of NPC cells via the *circHIPK2/HIPK2* axis, suggesting *circHIPK2* might be an oncogene to promote the process of NPC and could be a novel treatment target for its management.

Keywords: *circHIPK2*; nasopharyngeal carcinoma; homeodomain-interacting protein kinase 2 (*HIPK2*); proliferation

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Introduction

Nasopharyngeal carcinoma (NPC) originates from the epithelial tissue of the nasopharynx and is among the most common malignant tumors of the head and neck (1). NPC is very common in Southern China, and Southeast Asia (2). While radiotherapy or chemotherapy may successfully control local tumor development along with local recurrence, with a 5-year rate of survival of over 80%, distant metastasis coupled with radiotherapy

resistance contribute to the low survival rate (3). Thus, to develop more efficient therapy methods, further research into the pathogenesis of NPC along with the molecular mechanisms of metastasis and proliferation is required.

CircRNAs are "covalently closed single-stranded transcripts comprising many RNA species" (4). Originally thought to be a by-product of mRNA biosynthesis due to the processing of precursor mRNA (5), circRNAs have been proven to have pivotal roles in a range of biological processes and are linked with the onset and progress of many human diseases, including a multitude of malignant tumors (6-8). CircRNAs may work as sponges of microRNAs (miRNAs) (9), dock to proteins (10,11), or code for tiny peptides, leading to abnormal gene expression that could influence cancer biology (12). Although many circRNAs have been identified, their functions in NPC are still largely unknown.

Herein, we found a circRNA derived from the homodomain-interacting protein kinase 2 (*HIPK2*) gene, termed *circHIPK2* (circBase ID: *hsa_circ_0001756*), which was remarkably upregulated in NPC tissues. The proliferation of *circHIPK2* was explored in NPC cells via *in vitro* along with *in vivo* experiments. Further research illustrated *circHIPK2* repressed *HIPK2* protein contents in a post-transcriptional manner, lowering *β-catenin* expression and dampening NPC growth. Collectively, *circHIPK2* may serve as a possible target for NPC treatment. We present the following article in accordance with the ARRIVE reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-22-1645/rc).

Methods

Human NPC tissue specimens and cell culture

Pathological evaluation was utilized to diagnose NPC in normal along with nasopharyngeal tissues taken from patients at The First Affiliated Hospital of Soochow University. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The research protocol and the use of human samples were approved by the Ethics Committee of Soochow University (No. SUDA20210705A01) and all participants signed informed consent forms. The CNE, CNE-2, 5-8F, 6-10B, along with the immortalized nasopharyngeal epithelial cells NP69 were acquired from the Oncology Laboratory of the First Affiliated Hospital of Soochow University. CNE, CNE-2, 5-8F, along with 6-10B cells were inoculated in H1640 medium enriched with 10% fetal bovine serum (HyClone, Logan, UT, USA), whilst NP69 cells were grown in keratinocyte serum-free medium enriched with bovine pituitary extract (Invitrogen) (BD Biosciences). The cells were then kept under 37 ℃ along with 5% CO_2 conditions.

RNA extraction, preparation of complementary DNA (cDNA), and qRT-PCR analysis

Isolation of total RNA (tRNA) from NPC cell lines or tissues was performed with TRIzol reagent (Takara, Dalian, China), and generation of cDNA. tRNA was performed with the PrimeScript® RT Master Mix (Takara, Dalian, China) as described by the manufacturer. Afterwards, quantitative Real-Time polymerase chain reaction (qRT-PCR) was run on the Roche Light Cycler 96 Real-time PCR system (Roche Diagnostics, Basel, Switzerland) in triplicate. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was adopted as endogenous controls, and the comparative ΔΔCt approach was adopted to assess relative expression. The primer sequences were as follows: *circHIPK2*, Forward: AGGTCTTATCCACGCTGACC, Reverse: GAAGGGTGTGAGGGGAGAAA; *HIPK2*, Forward: CCACCTACTTGCAGTCCAGA, Reverse: AGCTCCTGGATATAACGGCC; *GAPDH*, Forward: AATCCCATCACCATCTTCC, Reverse: CATCACGCCACAGTTTCC.

Western blotting

Western blotting was completed as documented previously (13). Harvesting of proteins was performed as illustrated in the figures, fractionated via sodium dodecyl sulfate polyacrylamide gel electrophoresis, and blotted onto polyvinylidene fluoride membranes. The membranes were blocked in 1% Bovine Serum Albumin (BSA) for 20 minutes at room temperature (RT) then inoculated overnight with anti-*β-catenin*, anti-*HIPK2*, anti-*GAPDH* (Abgent, USA), and anti-*LaminB1* antibodies. Afterwards, the blots were probed for 1 hour at room temperature with horseradish peroxidase-linked secondary antibodies before being processed with the enhanced chemiluminescence plus Western Blotting Detection System. *GAPDH* was utilized as a loading control.

Plasmid construction and small interfering RNA (siRNA) interference assay

We cloned the human *circHIPK2* cDNA into the Plvx-

circ vector with a front circular frame and a rear circular frame to create *circHIPK2* over-expression plasmids, with an empty plasmid as a negative control. GenePharma produced siRNA for *circHIPK2* (Shanghai, China), and as a negative control, scrambled siRNA was generated. Lipofectamine 3000 (Invitrogen) was adopted in the transfection as described by the manufacturer, and 48 hours post transfection, total RNA along with proteins was collected.

CCK-8 cell proliferation assay

The cells after transfection were digested and planted at 5,000 cells/well in 96-well plates. Five duplicate wells were set for each sample in each group, and 100 μL of the growth medium was introduced to every well. The cells were inoculated with 10 μL of CCK-8 solution (Dojindo, Japan) at the given time points and incubated in the dark for an additional 2 hours. At a wavelength of 450 nm, the absorbance was measured. The experiment was repeated thrice.

Colony formation assay

The cells after transfection were digested and planted at 1,000 cells/well in 6-well plates for 2 weeks and the surviving colonies were fixed with 4% paraformaldehyde (PFA), stained with 0.05% crystal violet, then counted.

Fluorescence in situ hybridization (FISH)

The sub-cellular localization of *circHIPK2* in NPC cells was assessed with RNA FISH (RiboBio, Guangzhou, China) utilizing the RiboTMFluorescent *in situ* Hybridization Kit as documented by the manufacturer and a *circHIPK2* probe tagged with the Cy3 fluorescent dye.

Nucleocytoplasmic separation experiments

A Nuclear and Cytoplasmic Protein Extraction Kit (Beyotime, Shanghai, China) was utilized to separate of nuclear and cytosolic fractions of NPC cells as described by the manufacturer. *LamB1* and *GAPDH* served as the nuclear as well as the cytoplasmic control.

Animal studies

Animal experiments were performed under a project license (No. SUDA20210705A01) granted by the Ethics Committee of Soochow University, in compliance with the Ethics Committee of Soochow University guidelines for the care and use of animals. A protocol was prepared before the study without registration. The BALB/c nude mice (n=5 for each group) used in this investigation were five weeks old, male, and acquired from Beijing Vital River Laboratory Animal Technology Co., Ltd. Animals were kept in a 12-hour light/12-hour dark cycle at 22±2 ℃ with unrestricted access to food along with water. Cells transfected with the *circHIPK2* over-expression vector, *circHIPK2* short hairpin RNA (shRNA), or the empty vector and scrambled shRNA were injected into the armpit of the mice. After 4 weeks, the subcutaneous tumor size was measured, with the volume (V) of the tumor computed via the formula $V = (Width^2 \times Length)/2$.

Statistical analysis

Results are recorded as means ± standard error of the mean for at least three independent experiments. GraphPadPrism8 statistical software package was used for data analysis and the Student's *t*-test (two-tailed) was used for continuous variables. $P<0.05$ denotes statistical significance.

Results

circHIPK2 (hsa-circ-0001756) is considerably upregulated in NPC

Differentially expressed circRNAs were explored via RNA sequencing data in four NPC along with normal nasopharyngeal tissues to determine their significance in the onset and progress of the disease (unpublished data) (*Figure 1A*). The results showed elevated contents of circRNAs were more prevalent than down-regulated circRNAs among differentially expressed circRNAs. We categorized the up-regulated circRNAs via fold change after screening them using an average normal tissue read count of more than 100. qRT-PCR was adopted to confirm the three up-regulated circRNAs in 14 NPC along with normal nasopharyngeal tissues. The results showed that *hsa-circ-0001756* (*circHIPK2*), rather than *hsa-circ-0006006* and *hsa-circ-0057090*, was dramatically up-regulated in NPC tissues (*Figure 1B,1C*), and the head-to-tail splicing of exon 2 gave rise to *circHIPK2*, which was derived from the *HIPK2* gene (*Figure 1D*). Subsequently, the expression of *circHIPK2* was detected in NPC cell lines (CNE, CNE-2, 5-8F and 6-10B) by qRT-PCR, and the results revealed

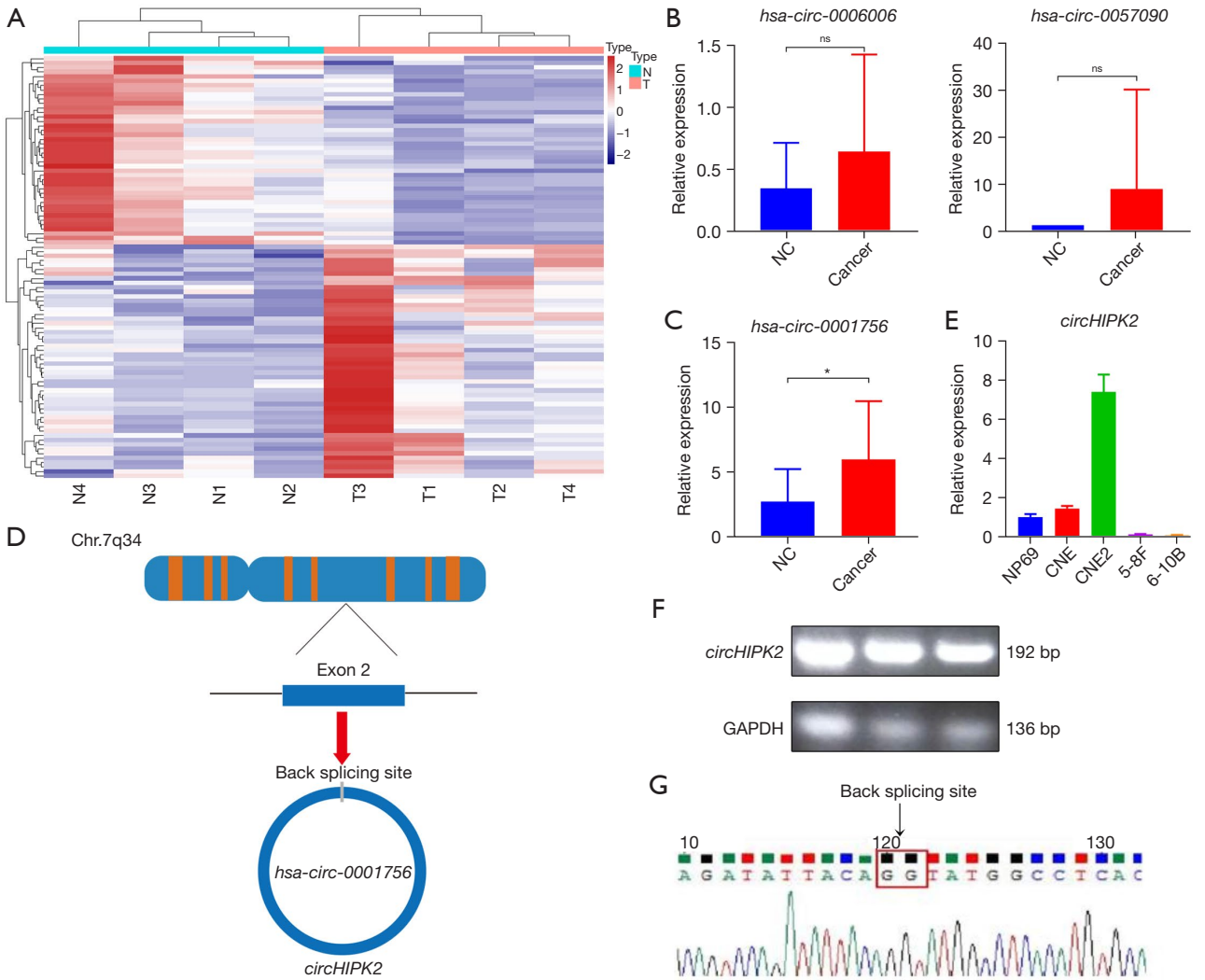

Figure 1 *circHIPK2* is significantly up-regulated in nasopharyngeal carcinoma tissues. (A) Clustered heat map of the differentially expressed circRNAs in four NPC (T1–T4) and normal nasopharyngeal tissues (N1–N4). Rows represent circRNAs while columns represent tissues. (B,C) *hsa-circ-0006006*, *hsa-circ-0057090* and *hsa-circ-0001756* were validated in 14 NPC and normal nasopharyngeal tissues using qRT-PCR (*P<0.05; ns, non-significant). (D) Schematic illustration showing the circularization of *HIPK2* exon 2 forming *circHIPK2* (*hsa-circ-0001756*). (E) The expression of *circHIPK2* in NPC cell lines were measured by qRT-PCR. (F,G) The existence of *circHIPK2* was proved by RT-PCR and its back splicing junction was verified by Sanger sequencing. Arrow indicates the special splicing junction of *circHIPK2*. NPC, nasopharyngeal carcinoma; circRNAs, circular RNAs; qRT-PCR, quantitative real-time polymerase chain reaction.

that *circHIPK2* showed higher expression in CNE along with CNE-2 cell lines, and lower expression in 5-8F, as well as 6-10B cell lines (*Figure 1E*). Moreover, Sanger sequencing verified head-to-tail splicing in the RT-PCR product of *circHIPK2* with the expected size (*Figure 1F,1G*). Collectively, these results illustrated that *circHIPK2* was presented in the NPC cell lines and was remarkably upregulated in NPC tissues.

Overexpression of circHIPK2 enhances the proliferation of NPC cells

To investigate the function of *circHIPK2* in NPC cells, we overexpressed it in 5-8F cells by transfecting with *circHIPK2* or control vector plasmids. The *circHIPK2* expression was remarkably increased in the 5-8F cells transfected with *circHIPK2* plasmids (*Figure 2A*). CCK-8 and clone formation assays were then performed in the

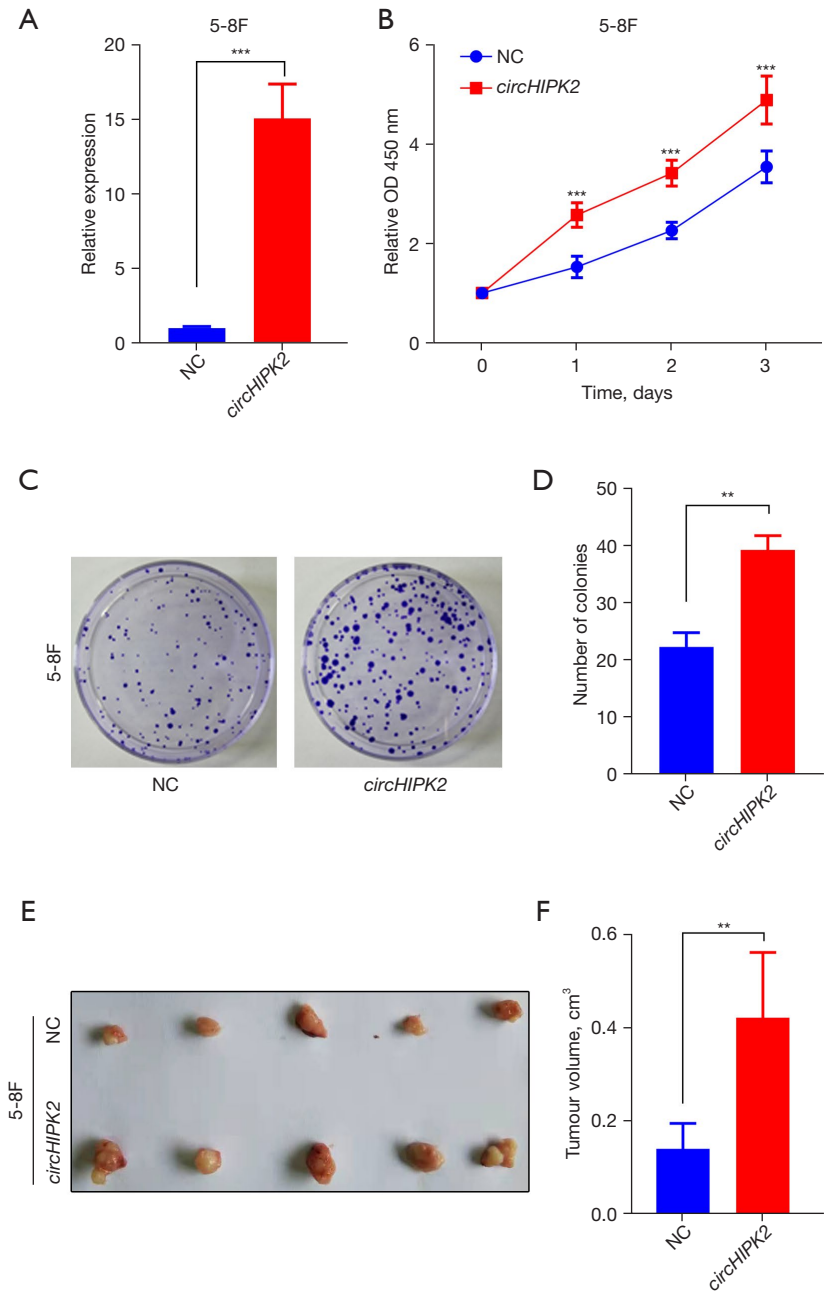

Figure 2 Overexpression of *circHIPK2* promotes the proliferation of NPC cells. (A) The expression levels of *circHIPK2* in 5-8F cells transfected with *circHIPK2* or control vector plasmids were detected by qRT-PCR (***P<0.001). (B) The cell proliferation ability of 5-8F cells transfected with *circHIPK2* or control vector plasmids at 24, 48, and 72 h (***P<0.001). (C,D) Representative results of colony formation assay of 5-8F cells transfected with *circHIPK2* or control vector plasmids (**P<0.01). Cells were stained with 0.05% crystal violet. Significance was determined from three independent experiments and assessed by Student's *t*-test. (E,F) Representation picture of tumor formation of the xenograft in nude mice (n=5 for each group) with *circHIPK2* overexpressed 5-8F cells. Compared with the vector group, the tumor volume significantly increased in *circHIPK2*-treated nude mice (**P<0.01). NPC, nasopharyngeal carcinoma; qRT-PCR, quantitative real-time polymerase chain reaction.

5-8F overexpression or control group, respectively, and the results illustrated over-expression of *circHIPK2* promoted the proliferation of 5-8F cells *in vitro* (*Figure 2B-2D*). 5-8F cell transfects of *circHIPK2* or control vector were inoculated subcutaneously into BALB/c nude mice to assess if over-expression of *circHIPK2* influenced tumor formation *in vivo*, and the *circHIPK2* over-expression group gradually enhanced tumor progress and volume in contrast with the controls (*Figure 2E,2F*). Over-expression of *circHIPK2* effectively stimulates the growth of NPC cells, as illustrated by these data.

Depletion of circHIPK2 dampens growth of NPC cells

Knockdown of the *circHIPK2* expression in CNE2 cells was then performed by transfection with *circHIPK2* siRNA or negative control, and the results revealed *circHIPK2* expression was remarkably decreased in the CNE2 cells transfected with *circHIPK2* siRNA (*Figure 3A*). Subsequently, CCK-8 and clone formation assays were performed in the CNE2 depletion or control group, and the results showed depletion of *circHIPK2* inhibits the proliferation of CNE2 cells *in vitro* (*Figure 3B-3D*). CNE2 cell transfects of *circHIPK2* shRNA or a negative control were inoculated subcutaneously into BALB/c nude mice to assess whether suppression of *circHIPK2* influences tumor growth *in vivo*. In contrast with the controls, the *circHIPK2* depletion group remarkably reduced tumor development along with volume (*Figure 3E,3F*). These findings illustrate *circHIPK2* depletion effectively blocks NPC cells from proliferating.

circHIPK2 inhibits the protein level of HIPK2 at the post-transcriptional level

To explore the potential mechanism of *circHIPK2* in NPC, the mRNA contents of *HIPK2* were detected in *circHIPK2* overexpressed 5-8F cells or depleted CNE2 cells. The results revealed the mRNA contents of *HIPK2* showed no significant difference upon *circHIPK2* upregulation or downregulation (*Figure 4A*). We then detected the protein contents of *HIPK2* in *circHIPK2* overexpressed or depleted 5-8F and CNE2 cells, and established that the protein contents of *HIPK2* were remarkably decreased in *circHIPK2* overexpressed 5-8F and CNE2 cells (*Figure 4B,4C*) and increased in *circHIPK2* depleted 5-8F and CNE2 cells (*Figure 4D,4E*), suggesting *circHIPK2* inhibited the protein level of *HIPK2* in a post-transcriptional pattern.

HIPK2 is a type of serine/threonine protein kinase and a member of the *HIPK* family (14). As a tumor suppressor gene, it reduced the amount of *β-catenin* protein in the nucleus to exert tumor inhibition (15). Subsequently, we detected the protein contents of *β-catenin*, a well-known molecule that promotes tumor cell proliferation, and established that the protein contents of *β-catenin* were remarkably increased in *circHIPK2* overexpressed 5-8F and CNE2 cells (*Figure 4B,4C*) and decreased in *circHIPK2* depleted 5-8F and CNE2 cells (*Figure 4D,4E*). This suggested *circHIPK2* inhibited the protein level of *HIPK2*, thus decreased the *β-catenin* expression and suppressed the proliferation of NPC. Moreover, RNA FISH assay and nucleocytoplasmic separation experiments were used to evaluate the localization of *circHIPK2* and *HIPK2* in NPC cells. The results revealed *circHIPK2* was mainly localized in the cytoplasm (*Figure 4F*), and *HIPK2* was observed in the nucleus and cytoplasm fractions of NPC cells (*Figure 4G*). Furthermore, we found *HIPK2* was remarkably decreased in both the nucleus and cytoplasm fraction upon *circHIPK2* overexpression (*Figure 4G*). As *HIPK2* was reported to play a cancer-inhibiting role in the nucleus in a previous study (16), we considered that overexpressed *circHIPK2* inhibited the protein level of *HIPK2* in the nucleus, leading to the upregulation of tumor promoting gene *β-catenin*. Overall, these results indicated *circHIPK2* inhibited the protein level of *HIPK2* in both the nucleus and cytoplasm by a post-transcriptional pattern, decreasing the *β-catenin* expression and suppressing the proliferation of NPC.

Discussion

Nasopharyngeal carcinoma with an insidious location is difficult to treat, and easily causes multiple complications in adjacent organs (17,18). The need for molecular targeted precision therapy is urgent. Due to advances made in next-generation sequencing technologies in recent years, a growing number of circRNAs have been discovered to be involved in the control of a range of human disease processes. Although recent studies have shown numerous circRNAs play an indispensable role in the occurrence and progress of NPC (19-23), their function in its onset and progress or specific mechanisms of circRNA in the disease are still insufficient.

Through RNA sequencing of NPC clinical samples, we uncovered a substantial number of circRNAs in this research. As a type of circRNA, *circHIPK2* has been found to synergize with SiO_2 to induce fibroblast activation in

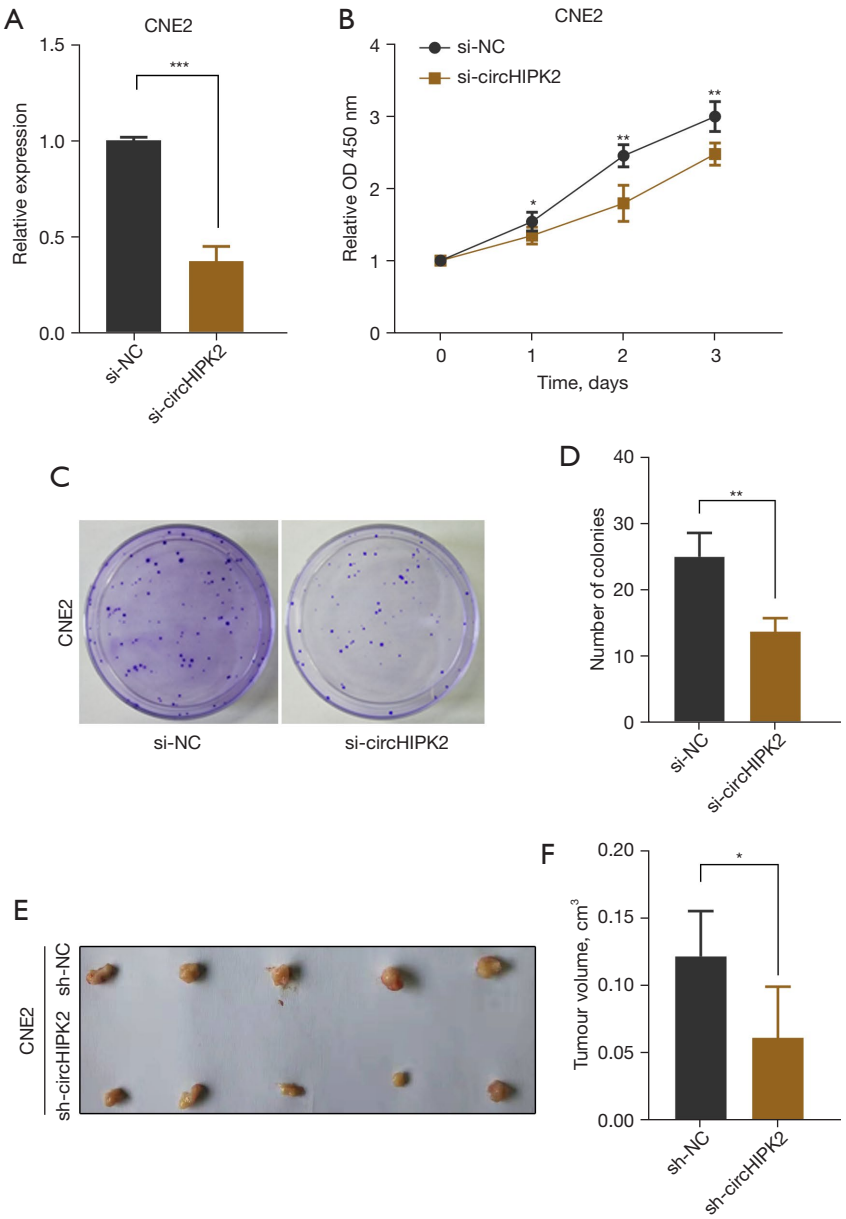

Figure 3 Depletion of *circHIPK2* inhibits proliferation of NPC cells. (A) The expression levels of *circHIPK2* in CNE2 cells transfected with *circHIPK2* siRNA or negative control (***P<0.001). (B) The cell proliferation ability of CNE2 cells transfected with *circHIPK2* siRNA or negative control at 24, 48, and 72 h (*P<0.05, **P<0.01). (C,D) Representative results of colony formation assay of CNE2 cells transfected with *circHIPK2* siRNA or control (**P<0.01). Cells were stained with 0.05% crystal violet. Significance was determined from three independent experiments and assessed by Student's *t*-test. (E,F) Representation picture of tumor formation of the xenograft in nude mice (n=5 for each group) with *circHIPK2* interference CNE2 cells. Compared with the control group, the tumor volume significantly decreased in *circHIPK2* depletion group (*P<0.05). NPC, nasopharyngeal carcinoma; siRNA, small interfering RNA.

silicosis (24), form a regulatory axis with *miR124-2HG* combined with autophagy and ER stress to promote astrocyte activation (25), regulate astrocytes and improve depressive behavior together with gut microbial bacteria in depression (26), and promote functional recovery by decreasing *circHIPK2* in neural stem cells in ischemic stroke (27). However, the role of *circHIPK2* in NPC has not yet been elucidated. We found it was remarkably

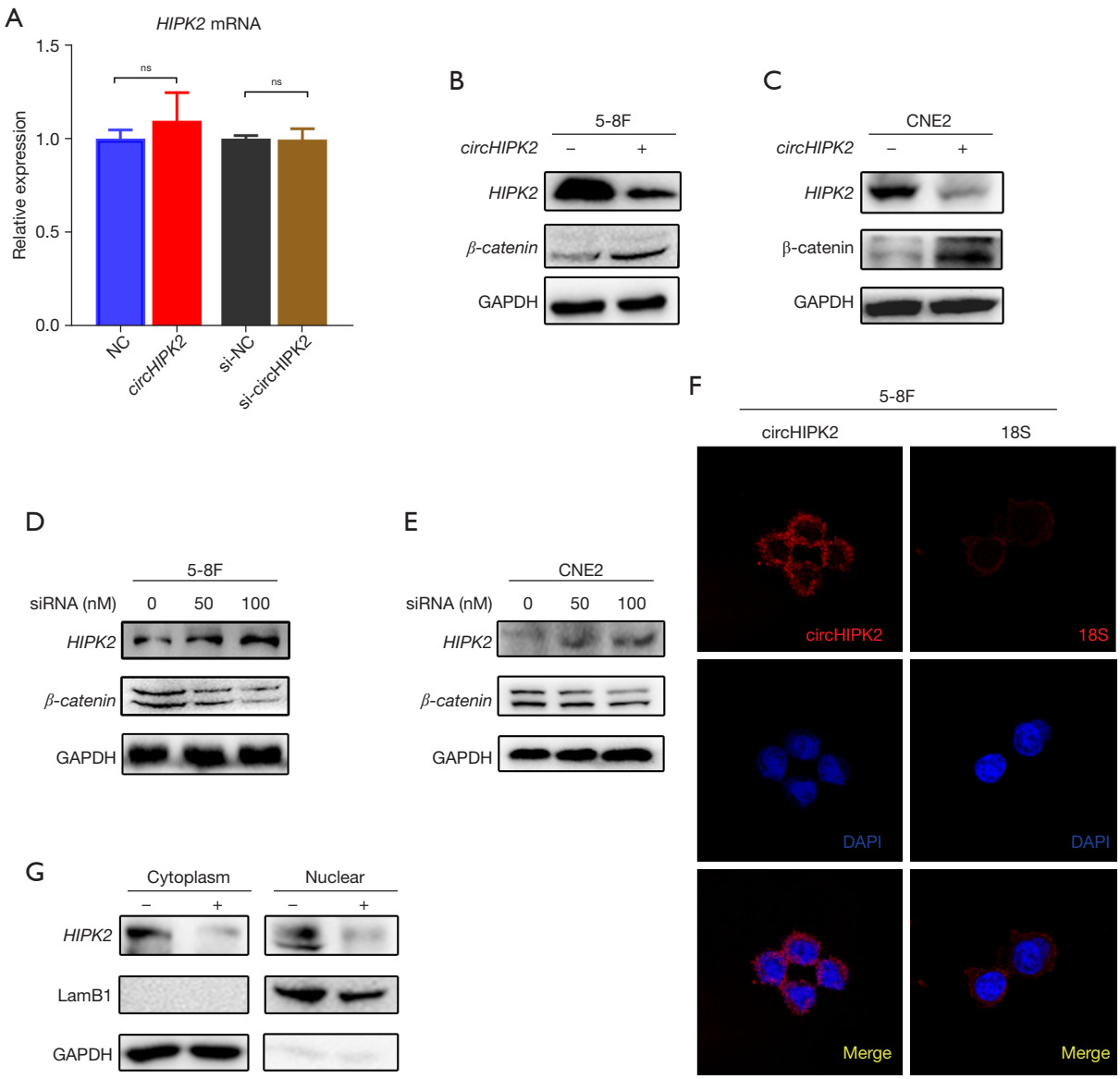

Figure 4 *circHIPK2* inhibits the protein level of *HIPK2* at the post-transcriptional level. (A) The mRNA levels of *HIPK2* in *circHIPK2* overexpressed 5-8F cells or depleted CNE2 cells (ns, non-significant). (B,C) The protein levels of *HIPK2* and *β-catenin* in *circHIPK2* overexpressed 5-8F and CNE2 cells. (D,E) The protein levels of *HIPK2* and *β-catenin* in *circHIPK2* depleted 5-8F and CNE2 cells. (F) RNA FISH for *circHIPK2* and 18S was detected in 5-8F cells. Nuclei was stained blue (DAPI), *circHIPK2* and 18S were stained red. Magnification: 200×. (G) Nuclear *HIPK2* protein levels decreased significantly after *circHIPK2* overexpression. FISH, fluorescence in situ hybridization; DAPI, 4',6-diamidino-2-phenylindole.

upregulated in NPC tissues (*Figure 1*), proving its overexpression enhanced the growth of NPC cells (*Figure 2*) and its depletion inhibited their growth (*Figure 3*). We also illustrated that *circHIPK2* inhibited the protein level of *HIPK2* in both the nucleus and cytoplasm by a post-transcriptional pattern, decreasing *β-catenin* expression and suppressing the proliferation of NPC (*Figure 4*).

HIPK2 is a multi-functional protein that uses its kinase activity to dampen tumor development and stimulate responsiveness to therapy by modulating critical molecular cascades in cancer. *HIPK2* inhibitions have recently been discovered in cancers, and are caused by a variety of

mechanisms, consisting of *HIPK2* cytoplasmic localization, protein degradation, as well as tumor development (28,29). Numerous investigations have shown *HIPK2* played a pivotal role as a tumor repressor in hepatocellular, colon, and prostate cancer (28,30,31). Recent research evidence has shown posttranslational modification of small ubiquitin-like modifier, phosphorylation, acetylation, and ubiquitination exert tight control over *HIPK2* (30,32). Because circRNAs may influence gene expression by docking to proteins, they may play a role in cancer biology, and we hypothesized *circHIPK2* might bind to specific protein to regulate the expression of *HIPK2* by a post-transcriptional pattern, although the precise mechanism by which *circHIPK2* regulates *HIPK2* might need to be explored in future investigations.

Conclusions

In summary, our study delineates a *circHIPK2/HIPK2* module with respect to functional implications in the tumor proliferation of NPC. Our results imply *circHIPK2* might play an indispensable role in tumor biology, and that the regulatory signaling might offer a novel therapeutic target for the management of NPC.

Acknowledgments

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Footnote

Reporting Checklist: The authors have completed the ARRIVE reporting checklist. Available at https://tcr.amegroups.com/article/view/10.21037/tcr-22-1645/rc

Data Sharing Statement: Available at https://tcr.amegroups.com/article/view/10.21037/tcr-22-1645/dss

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tcr.amegroups.com/article/view/10.21037/tcr-22-1645/coif). The authors have no conflicts of interest to declare.

Ethical 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). The research protocol and the use of human samples were approved by the Ethics Committee of Soochow University (No. SUDA20210705A01) and all participants signed informed consent forms. Animal experiments were performed under a project license (No. SUDA20210705A01) granted by the Ethics Committee of Soochow University, in compliance with the Ethics Committee of Soochow University guidelines for the care and use of animals.

References

1. Chen YP, Chan ATC, Le QT, et al. Nasopharyngeal carcinoma. Lancet 2019;394:64-80.
2. Tu C, Zeng Z, Qi P, et al. Identification of genomic alterations in nasopharyngeal carcinoma and nasopharyngeal carcinoma-derived Epstein-Barr virus by whole-genome sequencing. Carcinogenesis 2018;39:1517-28.
3. Ng WT, Corry J, Langendijk JA, et al. Current management of stage IV nasopharyngeal carcinoma without distant metastasis. Cancer Treat Rev 2020;85:101995.
4. Kristensen LS, Andersen MS, Stagsted LVW, et al. The biogenesis, biology and characterization of circular RNAs. Nat Rev Genet 2019;20:675-91.
5. Chen LL. The biogenesis and emerging roles of circular RNAs. Nat Rev Mol Cell Biol 2016;17:205-11.
6. Drula R, Braicu C, Harangus A, et al. Critical function of circular RNAs in lung cancer. Wiley Interdiscip Rev RNA 2020;11:e1592.
7. Naeli P, Pourhanifeh MH, Karimzadeh MR, et al. Circular RNAs and gastrointestinal cancers: Epigenetic regulators with a prognostic and therapeutic role. Crit Rev Oncol Hematol 2020;145:102854.

8. Luo N, Sulaiman Z, Wang C, et al. Hsa_circ_0000497 and hsa_circ_0000918 contributed to peritoneal metastasis of ovarian cancer via ascites. J Transl Med 2022;20:201.
9. Zhang LX, Gao J, Long X, et al. The circular RNA circHMGB2 drives immunosuppression and anti-PD-1 resistance in lung adenocarcinomas and squamous cell carcinomas via the miR-181a-5p/CARM1 axis. Mol Cancer 2022;21:110.
10. Du WW, Yang W, Liu E, et al. Foxo3 circular RNA retards cell cycle progression via forming ternary complexes with p21 and CDK2. Nucleic Acids Res 2016;44:2846-58.
11. Zhang Y, Zhang X, Xu Y, et al. Circular RNA and Its Roles in the Occurrence, Development, Diagnosis of Cancer. Front Oncol 2022;12:845703.
12. Yang Y, Gao X, Zhang M, et al. Novel Role of FBXW7 Circular RNA in Repressing Glioma Tumorigenesis. J Natl Cancer Inst 2018.
13. Cheng F, Zhao S, Li J, et al. Enhanced effect of recombinant adenoviruses co-expression of ING4 and OSM on anti-tumour activity of laryngeal cancer. J Cell Mol Med 2022;26:1556-66.
14. Zhang F, Qi L, Feng Q, et al. HIPK2 phosphorylates HDAC3 for NF-κB acetylation to ameliorate colitis-associated colorectal carcinoma and sepsis. Proc Natl Acad Sci U S A 2021;118:e2021798118.
15. Kim EA, Kim JE, Sung KS, et al. Homeodomain-interacting protein kinase 2 (HIPK2) targets beta-catenin for phosphorylation and proteasomal degradation. Biochem Biophys Res Commun 2010;394:966-71.
16. Kim DH, Park H, Choi YJ, et al. Exosomal miR-1260b derived from non-small cell lung cancer promotes tumor metastasis through the inhibition of HIPK2. Cell Death Dis 2021;12:747.
17. Ren WT, Li YX, Wang K, et al. Cerebral functional abnormalities in patients with nasopharyngeal carcinoma after radiotherapy: an observational magnetic resonance resting-state study. Chin Med J (Engl) 2019;132:1563-71.
18. Lin KT, Lee SY, Liu SC, et al. Risk of ocular complications following radiation therapy in patients with nasopharyngeal carcinoma. Laryngoscope 2020;130:1270-7.
19. Hong X, Liu N, Liang Y, et al. Circular RNA CRIM1 functions as a ceRNA to promote nasopharyngeal carcinoma metastasis and docetaxel chemoresistance through upregulating FOXQ1. Mol Cancer 2020;19:33.
20. Ke Z, Xie F, Zheng C, et al. CircHIPK3 promotes proliferation and invasion in nasopharyngeal carcinoma by abrogating miR-4288-induced ELF3 inhibition. J Cell Physiol 2019;234:1699-706.
21. Yin L, Chen J, Ma C, et al. Hsa_circ_0046263 functions as a ceRNA to promote nasopharyngeal carcinoma progression by upregulating IGFBP3. Cell Death Dis 2020;11:562.
22. Tang L, Xiong W, Zhang L, et al. circSETD3 regulates MAPRE1 through miR-615-5p and miR-1538 sponges to promote migration and invasion in nasopharyngeal carcinoma. Oncogene 2021;40:307-21.
23. Wang Y, Yan Q, Mo Y, et al. Splicing factor derived circular RNA circCAMSAP1 accelerates nasopharyngeal carcinoma tumorigenesis via a SERPINH1/c-Myc positive feedback loop. Mol Cancer 2022;21:62.
24. Cao Z, Xiao Q, Dai X, et al. circHIPK2-mediated σ-1R promotes endoplasmic reticulum stress in human pulmonary fibroblasts exposed to silica. Cell Death Dis 2017;8:3212.
25. Huang R, Zhang Y, Han B, et al. Circular RNA HIPK2 regulates astrocyte activation via cooperation of autophagy and ER stress by targeting MIR124-2HG. Autophagy 2017;13:1722-41.
26. Zhang Y, Huang R, Cheng M, et al. Gut microbiota from NLRP3-deficient mice ameliorates depressive-like behaviors by regulating astrocyte dysfunction via circHIPK2. Microbiome 2019;7:116.
27. Wang G, Han B, Shen L, et al. Silencing of circular RNA HIPK2 in neural stem cells enhances functional recovery following ischaemic stroke. EBioMedicine 2020;52:102660.
28. Di Segni M, Virdia I, Verdina A, et al. HIPK2 Cooperates with KRAS Signaling and Associates with Colorectal Cancer Progression. Mol Cancer Res 2022;20:686-98.
29. Sardina F, Monteonofrio L, Ferrara M, et al. HIPK2 Is Required for Midbody Remnant Removal Through Autophagy-Mediated Degradation. Front Cell Dev Biol 2020;8:572094.
30. Jin X, Qing S, Li Q, et al. Prostate cancer-associated SPOP mutations lead to genomic instability through disruption of the SPOP-HIPK2 axis. Nucleic Acids Res 2021;49:6788-803.
31. Chen P, Duan X, Li X, et al. HIPK2 suppresses tumor growth and progression of hepatocellular carcinoma through promoting the degradation of HIF-1α. Oncogene

2020;39:2863-76.
32. Liebl MC, Moehlenbrink J, Becker H, et al. DAZAP2 acts as specifier of the p53 response to DNA damage. Nucleic Acids Res 2021;49:2759-76.

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