CHN1 Promotes EMT via the Akt / GSK-3 β / Snail Pathway in Cervical Carcinoma

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Abstract

Background: Metastasis and invasion are key points to lead the mortality of cervical squamous cell carcinoma (CSCC). Epithelial–mesenchymal transition (EMT) is now a universal explanation for the mechanisms of tumor metastasis. A-chimeric protein (α -chimaerin, CHN1) plays an important role in the regulation of signal transduction and development. However, the molecular regulatory relationship between CHN1 and CSCC progression involved EMT has not been identified.

Methods: The expression of CHN1 in CSCC tissues, adjacent tissues, and lymph node metastases of CSCC patients was detected by immunohistochemistry assay. Upregulation and knockdown of CHN1 was achieved by transfecting the plasmid into CSCC cells. The effect of CHN1 on proliferation was determined by CCK-8 and plate clone formation assay. Changes in migration and invasion capabilities were evaluated by scratch migration assay and trans-well invasion assay. The effect of CHN1 overexpression and interference on xenograft tumor growth was determined by measuring tumor weight and pathological analysis. The expression of EMT-related mRNA was measured by qRT-PCR assay in transfected CSCC cells. EMT-related proteins and Akt / GSK-3 β / Snail signaling pathway-related proteins were also evaluated by using western blotting assay.

Results: CHN1 was overexpression in CSCC tissues and was associated with lymph node metastasis and low survival of CSCC patients. The overexpression CHN1 promoted cell proliferation, migration and invasion bility in CSCC cells. On the contrary, silencing CHN1 inhibited these phenomena. In vivo experiments, the overexpression of CHN1 promoted tumors formed in xenograft tumor mouse model, with increased volume and weight of xenograft tumor. In addition, CHN1 induced the expression of EMT related transcription factors, accompanied by the decreased expression of epithelial markers and the increased expression of mesenchymal markers. The Akt / GSK-3 β / Snail signaling pathway was activated by the overexpression of CHN1 in vitro, and the activation of the pathway was inhibited by signaling pathway inhibitor LY294002.

Conclusion: These results suggest that CHN1 promoted development and progression in cervical carcinoma via of the Akt / GSK-3 β / Snail pathway by inducing EMT.

Background

Cervical carcinoma is one of the main malignancy threatening the health and life of women worldwide [1]. With the development of early diagnosis and treatment in cervical carcinoma, the incidence of cervical carcinoma in developed countries has declined significantly [2], but the incidence and mortality of cervical carcinoma among young women in developing countries remain high [1, 3].

The metastatic spread of cancer cells from primary site to distant area, such as lymph nodes around the vessels of pelvic wall, is the major cause of unsuccessful treatment and ultimate death in cervical cancer [4, 5]. Accumulated evidence demonstrated that the epithelial-mesenchymal transition (EMT) is responsible for the invasion and metastasis of various carcinomas and had been related to elevated

resistance of chemotherapy and immunotherapy [6–8]. Despite enormous efforts verified that EMT reprogramming of tumor epithelial cells depends on the systematical occurrence at multiple regulation levels [6, 9], the exact underlying mechanisms connect EMT, metastasis and cervical carcinoma remain to dig for a better understanding of cancer progression and exploring the related therapeutic methods.

Chimaerin (CHN1) is one kind of ras-related Rho GTPase-activating protein (RhoGAPs) involves in cytoskeletal regulation [10–12]. Two chimaerin isoforms, α and β , exist in mammalian genomes and each of them consists of at least two splice variants: a full-length type 1 transcript and a truncated type 2 transcript. Although a cysteine-rich domain followed by the GAP domain is shared by the two transcripts, a SH2-domain encompassed N-terminal is characterized in type 2 isoforms [13–15]. CHN1 is widely investigated in neurobiology, and it is pivotal in neuronal signal-transduction, brain development, synaptogenesis and cognitive ability [12, 16–19]. Besides, it also involved in the regulation of T cell adhesion and chemotaxis [20], transmission of signals in tumor progression [21], maintenance of epithelial morphology [22], development of oculomotor projection [23], and regulation of Duane retraction syndrome [19, 24, 25]. However, the role of CHN1 in tumorigenesis has not been elucidated clearly, especially in cervical carcinoma.

The aim of this study is to investigate the relationship between differential expressed CHN1 and the progression and metastasis of cervical carcinoma, and meanwhile reveal the potential mechanism of how CHN1 contribute to the related effect. In the present study, we prove that CHN1 is highly expressed in cervical carcinoma with a correlation of long-term prognosis. CHN1 can accelerate the occurrence of tumor biological behaviors, and it may stimulate the activation of EMT through Akt / GSK-3 β / Snail pathway to promote the metastasis of cervical carcinoma.

Methods

Tissue samples and patients

Two paraffin-embedded tissue microarrays (TMA), one consisted of 62 paired SCC/adjacent non-carcinoma samples and another contained 84 CSCC and 8 lymph nodes from metastatic cervical carcinoma samples, were obtained from Shanghai Outdo Biotech (Shanghai, China) and Alena Bio (Xi'an, China), respectively. The other paraffin embedded CSCC samples were histopathologically diagnosed at PLA 251 Hospital. These patients did not undergo radiation and chemotherapy before the surgery and samples were stored at -80 °C.

Cell lines, cell culture and gene transfection

The CSCC cell line SiHa was preserved by our lab. The cells were cultured in DMEM/F12 medium (Thermo Fisher Scientific, USA) supplied with 10% fetal bovine serum (FBS) (Thermo Fisher Scientific, USA) and maintained at 37 °C in a 5% CO₂ contained humidified atmosphere. The recombinant full-length *CHN1* gene expression plasmid and *CHN1* shRNA interfered plasmid were synthesized and obtained from GenePharma Company (GenePharma, Shanghai, China). The cells were transfected with

empty vector plasmid as negative controls. Transfection was performed in the 24-well plate (Corning Life Science, USA) by Lipofectamine 3000 (Invitrogen, USA) following the instruction, and the efficacy of transfection was assessed through western blotting. Stable transfection clones were selected by 400 µg/ml G418 (Sigma, USA) for 14 days.

Immunohistochemistry (IHC) and hematoxylin & eosin (H&E) staining

The paraffin-embedded sections were de-waxed, hydrated, and antigen retrieved in 0.01M citrate solution at 95 °C for 20 min. Then they were treated with 3% hydrogen peroxide for 10 min to block the endogenous peroxidase. The mouse anti-human CHN1 monoclonal antibody (1:150, Abcam, UK) and rabbit anti-human Snail polyclonal antibody (1:500, Proteintech Group, USA) were incubated respectively followed by the serum blocking. A PV-6002 kit (ZSGB-Bio, Beijing, China) was used for the following steps. The specimens were observed and captured under an inverted microscope (Leica, Wetzlar, Germany). The tumors harvested from the xenograft nude mice were fixed and embedded in paraffin and their pathological features were analyzed through H&E staining (Solarbio Life Science, Beijing, China).

RNA extraction, reverse transcription and quantitative real-time PCR (qRT-PCR)

Total RNA was isolated using TRIzol reagent (Invitrogen, USA) from cultured cells and prepared according to the instruction manual. The complementary DNA was reverse transcribed according to the PrimeScript RT reagent Kit (TaKaRa, China) and qPCR was performed with the SYBR Premix Ex Taq II (Tli RNaseH Plus) Kit (TaKaRa, China) on an ABI 7500 Real-time PCR system (Applied Biosystems, USA). All genes were conducted in triplicate and relative expression was normalized to the housekeeping gene β -actin. The primers used here are listed in Supplementary Table1.

Western blotting

The extracted equal amounts of protein were separated through polyacrylamide SDS gels (SDS-PAGE) and transferred to polyvinylidene fluoride (PVDF) membranes (Thermo Fisher Scientific, USA). Then they were probed with different primary antibodies including CHN1 (Abcam, UK), Fibronectin (Invitrogen, USA), β -catenin, Snail (two from CST, USA), vimentin and E-cadherin (two from Proteintech Group, USA) and the blot was detected with peroxidase-conjugated secondary antibodies (ZSGB-Bio, China). The signals were acquired by chemiluminescence kit (Merckmillipore, Germany). β -tubulin and GAPDH (two from Transgen Biotech, China) were used as the internal control.

Immunofluorescence (IF) analysis

The cells were seeded on the poly-L-lysine (ZSGB-Bio, China) coated coverslips and then fixed post culturing. After fixation by adopting 4% paraformaldehyde solution at room temperature, the cells were washed with 0.01M PBS solution for 3 times. The cells were added 5% goat serum blocking liquid with 0.3% Triton X-100 for 30 minutes. The IF was performed in the wet box by overnight incubation at 4 °C with primary antibodies against CHN1 (1:150, Abcam, UK), E-cadherin (1:200, Proteintech Group, USA), and vimentin (1:300, Proteintech Group, USA), respectively. The Alexa Fluor® 597-conjugated secondary

antibody (1:300, ZSGB-Bio, China) was incubated at 37 °C for 1 h after the 0.01M PBS washing. The cell nuclei were stained with DAPI (0.2 µg/ml, Sigma, USA) and images were acquired by inverted fluorescence microscope (Leica, Germany).

Cell proliferation and migration assays

Cellular growth curves and colony-forming assay were used to evaluate the cell proliferation rate. Briefly, cells were seeded, cultured, and incubated with CCK-8 Kit (Dojindo, Japan) and the optical density (OD) value was analyzed by Synergy HT multi-modereader (BioTek, USA) at 450nm. Plate Colony-forming assay was performed by seeding 200/well cells in culture dish. The cells were fixed and stained with crystal violet (Beyotime, China) and counted under the microscope (>50 clones validated, Leica, Germany) post 14 days culture.

Cell migration was evaluated by wound healing and transwell assays. 24 h post the cell seeding and culturing, a scratch was made by a 200 µl sterile pipette tip. The cells were cultured by FBS free medium and wound confluence was observed after 24 and 48 h. The transwell assay was also used to confirm the cell migration. 2×10^4 single-cell suspension was added into the upper chambers of 24-well transwell plate (Corning Life Science, USA), and 0.1%FBS contained medium was added into the lower chambers. The migrated cells were fixed and stained by crystal violet (Beyotime, China) and counted under microscope post 24 h incubation.

In vivo detection

4 weeks old NU/NU female nude mice were used for the xenograft detection (purchased from Vital River Laboratory Animal Technology Co., Ltd, China). 5×10^7 cells were mixed with matrigel (Becton Dickinson) and subcutaneously injected into the oter of each mouse. 5 mice were used for each group. Mice were anesthetized, sacrificed after 8 weeks, and tumors were harvested, measured and stained. All the mice used here were bred at the SPF Animal Laboratory of Institute of Science and Technology, National Research Institute for Family Planning.

Statistical analysis

SPSS 18.0 statistical software (IBM Corporation, USA) was used for data analysis. Data were expressed as mean $\pm$ standard deviation (SD) after at least three independent experiments. Pearson *chi*-square test was assessed for clinical correlation analysis and Kaplan Meier method was performed to analyze the overall survival rate of cervical cancer patients. The difference between the two groups was tested by Student's *t*-test. As long as *P*-value < 0.05, the results were statistically significant.

Results

Overexpression of CHN1 in CSCC was related to metastasis and was negatively connected with the overall survival time of patients

In order to investigate the expression level of CHN1, western blotting was used for testing its expression in both CSCC and paracancerous tissues. It proved that CHN1 was highly expressed in tumors compared to the correlated non-carcinoma tissues in 5 pairs of CSCC and matched adjacent tissues (Fig. 1a). The location of CHN1 was mainly concentrated in the cytoplasm by IF staining (Fig. S1a) and IHC assays (Fig. S1b). IHC was further operated to confirm its expression status in a 62-paired CSCC/ adjacent non-carcinoma TMA and three pairs were shown as representatives (Fig. S1c). Among those samples, a strong positive signal of CHN1 was obviously observed in high, middle and low differentiated CSCC tissues (Fig. 1b) with an overall positive rate of 85.5% (53/62), which was significantly different from the 43.5% (27/62) proportion in non-carcinoma tissues (Table 1). However, CHN1 mainly expressed in the cervical epithelium base of adjacent non-carcinoma tissues due to the rapid cell division and active cell proliferation (Fig. 1b). To further study the patient survival time and CHN1 status, we collected another 65 samples of CSCC patients for the IHC detection, then 25 of them were participated in the follow-up study for the assessment of survival time. The overall survival rate was varied among the patients with differentially expressed CHN1. Compared to the negative expressed CHN1, both the strong positive and positive expression of CHN1 presented a shorter overall survival time, with the shortest time in the strong positive group (Fig. 1c). The median survival time of the strong positive patients was 48 months. These data demonstrated that the overexpressed CHN1 might negatively concerned with the overall survival time of patients.

Table 1
CHN1 expression levels in cervical squamous cell carcinoma and normal tissues

Tissues	N	CHN1 expression			Positive rate (%)
		-	+	++	
Normal	62	35	26	1	43.5
Tumor	62	9	31	22	85.5**
** $P<0.01$ VS normal tissue					
Pearson <i>chi</i> -square test was performed to determine the statistical significance of the level of expression of CHN1 in cervical squamous cell carcinoma and normal tissues.					

Subsequently, the clinicopathological significance of CHN1 expression was then analyzed by chi-square test in the 128 CSCC tissue assay. As shown, CHN1 level was connected with lymph node metastasis ($P = 0.003$), but not with the age, clinical stage, and histological differentiation of patients (Table 2). Since the pelvic lymph node metastasis is one of the common types during cervical cancer progression, we then detected the correlation between the metastatic lymph nodes and CHN1 expression by IHC. It was presented that CHN1 also highly expressed in the metastatic lymph nodes of cervical cancer tissue, which indicated that CHN1 might be connected with cervical cancer metastasis (Fig. 1d).

Table 2
Relationship between CHN1 expression and clinicopathologic feature

Features	Classification	N	CHN1expression		Pvalue
			Low (%)	High (%)	
Age (years)	< 50	83	15(18.1%)	68(87.9%)	0.79
	≥ 50	45	9(20.0%)	36(80.0%)	
TNM Stage	I	31	8(25.8%)	23(74.2%)	0.138
	II	42	10(23.8%)	32(76.2%)	
	III-IV	55	6(10.9%)	49(89.1%)	
Differentiation grade	Well	16	1(6.3%)	15(93.7%)	0.131
	Moderate	97	22(22.7%)	75(77.3%)	
	Poor	15	1(66.7%)	14(93.3%)	
Lymph node metastasis	No	78	21(26.9%)	57(65.4%)	0.003**
	Yes	50	3(6.0%)	47(94.0%)	
**Statistical significance (<i>p</i> < 0.01) was shown in bold					

Up-regulation of CHN1 promotes the proliferation of SiHa cell in vitro and enhances tumorigenicity in vivo

As CHN1 was highly expressed in cervical carcinoma tissues, the influence of its up regulation on cell proliferation was next tested by establishing a CHN1 stable transfected model in SiHa cell line. After confirmed by western blotting, the CHN1-overexpressed cells had been successfully acquired when compared to the vector-only transfected cells (Fig. 2a). Then these cells had been tested for their ability of proliferation both *in vitro* and *in vivo*. As presented, an increasing of CHN1 level, coupled with a significantly enhanced cell proliferation, was observed in the CHN1-overexpressed SiHa cells compared to the vector-transfected group by using the CCK-8 assay (Fig. 2b); the same as the above-mentioned results, the colony-forming assay further verified that the numbers of colonies in SiHa-CHN1 were much more than that in SiHa-vector (Fig. 2c). These data demonstrated that the overexpression of CHN1 may increase the proliferation capacity of SiHa cells *in vitro*. Besides, the tumorigenicity of SiHa-CHN1 and SiHa-vector was followed in nude mouse for testing their ability of proliferation *in vivo*. 8 weeks post xenograft, tumors could be observed in both of these two groups. However, the ones formed by CHN1 up-regulated SiHa cells showed a much bigger weight than the vector group with a statistical significance ($p = 0.001$, Fig. 2d). The H&E staining certified that the tumors consisted by these two kinds of cells presented an extinction of cell polarity in the parenchyma, enlarged cell nuclei, pathological mitoses, and apparent cell heteromorphism, all of which are the distinctive features of tumor cells (Fig. 2e, up panel). In contrast to SiHa-vector, the further IHC data showed that CHN1 was positively expressed in SiHa-CHN1

formed tumors (Fig. 2e, down panel), which implied that the xenograft tumors might be caused by the up-regulated CHN1. Taken together, those data demonstrated that the increased CHN1 protein level may promote cell proliferation both *in vitro* and *in vivo*.

Down-regulation of CHN1 inhibits the proliferation of SiHa cell *in vitro* and reduces tumorigenicity *in vivo*

Besides the up-regulation, *CHN1* was then interfered by using two *CHN1* targeted shRNAs, shCHN1-1 and shCHN1-2, a scrambled shRNA was involved as a negative control (shVector), and the ability of cell proliferation was again detected both *in vitro* and *in vivo*. Post western blotting detection, the protein level of CHN1 was strongly decreased both in shCHN1-1 and shCHN1-2 compared to shVector (Fig. 3a). Meanwhile, the proliferation of CHN1 down-regulated cells were significantly inhibited tested by the CCK-8 assay (Fig. 3b), as well as their ability of colony-forming (Fig. 3c). The *in vivo* tumorigenicity assay further confirmed that, even though the tumors developed in all the three groups, the ones observed in shCHN1-1 and shCHN1-2 were significantly smaller than that in shVector (Fig. 3d).

Interference of CHN1 inhibits invasion and migration ability of SiHa cells

The capacity of cell migration was tested simultaneously in SiHa cells with *CHN1* knockdown. The transwell assay proved that *CHN1* knockdown could significantly inhibit the cell invasion (Fig. 4a); consistently, this inhibition of migration was also observed in wound-healing assay (Fig. 4b).

Taken together, these results demonstrated that the invasion and migration of SiHa cells could be inhibited by the down regulation of CHN1 *in vitro*, as well as the tumorigenicity *in vivo*.

CHN1 induces EMT through AKT / GSK-3 β / Snail pathway

Since EMT is one of the key elements for cancer metastasis, as mentioned before, the inhibition of cell migration was correlated with *CHN1* knockdown, the expression of two crucial markers of EMT, epithelial marker E-cadherin and mesenchymal marker Vimentin, were then detected to reveal the connection between EMT and down-regulated CHN1. Immunofluorescent staining showed that, in contrast to the control, increased E-cadherin and decreased Vimentin were observed in CHN1 interfered cells (Fig. 5a). In addition, the level of CHN1 and Snail, another EMT relevant transcription factor, was tested by IHC in serial sliced 24 CSCC samples to further verify the relativity of their expression. It could be seen that both of them were brown stained with the similar localization in tumor samples (Fig. 5b), which implied that the level Snail might related to the expression of CHN1 during the cervical cancer development.

The qRT-PCR analysis showed that an increased *CHN1* gene level could decrease the level of *E-cadherin* and *β -catenin*, but increased the level of *Vimentin*, *Fibronectin*, and *Snail* (Fig. 5c). Western blotting on the other hand, presented the same tendency in protein level that overexpressed CHN1 not only increased the expression of Snail, Fibronectin, Vimentin, but also decreased the expression of E-cadherin and β -catenin (Fig. 5d). But when CHN1 was interfered, the expression of those EMT related markers showed a reversed pattern (Fig. 5e).

It was published that activated AKT is essential for the induction of EMT through the inhibition of GSK-3 β , which leads to the stabilization and nuclear localization of Snail to trigger the migration of cells and EMT [26]. In that case, several key factors involved in the ATK / GSK-3 β / Snail pathway were detected by western blotting and the results showed that the expression of phosphorylated AKT, phosphorylated GSK-3 β , and Snail was enhanced when CHN1 was up-regulated (Fig. 5f). To further make sure the effect of CHN1 was via the activation of the ATK / GSK-3 β / Snail pathway, the expression of those three proteins were tested again under the presence of the PI3K inhibitor LY294002. It demonstrated that the level of phosphorylated AKT, phosphorylated GSK-3 β , and Snail were effectively reduced with the attendance of LY294002 (Fig. 5f).

Discussion

The progression of cervical cancer is a multifactor and multistep involved process. Even though the techniques of cervical screening and diagnostic have made some progress, as well as the improvement of its vaccine, it is now still one of the gynecologic malignant tumors worldwide that the effective way to restrain its development and to inhibit its relapse and metastasis still needs to be figured out.

In this study, the carcinogenesis of CHN1 is investigated to figure out its role during the occurrence and development of cervical carcinogenesis. We found that the overexpression of CHN1 in cervical carcinogenesis was related to a higher metastasizing degree and pathological stage, as well as total survival rate. The further in vitro functional study demonstrated that the metastasis and tumorigenesis of SiHa cells could be significantly enhanced by the increased level of CHN1. In contract, an inhibited effect was observed when its expression was interrupted. The nude mice in vivo study also supported the same impacts that higher level of CHN1 promoted the tumorigenesis while lower level inhibited it. One of the main reasons to cause the recurrence and death of CSCC patients is due to the distant metastasis, for example, pelvic lymph node [27–29]. EMT plays a crucial role during the lymph node metastasis of cervical cancer [30]. It has been reported that an increased tumor progression, invasion, metastasis, and distortion of epithelial integrity could be observed if the primary cervical cancer coupled with the occurrence of EMT [31]. Then it is important to test whether EMT is involved in the tumorigenesis of CHN1. According to our data, SiHa cells, with an increased expression of CHN1, promoted the down regulation of epithelial markers, but induced the up regulation of mesenchymal markers in both RNA and protein levels. However, the expression of these EMT markers was significantly reserved when CHN1 level was disrupted in SiHa cells.

In addition, the location of CHN1 was found related to the position of Snail, a key EMT principal transcription factor, by the IHC staining of lymph nodes with metastases of CSCC. The expression of Snail is regulated by the level of CHN1, an increased expression of CHN1 correlated with a high level of Snail while a decreased expression of CHN1 was relevant to a low level of Snail.

It is published that the activation of Akt / GSK-3 β / Snail pathway involves in the occurrence of EMT in cervical cancer [32, 33]. Our further results showed that the phosphorylated level of Akt and GSK-3 β , as

well as the expression of Snail, could be raised when the protein level of CHN1 was strongly enhanced, and the same tendency happened when CHN1 was attenuated, which suggesting that CHN1 might be required for the activation of Akt / GSK-3 β / Snail to promote the metastasis of cervical carcinoma.

Conclusions

Taken together, overexpression of CHN1 promoted the proliferation, migration and invasion of CSCC cells in vitro. In addition, CHN1 overexpression was significantly relevant to high metastatic degree, low survival rate and poor prognosis in CSCC patients. CHN1 could induce EMT through the Akt / GSK-3 β / Snail pathway, as well as enhance metastasis and progression of cervical carcinoma. CHN1 might be considered as a novel marker of cervical carcinoma for the clinical diagnosis of metastasis and poor prognosis. It could be a potential target for a better cervical carcinoma management with a further digging of its characters.

Abbreviations

CHN1, α -chimaerin gene; EMT, epithelial-mesenchymal transition; CSCC, cervical squamous cell carcinoma; TMA, tissue microarray; H&E, Hematoxylin and Eosin; IF, immunofluorescence; IHC, immunohistochemistry; qRT-PCR, quantitative reverse transcription PCR; shRNA, short hairpin RNA.

Declarations

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Authors' contributions

HZ conceived and operated the experiments. LW, SW, XC, ML and XZ participated in the experimental design. HZ and LW wrote the manuscript. HZ, JW and XX performed the statistical analysis. All authors read and approved the final manuscript.

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Availability of data and material

The data generated during this study are included in this published article.

Ethics approval and consent to participate

All experiments on animals were approved by the Animal Ethics Committee of the National Research Institute for Family Planning. Not applicable for human data or tissue.

Consent for publication

We agree to publish this study.

Competing interests

The authors declare that there are no conflicts of interest in this work.

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2021; doi: 10.3322/caac.21660.
2. Mohanty G, Ghosh SN. Risk factors for cancer of cervix, status of screening and methods for its detection. *Arch Gynecol Obstet*. 2015;291(2):247-9; doi: 10.1007/s00404-014-3492-1.
3. Del Pilar Estevez-Diz M, Bonadio RC, Miranda VC, Carvalho JP. Management of cervical cancer patients during the COVID-19 pandemic: a challenge for developing countries. *Ecancermedicalscience*. 2020;14:1060; doi: 10.3332/ecancer.2020.1060.
4. Tian Y, Qi P, Niu Q, Hu X. Combined Snail and E-cadherin Predicts Overall Survival of Cervical Carcinoma Patients: Comparison Among Various Epithelial-Mesenchymal Transition Proteins. *Front Mol Biosci*. 2020;7:22; doi: 10.3389/fmolb.2020.00022.
5. Kocian R, Slama J, Fischerova D, Germanova A, Burgetova A, Dusek L, et al. Micrometastases in Sentinel Lymph Nodes Represent a Significant Negative Prognostic Factor in Early-Stage Cervical Cancer: A Single-Institutional Retrospective Cohort Study. *Cancers (Basel)*. 2020;12(6); doi: 10.3390/cancers12061438.
6. Dongre A, Rashidian M, Eaton EN, Reinhardt F, Thiru P, Zagorulya M, et al. Direct and Indirect Regulators of Epithelial-Mesenchymal Transition (EMT)-mediated Immunosuppression in Breast Carcinomas. *Cancer Discov*. 2020; doi: 10.1158/2159-8290.CD-20-0603.
7. Liu T, Zhao X, Zheng X, Zheng Y, Dong X, Zhao N, et al. The EMT transcription factor, Twist1, as a novel therapeutic target for pulmonary sarcomatoid carcinomas. *Int J Oncol*. 2020;56(3):750-60; doi: 10.3892/ijo.2020.4972.
8. Rasti A, Madjd Z, Abolhasani M, Mehrazma M, Janani L, Saeednejad Zanjani L, et al. Cytoplasmic expression of Twist1, an EMT-related transcription factor, is associated with higher grades renal cell carcinomas and worse progression-free survival in clear cell renal cell carcinoma. *Clin Exp Med*. 2018;18(2):177-90; doi: 10.1007/s10238-017-0481-2.

9. Rajabi H, Kufe D. MUC1-C Oncoprotein Integrates a Program of EMT, Epigenetic Reprogramming and Immune Evasion in Human Carcinomas. *Biochim Biophys Acta Rev Cancer*. 2017;1868(1):117-22; doi: 10.1016/j.bbcan.2017.03.003.
10. Leung T, How BE, Manser E, Lim L. Cerebellar beta 2-chimaerin, a GTPase-activating protein for p21 ras-related rac is specifically expressed in granule cells and has a unique N-terminal SH2 domain. *J Biol Chem*. 1994;269(17):12888-92.
11. Hall C, Sin WC, Teo M, Michael GJ, Smith P, Dong JM, et al. Alpha 2-chimerin, an SH2-containing GTPase-activating protein for the ras-related protein p21rac derived by alternate splicing of the human n-chimerin gene, is selectively expressed in brain regions and testes. *Mol Cell Biol*. 1993;13(8):4986-98; doi: 10.1128/mcb.13.8.4986.
12. Ip JP, Shi L, Chen Y, Itoh Y, Fu WY, Betz A, et al. alpha2-chimaerin controls neuronal migration and functioning of the cerebral cortex through CRMP-2. *Nat Neurosci*. 2011;15(1):39-47; doi: 10.1038/nn.2972.
13. Areces LB, Kazanietz MG, Blumberg PM. Close similarity of baculovirus-expressed n-chimaerin and protein kinase C alpha as phorbol ester receptors. *J Biol Chem*. 1994;269(30):19553-8.
14. Caloca MJ, Garcia-Bermejo ML, Blumberg PM, Lewin NE, Kremmer E, Mischak H, et al. beta2-chimaerin is a novel target for diacylglycerol: binding properties and changes in subcellular localization mediated by ligand binding to its C1 domain. *Proc Natl Acad Sci U S A*. 1999;96(21):11854-9; doi: 10.1073/pnas.96.21.11854.
15. Colon-Gonzalez F, Leskow FC, Kazanietz MG. Identification of an autoinhibitory mechanism that restricts C1 domain-mediated activation of the Rac-GAP alpha2-chimaerin. *J Biol Chem*. 2008;283(50):35247-57; doi: 10.1074/jbc.M806264200.
16. Bruinsma SP, Cagan RL, Baranski TJ. Chimaerin and Rac regulate cell number, adherens junctions, and ERK MAP kinase signaling in the Drosophila eye. *Proc Natl Acad Sci U S A*. 2007;104(17):7098-103; doi: 10.1073/pnas.0701686104.
17. Wegmeyer H, Egea J, Rabe N, Gezelius H, Filosa A, Enjin A, et al. EphA4-dependent axon guidance is mediated by the RacGAP alpha2-chimaerin. *Neuron*. 2007;55(5):756-67; doi: 10.1016/j.neuron.2007.07.038.
18. Brown M, Jacobs T, Eickholt B, Ferrari G, Teo M, Monfries C, et al. Alpha2-chimaerin, cyclin-dependent Kinase 5/p35, and its target collapsin response mediator protein-2 are essential components in semaphorin 3A-induced growth-cone collapse. *J Neurosci*. 2004;24(41):8994-9004; doi: 10.1523/JNEUROSCI.3184-04.2004.
19. Miyake N, Chilton J, Psatha M, Cheng L, Andrews C, Chan WM, et al. Human CHN1 mutations hyperactivate alpha2-chimaerin and cause Duane's retraction syndrome. *Science*. 2008;321(5890):839-43; doi: 10.1126/science.1156121.
20. Siliceo M, Garcia-Bernal D, Carrasco S, Diaz-Flores E, Coluccio Leskow F, Teixido J, et al. Beta2-chimaerin provides a diacylglycerol-dependent mechanism for regulation of adhesion and chemotaxis of T cells. *J Cell Sci*. 2006;119(Pt 1):141-52; doi: 10.1242/jcs.02722.

21. Bruinsma SP, Baranski TJ. Beta2-chimaerin in cancer signaling: connecting cell adhesion and MAP kinase activation. *Cell Cycle*. 2007;6(20):2440-4; doi: 10.4161/cc.6.20.4786.
22. Yagi S, Matsuda M, Kiyokawa E. Chimaerin suppresses Rac1 activation at the apical membrane to maintain the cyst structure. *PLoS One*. 2012;7(12):e52258; doi: 10.1371/journal.pone.0052258.
23. Clark C, Austen O, Poparic I, Guthrie S. alpha2-Chimaerin regulates a key axon guidance transition during development of the oculomotor projection. *J Neurosci*. 2013;33(42):16540-51; doi: 10.1523/JNEUROSCI.1869-13.2013.
24. Nugent AA, Park JG, Wei Y, Tenney AP, Gilette NM, DeLisle MM, et al. Mutant alpha2-chimaerin signals via bidirectional ephrin pathways in Duane retraction syndrome. *J Clin Invest*. 2017;127(5):1664-82; doi: 10.1172/JCI88502.
25. Murillo-Correa CE, Kon-Jara V, Engle EC, Zenteno JC. Clinical features associated with an I126M alpha2-chimaerin mutation in a family with autosomal-dominant Duane retraction syndrome. *J AAPOS*. 2009;13(3):245-8; doi: 10.1016/j.jaapos.2009.03.007.
26. Zhou BP, Deng J, Xia W, Xu J, Li YM, Gunduz M, et al. Dual regulation of Snail by GSK-3beta-mediated phosphorylation in control of epithelial-mesenchymal transition. *Nat Cell Biol*. 2004;6(10):931-40; doi: 10.1038/ncb1173.
27. Kodama J, Seki N, Masahiro S, Kusumoto T, Nakamura K, Hongo A, et al. Prognostic factors in stage IB-IIb cervical adenocarcinoma patients treated with radical hysterectomy and pelvic lymphadenectomy. *J Surg Oncol*. 2010;101(5):413-7; doi: 10.1002/jso.21499.
28. Lee MY, Shen MR. Epithelial-mesenchymal transition in cervical carcinoma. *Am J Transl Res*. 2012;4(1):1-13.
29. Chen Y, Zhang L, Tian J, Fu X, Ren X, Hao Q. Significance of the absolute number and ratio of metastatic lymph nodes in predicting postoperative survival for the International Federation of Gynecology and Obstetrics stage IA2 to IIA cervical cancer. *Int J Gynecol Cancer*. 2013;23(1):157-63; doi: 10.1097/IGC.0b013e3182778bcf.
30. Noordhuis MG, Fehrmann RS, Wisman GB, Nijhuis ER, van Zanden JJ, Moerland PD, et al. Involvement of the TGF-beta and beta-catenin pathways in pelvic lymph node metastasis in early-stage cervical cancer. *Clin Cancer Res*. 2011;17(6):1317-30; doi: 10.1158/1078-0432.CCR-10-2320.
31. Lee MY, Chou CY, Tang MJ, Shen MR. Epithelial-mesenchymal transition in cervical cancer: correlation with tumor progression, epidermal growth factor receptor overexpression, and snail up-regulation. *Clin Cancer Res*. 2008;14(15):4743-50; doi: 10.1158/1078-0432.CCR-08-0234.
32. Zhao W, Zhou Y, Xu H, Cheng Y, Kong B. Snail family proteins in cervical squamous carcinoma: expression and significance. *Clin Invest Med*. 2013;36(4):E223-33; doi: 10.25011/cim.v36i4.19956.
33. Li Z, Yu CP, Zhong Y, Liu TJ, Huang QD, Zhao XH, et al. Sam68 expression and cytoplasmic localization is correlated with lymph node metastasis as well as prognosis in patients with early-stage cervical cancer. *Ann Oncol*. 2012;23(3):638-46; doi: 10.1093/annonc/mdr290.

Figures

Figure 1

CHN1 is overexpressed in CSCC tissues. a Expression levels of CHN1 in CSCC and matched adjacent normal tissues. Three independent experiments were performed and GAPDH was used as the internal control. b IHC staining assays were performed to analysis the expression of CHN1 in CSCC with various differentiation status and matched non-carcinoma tissues (original magnification, 200×). c Kaplan-Meier analysis indicating CSCC patients for high-expression of CHN1 displayed low overall survival. d CHN1

highly expressed in the metastatic lymph nodes of cervical carcinoma tissue (original magnification, 200×).

Figure 2

CHN1 overexpression promotes tumorigenicity of SiHa cells. a CHN1 was overexpressed in SiHa cells after transfection. GAPDH was used as the internal control. b Cell proliferation was enhanced after the overexpression of CHN1 in SiHa cells (data represent means $\pm$ SD, $n=3$, $^{**}p<0.01$). c Foci formation was

effectively promoted after CHN1 overexpressed in SiHa cells (data represent means $\pm$ SD, $n=3$ ** $p<0.01$). d Xenograft tumors were formed after CHN1 and empty vector transfection in nude mice. Weight assessment of xenograft tumors (right, $n=5$ for each group, ** $p<0.01$). e Representative H&E and IHC staining of CHN1 expression in CHN1- and empty vector-transfected xenograft tumors (original magnification, 400 $\times$).

Figure 3

CHN1 interference inhibits the tumorigenicity of SiHa cells. a CHN1 expression was reduced after shRNA knockdown. b Cell proliferation was inhibited after CHN1 knockdown in SiHa cell line (data represent means $\pm$ SD, $n=3$ ** $p<0.01$). c Foci formation was inhibited after the interference of CHN1 expression in SiHa cells (data represent means $\pm$ SD, $n=3$ ** $p<0.01$). d Smaller xenograft tumors were generated after

the injection of SiHa-shCHN1 cells in the nude mice. Weight assessment of xenograft tumors (right, n=5 for each group, **p<0.01).

Figure 4

CHN1 interference decreases the invasion and migration of SiHa cells. a Transwell invasion assay detected an inhibited invasion capacity of SiHa cells after CHN1 interference. b Wound-healing assays detected a reduced migration rates after down-regulation of CHN1. Magnification? All data represent means $\pm$ SD, n=3**p<0.01.

Figure 5

CHN1 expression induces EMT through activating the Akt / GSK-3 β / Snail pathway. a Increased expression of E-cadherin and decreased expression of Vimentin were detected after down-regulating CHN1 expression in SiHa cells. DAPI (blue) was added to stain the nuclei (magnification?). b CHN1 and Snail were located in the similar localization in samples of CSCC patients (original magnification, 200 $\times$). c The expression of EMT markers and EMT-related transcription factors were affected after up regulation

of CHN1 in SiHa cells. The results represent means $\pm$ SD, n=3. Overexpression (d) and interference (e) of CHN1 affected the expression of epithelial and mesenchymal markers. f Overexpression of CHN1 increased the expression of p-Akt, p-GSK-3 β and Snail. The attendance of AKT inhibitor LY29402, reversed the impact of overexpressed CHN1 on the expression of p-Akt, p-GSK-3 β and Snail. Three independent experiments were performed and GAPDH and β -tubulin were used as the internal control.

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