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Downregulation of lncRNA CCHE1 inhibits cell proliferation, migration and invasion by suppressing MEK/ERK/c-MYC pathway in nasopharyngeal carcinoma

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Abstract. – **OBJECTIVE:** The current study was designed to investigate the functionality of lncRNA CCHE1 in nasopharyngeal carcinoma.

MATERIALS AND METHODS: MiRNA levels of lncRNA CCHE1 were examined by RT-qPCR. CCK8 assay and colony formation assay were together performed to detect cell proliferation viability. Furthermore, wound healing assay and transwell assay were respectively conducted to assess cell migration and invasion. In addition, proteins related to MEK/ERK/c-MYC pathway were detected by Western blot.

RESULTS: Elevated levels of CCHE1 were verified in NPC cell lines. Downregulation of CCHE1 significantly inhibited tumor growth and suppressed A549 cell proliferation, migration and invasion. MEK/ERK/c-MYC pathway was activated in nasopharyngeal carcinoma. Treatment of PD98059 (MEK inhibitor) or SCH772984 (ERK inhibitor) reversed the effects of CCHE1 on cell proliferation, migration and invasion in NPC.

CONCLUSIONS: The present study suggested that downregulation of lncRNA CCHE1 could inhibit cell proliferation, migration and invasion by suppressing MEK/ERK/c-MYC pathway in nasopharyngeal carcinoma.

Key Words:
LncRNA CCHE1, Proliferation, Migration, Invasion, MEK/ERK/c-MYC pathway, Nasopharyngeal carcinoma.

Introduction

Nasopharyngeal carcinoma (NPC) is a malignant tumor derived from nasopharyngeal mucosa, with uneven worldwide distribution and high prevalence in Southern China and Southeast Asia[1]. According to statistics, approximately 86,500 new cases and more than 40,000 deaths of NPC arise every year in Asia alone[2]. Due to the anatomical limitations of NPC, radiotherapy combined with chemotherapy is the main therapy for NPC at present[3]. However, these therapeutics have higher recurrence rate and more complications[4]. Thus, it is urgent to exploit better therapeutic strategies for NPC.

Accompanied by further study in NPC pathogenesis and molecular biology, the inhibitors targeting the key opponents in deregulated signal pathway is one of the highlights in new drug development[5,6]. The mitogen-activated extracellular signal-regulated kinase (MEK) is a dual specificity kinase. It can activate extracellular signal-regulated kinases (ERK) by phosphorylating the threonine (Thr) and tyrosine (Tyr) residues[7]. MEK/ERK signal pathway plays an important role in the regulation of tumor biological behaviors such as proliferation, survival and migration. The hyperactivation of this signal pathway is commonly found in solid tumors as well as hematopoietic malignancies[8]. Wu et al[9] reported that MEK1 was markedly elevated in NPC tissue. Downregulation of MEK1 and p-ERK1/2 could attenuate NPC cell proliferation and enhance cell apoptosis. C-MYC is known as the substrate of p-ERK1/2. Chen et al[10] reported that enhancing the ERK/c-MYC pathway could downregulate p21 and promote tumor progression. Therefore, we considered MEK/ERK/c-MYC pathway as a significant direction for anti-NPC drug research.

Long non-coding RNAs (lncRNAs) are confirmed to be involved in the regulation of gene expression and genetic modification at transcription and post-transcription[11]. Some lncRNAs play important roles in the pathological process of various tumors including NPC[12,13]. LncRNA CCHE1 is a new transcriptional regulator in the

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process of some cancers. Gaballah et al[14] studied that lncRNA CCHE1 could affect development and progression of colorectal cancer by modulating ERK/COX-2 pathway. Meanwhile, CCHE1 knockdown could inhibit the activation of ERK/MAPK pathway and promote hepatocellular carcinoma cell apoptosis[15]. Based on these studies above, we suggest that lncRNA CCHE1 may play an important role in the pathogenesis of NPC by regulating MEK/ERK/c-MYC pathway.

In the present study, we intended to investigate the role of lncRNA CCHE1 in NPC cell lines and demonstrated whether lncRNA CCHE1 could regulate NPC cell proliferation and metastasis through MEK/ERK/c-MYC pathway.

Materials and Methods

Cell Culture and Transfection

NPC cell lines (6-10B, 5-8F, C666-1 and SUNE-1) and nasopharyngeal epithelial cell line (NP69) were obtained from Southern Medical University (Guangzhou, China) and cultured in Roswell Park Memorial Institute-1640 (RPMI-1640, Sigma-Aldrich, St. Louis, MO, USA) supplemented with 10% fetal bovine serum (FBS, Sigma-Aldrich, St. Louis, MO, USA), 100 Ul/ml penicillin and 100 μg/ml streptomycin in a standard atmosphere with 5% CO_2 and 95% humidity at 37°C.

Cells were transfected with shRNA-CCHE1-1, shRNA-CCHE1-2 and pcDNA-CCHE1 (GeneChem, Shanghai, CN) using Lipofectamine2000 transfection reagent (Invitrogen, Carlsbad, CA, USA) in accordance with the manufacturer's instructions. Transfection efficiency was detected by RT-qPCR. Following incubation for 48 h, cells were used for further analysis.

RNA Extraction and Reverse Transcription (RT)-qPCR

Total RNA was isolated using TRIzol reagents (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) according to the manufacturer's instructions. RNA was converted into cDNA using a reverse transcription kit (TaKaRa, Otsu, Shiga, Japan). Quantify analysis with real-time PCR assay was performed to detect the expression level of CCHE1 using SYBR-Green Supermix (Invitrogen Life Technologies) on ABI PRISM 7000 Sequence Detection System (ABI/Perkin Elmer, Foster City, CA, USA). Data were quantified using the classic $2^{-\Delta\Delta Ct}$ method.

Measurement of Cell Viability

Cell Counting Kit-8 (CCK-8) assay (Sigma-Aldrich, St. Louis, MO, USA) was used to measure cell proliferation. Cells were seeded onto 96-well plates at a density of 1×10^4 cells per well and incubated in appropriate medium at 37°C for 24 h. Then, CCK-8 solution (10 ul/well) was added into each well after indicated treatments. After incubation for 4 h at 37°C, absorbance (Optical Density, OD) at 450 nm was determined on an enzyme immunoassay analyzer (Bio-Rad, Hercules, CA, USA).

Colony Formation Assay

Colony-formation assays were performed to evaluate cell proliferation ability. Cell suspensions at a density of 5×10^3 cells were seeded into 6-well plates and maintained in RPMI-1640 medium to analyze the effects of transfections on cell proliferation. Cells were fixed in 20% methanol for 15 min and stained with 0.5% crystal violet for 15 min at room temperature. Cell colonies representing cell proliferation ability were counted under a microscope.

Wound Healing Assay

The transfected cell lines were seeded into 6-well plates and cultured at 37°C in a humidified chamber with 5% CO_2. When the cells grew to 80% confluence, a sterile 10 μL pipette tip was used to scrape a linear wound. The cell debris was washed with phosphate-buffered saline (PBS). The wound images were observed and recorded to assess the migration distance in the same position at 0, 24 h using a light microscope (DM4000B; Leica Microsystems GmbH, Wetzlar, Germany).

Transwell Assay

Cells after 24 h post-transfection were harvested and counted. Cell suspensions at a density of 5×10^3 cells were applied to analyze the effects of transfections on cell invasion. Transwell chambers (Corning, Corning, NY, USA) was used to carry out the transwell assay. The upper chamber was placed with transfected cell lines cultured with FBS free medium and the lower chamber was filled with RPMI-1640 medium containing 10% FBS. After incubation for 24 h at 37°C, 5% CO_2 and 95% humidity, the invaded cells were fixed with 4% paraformaldehyde and then stained with 0.5% crystal violet (Solarbio, Beijing, CHN) for 25 min. Evaluation of invasive capacity was performed by counting invading cells under an inverted microscope (Leica, Wetzlar, Germany).

Western Blot Analysis

Total protein was extracted from cells lysed using radio-immunoprecipitation buffer (Thermo Fisher Scientific, Waltham, MA, USA) containing protease and phosphatase inhibitors. Protein concentrations were quantitated by bicinchoninic acid (BCA) method in strict accordance with the instructions of kits. Protein samples (30 μg per lane) were, then, separated on 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene difluoride (PVDF) membranes. Then, PVDF membranes were blocked with 5% skimmed milk or bovine serum albumin (BSA) solution for 1 h at room temperature. PVDF membranes were probed with primary antibody: MMP2 (Abcam, ab97779, 1:3000), MMP9 (Abcam, ab219372, 1:10000), p-MEK (Abcam, ab96379, 1:5000), MEK (Abcam, ab32091, 1:5000), p-ERK (Abcam, ab131438, 1:1000), ERK (Abcam, ab184699, 1:10000), c-MYC (Abcam, ab32072, 1:1000), cyclinD1 (Abcam, ab226977, 1:5000) and p21 (Abcam, ab227443, 1:3000) overnight at 4°C and incubated with horseradish peroxidase (HRP)-conducted secondary antibody (Abcam, ab205718, 1:20000) for 1 h at room temperature. Band signals were developed and visualized by enhanced chemiluminescence (ECL; Sigma-Aldrich, St. Louis, MO, USA). GAPDH (Abcam, ab181602, 1:10000) severed as the endogenous control.

Statistical Analysis

Statistical analysis was performed using the SPSS 16.0 statistical package (SPSS Inc., Chicago, IL, USA). Data from repeated three experiments are presented as mean ± standard deviation (SD). Difference among multiple groups was compared using one-way analysis of variance followed by Scheffè post-hoc test. $p < 0.05$ was considered as statistically significant difference.

Results

CCHE1 Expression Levels Are Upregulated in NPC Cell Lines

The expression levels of CCHE1 in NPC cell lines (6-10B, 5-8F, C666-1, SUNE-1) and normal nasopharyngeal epithelial cell line (NP69) were measured by performing qPCR. CCHE1 expression levels in all NPC cell lines were higher than it in NP69 cells, representing the highest level in C666-1 cells (Figure 1).

CCHE1 Affects the Proliferation of NPC Cells

To examine the role of CCHE1 in the growth of NPC cells, C666-1 cells were transfected with shRNA-NC, shRNA-CCHE1, pcDNA-NC or pcDNA-CCHE1. CCHE1 expression in C666-1 cells was markedly downregulated following transfection with shRNA-CCHE1 and upregulated by transfecting with pcDNA-CCHE1 (Figure 2A, 2B). Furthermore, the effect of CCHE1 on the proliferative capacity of NPC cells was checked by CCK-8 and colony formation assay. CCHE1 suppression significantly inhibited the proliferation of NPC cells, while upregulation of CCHE1 promoted NPC cells growth (Figure 2C, 2D).

CCHE1 Affects the Migration and Invasion of NPC Cells

The effects of CCHE1 on the migration and invasion of C666-1 cells were respectively analyzed by wound healing and transwell assay. The migratory and invasive ability of NPC cells was significantly inhibited following transfection with shRNA-CCHE1 and upregulated by transfecting with pcDNA-CCHE1 (Figure 3A-3D). The levels of MMP2 and MMP9 proteins were also examined to evaluate the migration and invasion of NPC cells. The results revealed that MMP2 and MMP9 levels were significantly decreased in NPC cells transfected with shRNA-CCHE1, while upregulation of CCHE1 reversed this result (Figure 3E).

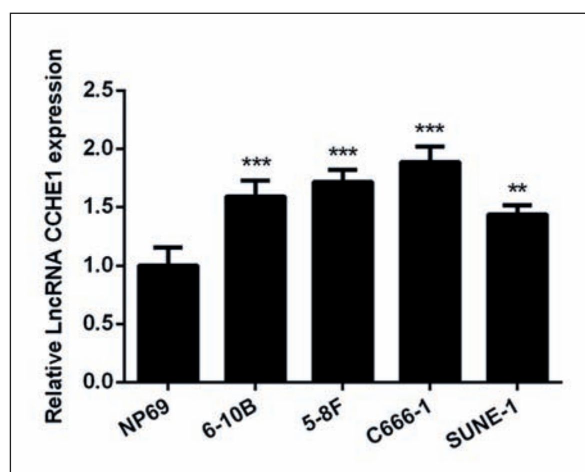

Figure 1. CCHE1 expression levels are upregulated in NPC cell lines. CCHE1 expression levels in NPC and normal nasopharyngeal epithelial cell line were measured by RT-qPCR. $^{**}p<0.01$, $^{***}p<0.001$ *vs.* NP69 group.

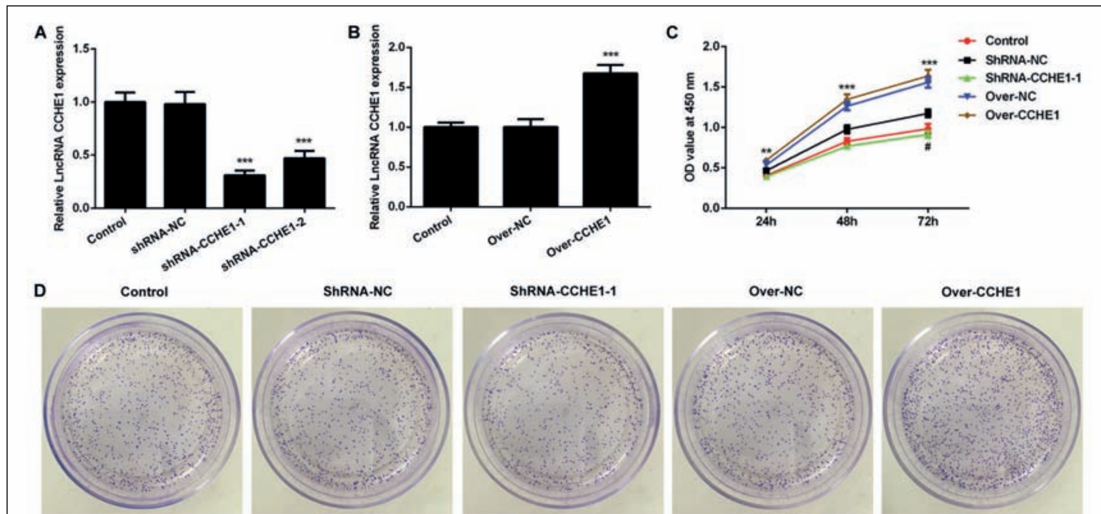

Figure 2. CCHE1 affects the proliferation of NPC cells. **A-B,** CCHE1 expression in C666-1 cells was measured by RT-qPCR. **C,** The proliferative capacity of C666-1 cells was measured by CCK8 assay. **D,** The colony formation efficiency of C666-1 cells was determined using colony formation assay. $^{**}p<0.01$, $^{***}p<0.001$ *vs.* Control group.

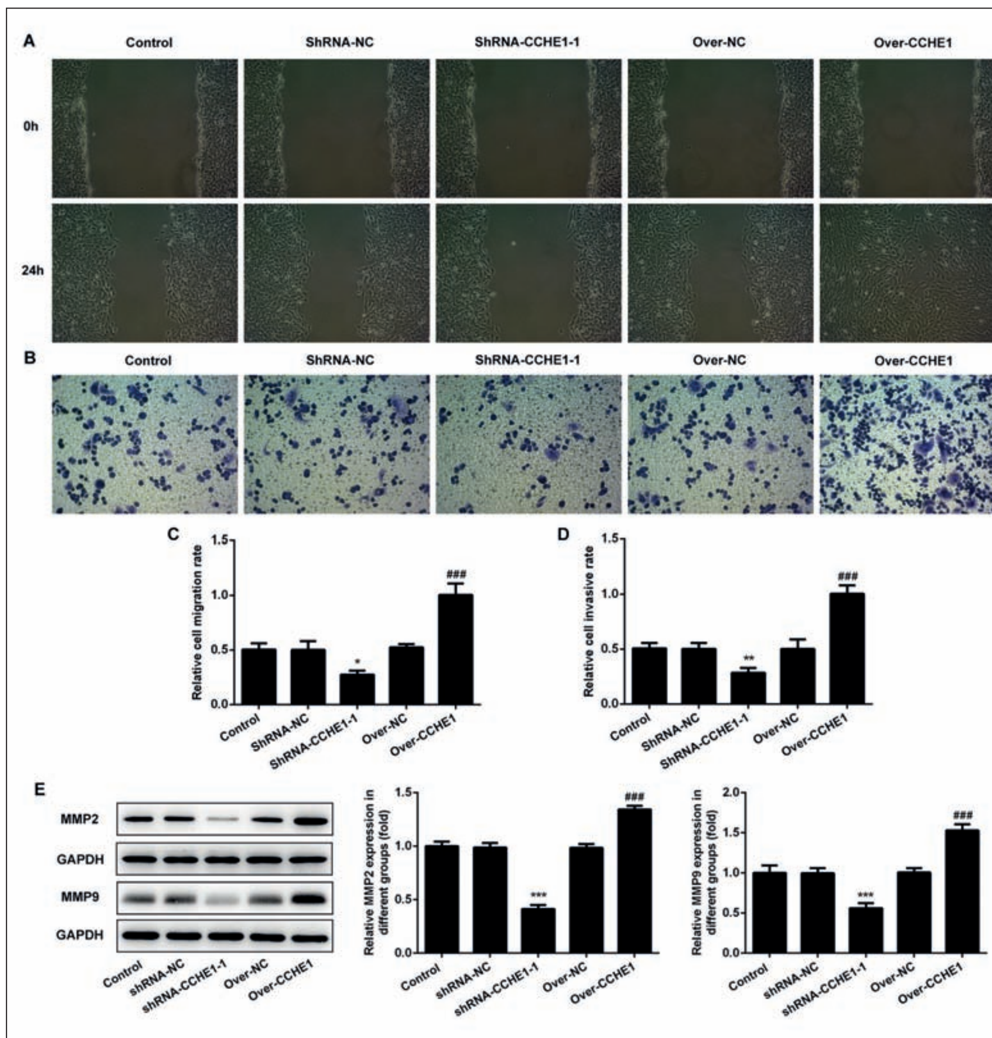

Figure 3. CCHE1 affects the migration and invasion of NPC cells. **A-C,** The migration of C666-1 cells was measured by wound healing assay. **B-D,** The invasion of C666-1 cells was measured by transwell assay (100×). **E,** The protein levels of MMP2 and MMP9 were detected by Western blotting. $^{*}p<0.05$, $^{***}p<0.001$, $^{\#\#\#}p<0.001$ *vs.* Control group.

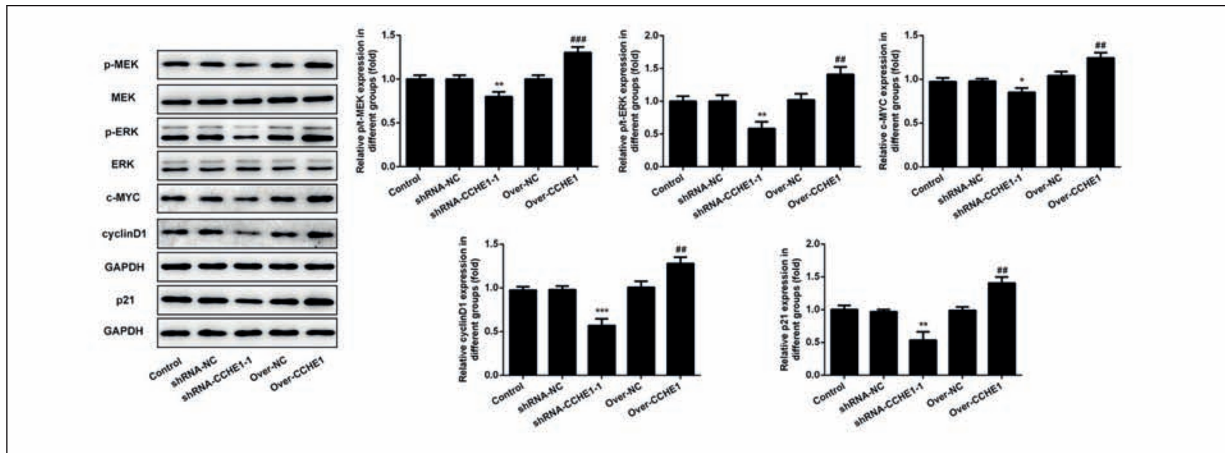

Figure 4. CCHE1 affects the MEK/ERK/c-Myc pathway in NPC cells. The protein levels of MEK, ERK, p-MEK, p-ERK, c-Myc, cyclinD1 and p21 were detected by Western blotting. *$p<0.05$, **$p<0.01$, ***$p<0.001$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ *vs*. Control group.

CCHE1 Affects the MEK/ERK/c-Myc Pathway in NPC Cells

To evaluate whether MEK/ERK/c-Myc pathway was affected by CCHE1 downregulation or upregulation, the protein levels of MEK, ERK, p-MEK, p-ERK, c-Myc, cyclinD1 and p21 were examined by Western blotting. The protein levels of p-MEK, p-ERK, c-Myc, cyclinD1 and p21 in C666-1 cells transfected with shRNA-CCHE1 were lower than those in the control group. Meanwhile, upregulation of CCHE1 leads to increased expression levels of p-MEK, p-ERK, c-Myc, cyclinD1 and p21 in NPC cells (Figure 4).

MEK or ERK Inhibitor Affects the Influence of CCHE1 on MEK/ERK/c-Myc Pathway

To evaluate the role of MEK/ERK/c-Myc pathway on NPC cells, we comparatively analyzed MEK, ERK, p-MEK, p-ERK, c-Myc, cyclinD1 and p21 protein expression levels by Western blotting. Treatment of PD98059 (MEK inhibitor) or SCH772984 (ERK inhibitor) for 1 h evidently suppressed p-MEK, p-ERK, c-Myc, cyclinD1 and p21 protein expression in NPC cells following shRNA-CCHE1 or pcDNA-CCHE1 transfection (Figure 5).

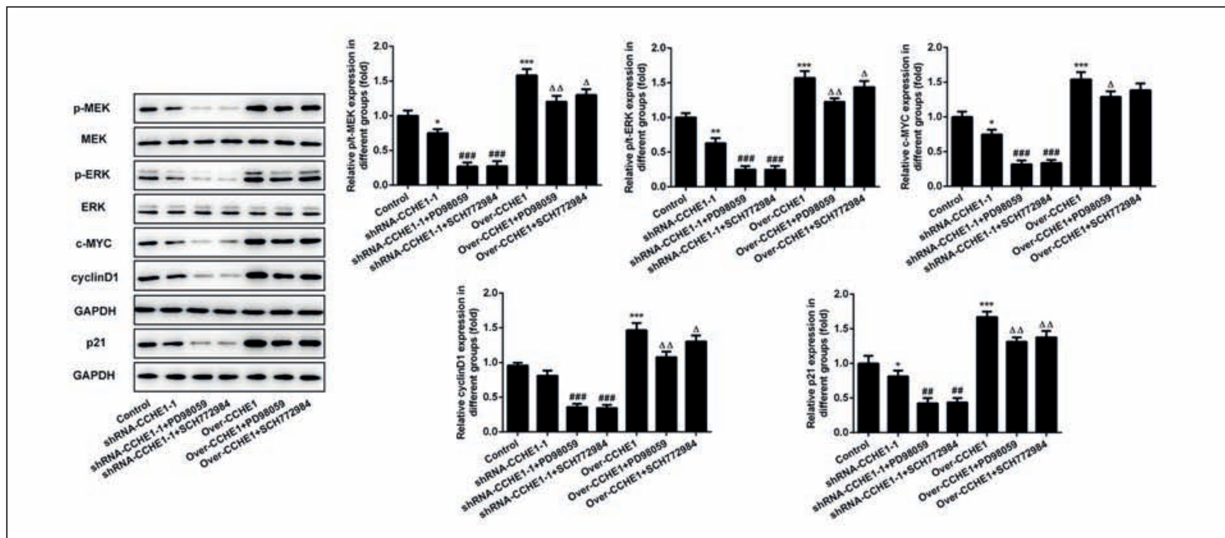

Figure 5. CCHE1 affects the migration and invasion of NPC cells. The protein levels of MEK, ERK, p-MEK, p-ERK, c-Myc, cyclinD1 and p21 were detected by Western blotting. *$p<0.05$, **$p<0.01$, ***$p<0.001$ *vs*. Control group, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ *vs*. shRNA-CCHE1-1 group, $^{\Delta}p<0.05$, $^{\Delta\Delta}p<0.01$ *vs*. Over-CCHE1 group.

CCHE1 Affects the Proliferation of NPC Cells through MEK/ERK/c-Myc Pathway

The effect of MEK/ERK/c-Myc pathway on the proliferative capacity of NPC cells was checked by CCK-8 and colony formation assay. The proliferative capacity and colony formation efficiency of transfected NPC cells were all inhibited after treatment of PD98059 or SCH772984 for 1 h (Figure 6A, 6B).

CCHE1 Affects the Migration and Invasion of NPC Cells through MEK/ERK/c-Myc Pathway

To further identify the role of MEK/ERK/c-Myc pathway on the migratory and invasive ability of NPC cells, wound healing and transwell assay were performed. PD98059 or SCH772984 treatment significantly inhibited the migration and invasion of transfected NPC cells (Figure 7A-7D). Furthermore, the protein levels of MMP2 and MMP9 were also downregulated through PD98059 or SCH772984 treatment in transfected NPC cells (Figure 7E).

Discussion

NPC is one of the most common malignancies of head and neck, which is caused by EBV (Epstein-Barr virus) infection, genetic and environmental factors[16,17]. It is characterized by high malignancy, prone to recurrence and metastasis, leading to extreme difficulty in the therapy of nasopharyngeal cancer[18]. Despite the diagnosis and treatment of NPC have been improved in recent years, the mortality still remains high[19].

With the development of molecular biology, many new molecular markers have been discov-

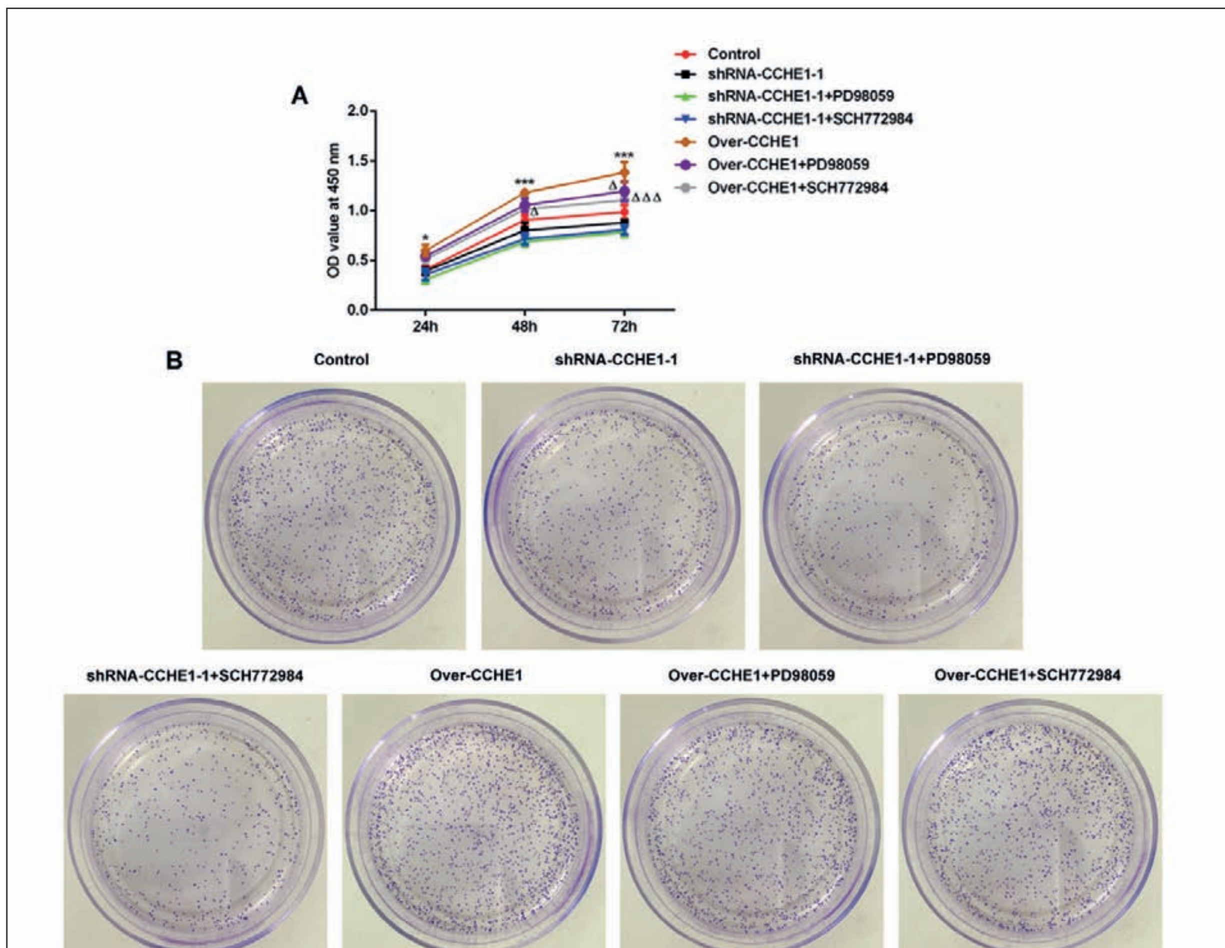

Figure 6. CCHE1 affects the proliferation of NPC cells through MEK/ERK/c-Myc pathway. **A,** The proliferative capacity of C666-1 cells was measured by CCK8 assay. **B,** The colony formation efficiency of C666-1 cells was determined using colony formation assay. $^{*}p<0.05$, $^{***}p<0.001$ *vs.* Control group, $^{\Delta}p<0.05$, $^{\Delta\Delta\Delta}p<0.001$ *vs.* Over-CCHE1 group.

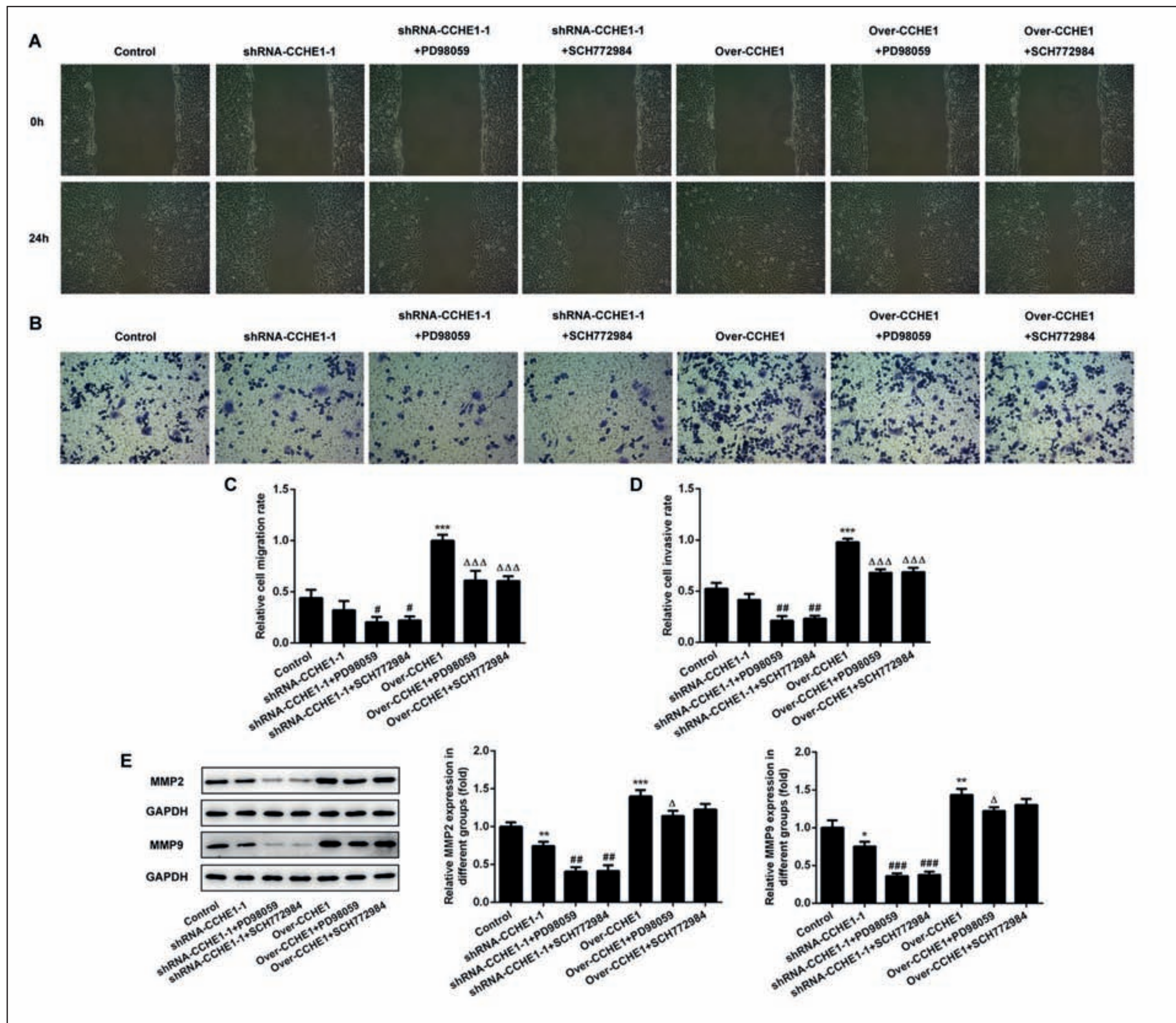

Figure 7. CCHE1 affects the migration and invasion of NPC cells through MEK/ERK/c-Myc pathway. **A-C,** The migration of C666-1 cells was measured by wound healing assay. **B-D,** The invasion of C666-1 cells was determined using transwell assay (100X). **E,** The protein levels of MMP2 and MMP9 were measured by Western blotting. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ *vs.* Control group, $^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ *vs.* shRNA-CCHE1-1 group, $^{\Delta}p<0.05$, $^{\Delta\Delta\Delta}p<0.001$ *vs.* Over-CCHE1 group.

ered, bringing new hope to the prevention, diagnosis, treatment and prognosis of NPC[20]. Some lncRNAs were closely related to the occurrence and development of NPC. Hu et al[12] reported that silencing lncRNA NCK1-AS1 could inhibit NPC cell migration and invasion by interacting with miR-135a. LncRNA CCHE1 played a certain regulatory role in the pathogenesis and development of some cancers[21,22], while the role of CCHE1 in NPC has not been clarified so far. Matrix metalloproteinase (MMP)-2 and matrix metalloproteinase (MMP)-9 belong to the gelatinases in MMP, which are closely related to the invasion and metastasis of cancer cells[23]. Following activation, MMP2 and MMP-9 can develop into type IV collagenase, then degrade extracellular matrix and destroy the complete basement membrane. Subsequently, cancer cells could easily infiltrate the surrounding tissue, invade blood vessels and lymphatic vessels[24]. The current study exerted elevated level of CCHE1 in NPC cell lines and confirmed the significantly positive correlation among CCHE1 levels and proliferation, migration and invasion of NPC.

MEK/ERK pathway played a critical role in the regulation of diverse cellular processes, such as cell proliferation, differentiation, motility and survival, etc.[8]. MEK/ERK are now considered as

significant targets for anticancer drug. Several MEK and ERK inhibitors had exerted promising therapeutic effects in different types of solid tumors[25,26]. Meanwhile, studies had proved that lncRNA CCHE1 could regulate ERK pathway in colorectal cancer and hepatocellular carcinoma[14,15]. In this study, our results suggested that downregulation of CCHE1 could inhibit the activation of MEK/ERK/c-Myc pathway. Besides, we proved that the specific effects and underlying mechanism of CCHE1 on NPC growth and metastasis were distinctly related to MEK/ERK/c-Myc pathway.

Conclusions

Our research was the first to identify that lncRNA CCHE1 was upregulated in NPC cell lines. Also, we indicated that downregulation of CCHE1 could inhibit the growth and metastasis of NPC cells through inhibiting MEK/ERK/c-Myc pathway. The molecular mechanism is researched to provide reference for exploiting novel ideas for clinical diagnosis and treatment of NPC. These results suggest that lncRNA CCHE1 maybe serve as a new therapeutic target in NPC.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Authors' Contribution

All authors made substantial contributions to the design of the study, performed the experiments and analyzed the data. All authors read and approved the final version of the manuscript.

References

1) Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018; 68: 394-424.
2) Mahdavifar N, Ghoncheh M, Mohammadian-Hafshejani A, Khosravi B, Salehiniya H. Epidemiology and Inequality in the incidence and mortality of nasopharynx cancer in Asia. Osong Public Health Res Perspect 2016; 7: 360-372.
3) Gou XX, Jin F, Wu WL, Long JH, Li YY, Gong XY, Chen GY, Chen XX, Liu LN. Induction chronomodulated chemotherapy plus radiotherapy for nasopharyngeal carcinoma: a Phase II prospective randomized study. J Cancer Res Ther 2018; 14: 1613-1619.
4) Fangzheng W, Chuner J, Haiyan Q, Quanquan S, Zhimin Y, Tongxin L, Jiping L, Peng W, Kaiyuan S, Zhenfu F, Yangming J. Survival without concurrent chemotherapy for locoregionally advanced nasopharyngeal carcinoma treated with induction chemotherapy plus intensity-modulated radiotherapy: single-center experience from an endemic area. Medicine (Baltimore) 2019; 98: e18484.
5) Lyu X, Wang J, Guo X, Wu G, Jiao Y, Faleti OD, Liu P, Liu T, Long Y, Chong T, Yang X, Huang J, He M, Tsang CM, Tsao SW, Wang Q, Jiang Q, Li X. EBV-miR-BART1-5P activates AMPK/mTOR/HIF1 pathway via a PTEN independent manner to promote glycolysis and angiogenesis in nasopharyngeal carcinoma. PLoS Pathog 2018; 14: e1007484.
v6) Zhang W, Guo Q, Liu G, Zheng F, Chen J, Huang D, Ding L, Yang X, Song E, Xiang Y, Yao H. NKILA represses nasopharyngeal carcinoma carcinogenesis and metastasis by NF-κB pathway inhibition. PLoS Genet 2019; 15: e1008325.
7) Iwamoto N, D'Alessandro LA, Depner S, Hahn B, Kramer BA, Lucarelli P, Vlasov A, Stepath M, Böhm ME, Deharde D, Damm G, Seehofer D, Lehmann WD, Klingmüller U, Schilling M. Context-specific flow through the MEK/ERK module produces cell- and ligand-specific patterns of ERK single and double phosphorylation. Sci Signal 2016; 9: ra13.
8) Tsubaki M, Itoh T, Satou T, Imano M, Komai M, Ogawa N, Mukai J, Nishida S. Nitrogen-containing bisphosphonates induce apoptosis of hematopoietic tumor cells via inhibition of Ras signaling pathways and Bim-mediated activation of the intrinsic apoptotic pathway. Biochem Pharmacol 2013; 85: 163-172.
9) Wu RS, Qiu EH, Zhu JJ, Wang JR, Lin HL. MiR-101 promotes nasopharyngeal carcinoma cell apoptosis through inhibiting Ras/Raf/MEK/ERK signaling pathway. Eur Rev Med Pharmacol Sci 2018; 22: 150-157.
10) Chen Y, Lin C, Liu Y, Jiang Y. HMGB1 promotes HCC progression partly by downregulating p21 via ERK/c-Myc pathway and upregulating MMP-2. Tumour Biol 2016; 37: 4399-4408.
11) Xuan W, Yu H, Zhang X, Song D. Crosstalk between the lncRNA UCA1 and microRNAs in cancer. FEBS Lett 2019; 593: 1901-1914.
12) Hu H, Li H, Feng X. Downregulation of lncRNA NCK1-AS1 inhibits cancer cell migration and invasion in nasopharyngeal carcinoma by upregulating miR-135a. Cancer Manag Res 2019; 11: 10531-10537.
13) Peng J, Liu F, Zheng H, Wu Q, Liu S. Long noncoding RNA ZFAS1 promotes tumorigenesis and metastasis in nasopharyngeal carcinoma by sponging miR-892b to up-regulate LPAR1 expression. J Cell Mol Med 2020; 24: 1437-1450.
14) Gaballah HH, Gaber RA, Elrashidy MA, Elshahat DA, Hablus MA, Ebeid AM. Expression of long non-coding RNA CCHE1 in colorectal carcinoma: correla-

tions with clinicopathological features and ERK/COX-2 pathway. Mol Biol Rep 2019; 46: 657-667.

15) Peng W, Fan H. Long noncoding RNA CCHE1 indicates a poor prognosis of hepatocellular carcinoma and promotes carcinogenesis via activation of the ERK/MAPK pathway. Biomed Pharmacother 2016; 83: 450-455.
16) Wu L, Li C, Pan L. Nasopharyngeal carcinoma: a review of current updates. Exp Ther Med 2018; 15: 3687-3692.
17) Roy Chattopadhyay N, Chakrabarti S, Chatterjee K, Deb Roy S, Kumar Sahu S, Reddy RR, Das P, Bijay Kanrar B, Kumar Das A, Tsering S, Puii Z, Zomawia E, Singh YI, Suryawanshi A, Choudhuri T. Histocompatibility locus antigens regions contribute to the ethnicity bias of Epstein-Barr virus-associated nasopharyngeal carcinoma in higher-incidence populations. Scand J Immunol 2019; 90: e12796.
18) Stoker SD, van Diessen JN, de Boer JP, Karakullukcu B, Leemans CR, Tan IB. Current treatment options for local residual nasopharyngeal carcinoma. Curr Treat Options Oncol 2013; 14: 475-491.
19) García-Lorenzo J, Farre N, Codina A, Gallego O, De Vega M, León X. Nasopharyngeal carcinoma: 30-year experience of a single institution in a non-endemic area. Clin Transl Oncol 2017; 19: 777-783.
20) Jiang H, Li J, Zhang B, Huang R, Zhang J, Chen Z, Shang X, Li X, Nie X. Intestinal flora disruption and novel biomarkers associated with nasopharyngeal carcinoma. Front Oncol 2019; 9: 1346.
21) Wang Y, Tong J, Lin H, Ma L, Xu Y. CCHE1 accelerated the initiation of oral squamous cell carcinoma through enhancing PAK2 expression by sponging miR-922. J Oral Pathol Med. 2020 Jan 24. doi: 10.1111/jop.12995. Epub ahead of print.
22) Jin X, Ye L, Lin M, Gu B, Wang J, He Y, Li W. lncRNA-CCHE1 is involved in migration and invasion but not in proliferation of pancreatic adenocarcinoma cells possibly by interacting with ROCK1. Oncol Lett 2019; 18: 1218-1224.
23) Luo S, Huang G, Wang Z, Wan Z, Chen H, Liao D, Chen C, Li H, Li B, Chen L, Huang Z, He Z. Niflumic acid exhibits anti-tumor activity in nasopharyngeal carcinoma cells through affecting the expression of ERK1/2 and the activity of MMP2 and MMP9. Int J Clin Exp Pathol 2015; 8: 9990-10001.
24) Basilico N, Magnetto C, D'Alessandro S, Panariti A, Rivolta I, Genova T, Khadjavi A, Gulino GR, Argenziano M, Soster M, Cavalli R, Giribaldi G, Guiot C, Prato M. Dextran-shelled oxygen-loaded nanodroplets reestablish a normoxia-like pro-angiogenic phenotype and behavior in hypoxic human dermal microvascular endothelium. Toxicol Appl Pharmacol 2015; 288: 330-338.
25) Iezzi A, Caiola E, Scagliotti A, Broggini M. Generation and characterization of MEK and ERK inhibitors- resistant non-small-cells-lung-cancer (NSCLC) cells. BMC Cancer 2018; 18: 1028.
26) Li Y, Dong Q, Mei T, Zheng M, Kumar RR, Yu B, Wu C, Zhang H, An F. Nanosized modification strategies for improving the antitumor efficacy of MEK inhibitors. Curr Drug Targets 2020; 21: 228-251.