WDR5 drives the development of cervical squamous cell carcinoma by inducing epithelial-mesenchymal transition and cancer-associated fibroblasts formation

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Research Article

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Abstract

WD repeat domain 5 (WDR5) has been indicated to be involved in tumor progression, however, its role in cervical cancer (CC) has not been investigated yet. A total of 350 pairs of CC tissues and para-carcinoma tissues (PCT) were collected. Primary human cervical epithelial cells (hCECs) and cancer-associated fibroblasts (CAFs) were isolated from PCT and cancer tissues. MM102 was used to block the interaction between WDR5 and mixed lineage leukemia protein-1 (MLL1), and it was used in vivo to investigate its therapeutic value. WDR5 was up-regulated in cervical squamous cell carcinoma (CSCC) tissues compared to that in PCT. C-X-C motif chemokine ligand 8 (CXCL8) was indicated to be the target gene of WDR5. Highly expressed CXCL8 promoted epithelial-mesenchymal transition (EMT) to form CAFs, and enhanced the cytokine secretions in CAFs to promote CSCC progression. CXCL8 expression was regulated by the interaction between WDR5 and MLL1, and blocking the interaction between these two proteins using MM102 significantly suppressed tumor growth in mice models. WDR5 plays a key role in CSCC progression by inducing CXCL8 expression and promoting the transformation of CAFs from epithelial cells.

Introduction

Cervical cancer (CC) is the third most common cancer and the fourth leading cause of cancer death in women [1]. Cervical squamous cell carcinoma (CSCC) is the most common pathology subtype, accounting for about 80–90% of total CC [1]. The incidence of CC is much higher in developing countries due to the unavailability of HPV vaccines and unprotected sexuality [2]. It is estimated that more than 85% of CC and deaths were found in developing countries, including China [2]. Although early diagnosis can increase the survival rate of CC, the overall treatment efficacy is still poor because of its unclarified pathogenesis [3].

Cancer-associated fibroblasts (CAFs) are one of the most abundant stromal components in tumor stroma and have prominent roles in the pathogenesis of many, if not all, solid tumors [4]. Mechanistically, CAFs build up and remodel the tumor microenvironment (TME) by secreting growth factors and cytokines [5]. Recent studies indicate that CAFs are involved in the progression of CC [6, 7], however, limited study has investigated how the formation of CAFs in CC tissues is regulated.

WD repeat domain 5 (WDR5) is an important component of histone methyltransferase (HMT), which is responsible for the catalyzation of trimethylation of histone 3 lysine 4 (H3K4me3) [8]. WDR5 has been proved to play crucial roles in cancer pathogenesis [9], however, no study has explored its role in CC development. In this study, we proposed a hypothesis that WDR5 may drive the development of CC by promoting CAFs formation. Based on this hypothesis, we analyzed the expression pattern of WDR5 in CC tissues and the para-carcinoma tissues (PCT) using clinical samples. We also investigated the function of WDR5 by in vitro studies and explored the therapeutic value by targeting the WDR5 function using a mouse model.
Materials And Methods

Ethics approval

The usage of human tissues was approved by the Ethics Committee of the Guangxi Medical University Cancer Hospital (No.: H2019018.v03). The experiments were undertaken with the understanding and written consent of each subject, and that the study conforms with The Code of Ethics of the World Medical Association (Declaration of Helsinki), printed in the British Medical Journal (18 July 1964).

Humane care was given during the experimental animal breeding and experimental procedures in accordance with the 3R principle of experimental animals. Experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC). All animal treatments were approved by the Ethics Committee of the Guangxi Medical University Cancer Hospital (No.: KA190114.v02).

Human tissue collection

From April 8, 2019 to August 1, 2021, CC tissues and the corresponding PCT (defined as tissues ≥ 2 cm away from cancer tissues) from 350 cases of CC patients (250 cases of CSCC, 75 cases of cervical adenocarcinoma, and 25 cases of cervical adenosquamous cell carcinoma) were collected from the Department of Gynecologic Oncology, Guangxi Medical University Cancer Hospital. Basic information on patients was also gathered. All patients were diagnosed for the first time and had not received treatment before. Patients who received treatments before or complicated with other systemic diseases such as diabetes, cardiovascular, or cerebrovascular diseases were excluded from this study.

Isolation and culture of cells

The primary normal cervical epithelial cells (hCECs) were isolated as the prior report [10]. Briefly, the fresh PCT was minced and digested with type I collagenase at 37 °C for 60 min. The digested mixture was then filtrated through a stainless-steel strainer (0.5-1.0mm). Tell suspension was centrifuged at 1,500 rpm for 5 min, and hCECs were collected and cultured in Dulbecco's Modified Eagle's Medium (DMEM, Thermo Fisher Scientific, USA) supplemented with 10% (v/v) fetal bovine serum (FBS) (Clark, USA). The purity of hCECs was identified by > 90% positive immunofluorescence staining for cytokeratin 7 (Supplementary Figure 1A). Cells with passages > 10 were used in the following cell experiments.

We also isolated primary CAFs from cancer tissues of CSCC as previously described [11]. Briefly, fresh cancer tissues were minced and digested with type I collagenase, and the digested mixture was filtrated through a nylon mesh of 38 um pore size. The CAFs were in the filtrate. The filtrate was centrifuged at 1000 g for 5 min, and CAFs were resuspended in DMEM supplemented with 10% FBS, and were then incubated in a fresh culture medium for 4 weeks to allow cell attachment and grow out. The purity of CAFs was identified by > 90% positive immunofluorescence staining for fibroblast activation protein (FAP) (Supplementary Figure 1B). Cells with passages > 10 were used in the following cell experiments. CSCC cell line CaSki was also used in this study. CaSki cells were purchased from the...
American Type Culture Collection (ATCC) and were cultured in DMEM supplemented with 10% FBS. Cells were all placed in the 37 °C incubators supplemented with 20% oxygen and 5% carbon dioxide.

**Cell transfections**

Small interfering RNAs (siRNAs) for WDR5 (si-WDR5) and CXCL8 (si-CXCL8) (VectorBuilder, China) were used to knock down the target transcripts. siRNAs having no target (si-NC) were served as the control. Plasmid overexpressing WDR5 (Vector-WDR5) or CXCL8 (Vector-CXCL8) were also used to express exogenous proteins, and the empty vectors (Vector-NC) were used as the control. The transfection was conducted using Lipofectamine 3000® Transfection Reagent (Invitrogen, USA) according to the manufacturers’ instructions. The sequence of siRNAs was provided in Table 1.

**Chromatin immunoprecipitation (ChIP) and ChIP-sequencing (ChIP-seq)**

ChIP assays were performed as described previously [12]. Briefly, cells were cross-linked, lysed, and sheared by sonication to produce DNA fragments with an average length of approximately 500 bp. Then, 1% of the chromatin fragments were used as the input. Chromatin was immunoprecipitated using antibodies against WDR5 (13105, Cell Signaling Technology, USA), H3K4me3 (ab8580, Abcam, USA), and MLL1 (34907, Cell Signaling Technology, USA). Normal rabbit IgG was added as the control. Then DNA fragments immunoprecipitated were purified and were further analyzed by quantitative real-time PCR (RT-qPCR). The sequence of primers used is provided in Table 1. ChIP-seq analyses were performed using the ChIP-IT High Sensitivity Kit (ab185908, Abcam, USA). The model-based analysis of the ChIP-Seq peak-finding algorithm was used to normalize ChIP against the input control.

**Immunohistochemistry**

Immunohistochemistry was performed on paraffin-embedded tissue sections. Tissues on the sections were blocked with 5% BSA, and were then incubated overnight at 4 °C with primary antibodies for the detection of the following: WDR5 (ab178410, Abcam, USA), CXCL8 (ab106350, Abcam, USA), and FAP (ab207178, Abcam, USA). After being incubated with the secondary antibodies, slides were visualized using DAB-Substrate (Beyotime, China) and photographed using the Aperio ePathology Scanner (Leica, Germany). Protein expression was quantified by H-score, which was calculated by the formula: H-score = Pi (i), where i is the intensity of staining with a value of 1, 2, or 3 (weak, moderate, or strong, respectively) and Pi is the percentage of stained cells for each intensity in the range of 0-100%.

**Immunofluorescence**

Cells were fixed with 4% formaldehyde and incubated overnight at 4°C with primary antibodies anti-vimentin (ab92547, Abcam, USA), anti-cytokeratin 7 (ab68459, Abcam, USA), anti-FAP (66562, Cell Signaling Technology, USA), and anti-tubulin (ab18207, Abcam, USA). Cells were then incubated with the goat anti-rabbit secondary antibody (BA1031, BOSTER, China) at 37°C for another 30 min. The nucleus was stained by 4,6-diamidino-2-phenylindole (Beyotime, China). Cells were observed by Laser Scanning
Confocal Microscope (Leica, Germany) and results were analyzed by Leica Application Suite X (Leica, Germany).

**Western blotting (WB)**

Cells lysates containing total proteins were collected and prepared using a loading buffer. An equal amount of protein from each group was loaded into SDS-PAGE (10% gel) and separated by electrophoresis. Proteins in the gel were then transferred to a polyvinyl difluoride membrane. After immersion in quick blocking buffer (Beyotime, China) for 30 min, membranes were incubated overnight at 4 °C with the primary antibodies for the detection of the following: E-cadherin (ab40772, Abcam, USA), N-cadherin (ab76011, Abcam, USA), β-catenin (ab32572, Abcam, USA), snail (ab216347, Abcam, USA), and tubulin (ab215037, Abcam, USA). Membranes were then incubated with the secondary antibodies (ab6721, Abcam, USA) at room temperature for another 30 min. Protein bands in the membrane were visualized using ECL plus kit (Beyotime, China), and the relative expressions of target proteins were quantified using Image J (National Institutes of Health, USA).

**RT-qPCR**

Total RNA was extracted by the Trizol method (Thermo Fisher Scientific, USA) and were reversely transcript into cDNA using PrimeScriptTM RT Master Mix (Takara, Japan). RT-qPCR was performed on the Bio-Rad CFX96 (Bio-Rad Laboratories, USA) using SYBR Premix Ex TaqTM (Takara, Japan). The sequence of primers was provided in Table 1. The expression level of each transcript was calculated using the comparative threshold cycle (Ct), based on the using the $2^{-\Delta\Delta Ct}$ formula.

**Invasion Assay**

Invasion chambers (Corning, USA) were used to perform invasion assay. Briefly, 100 ul of Matrigel (BD, USA) was used to coat the inner side of the chamber, and then cells suspended in a culture medium were loaded. DMEM containing 20% FBS was added to the lower chamber as the chemoattractant. The chambers were incubated at 37°C for 48 hours. The non-invasive cells on the upper side of the chamber were removed gently, and chambers were stained using crystal violet to locate the invaded cells. The number of invaded cells was observed and photographed under an inverted optical microscope (Olympus, Japan).

**Enzyme-linked immunosorbent assay (ELISA)**

Levels of growth factors [granulocyte–macrophage colony-stimulating factor (GM-CSF), interleukin-6 (IL-6), CC-chemokine ligand 2 (CCL2), and transforming growth factor-β (TGF-β)] in cancer tissues and cell cultures were analyzed using ELISA kits (mlbio, China) according to the manufacturer's protocols. The intra- and inter-assay coefficients of variation were all less than 10%. Samples were adjusted for total protein concentrations before detection to ensure that the amounts of total protein in each group were equal.
Tumor xenograft mouse model

Four-week male nude BALB/c mice (Cyagen, China) were housed in a facility with a 12 h light/dark cycle maintained at 25 ± 0.5°C and 50% to 60% humidity. The xenograft CC model was established as described before [13]. Briefly, CaSki cells were prepared as a single-cell suspension in Matrigel, and 1×10^6 prepared cells were injected subcutaneously into the right axillary fossa. Mice were euthanized 14 days after modeling (pellitobarbitalum natricum, 100mg/kg, intravenous administration), and tumor lesions were enucleated for further analysis.

Statistical Analysis

Statistical analyses were performed using GraphPad Prism 6.0 (GraphPad Software, USA). The student's t-test was used to analyze the difference between two groups, and one-way ANOVA was used for the comparison among multiple groups. The Chi-square test was used to analyze the categorical variables. In this study, results were obtained from three independent experiments, and \( P<0.05 \) was considered to be statistically significant.

Results

WDR5 is up-regulated in CSCC tissues compared to that in PCT

We first analyzed the expression pattern of WDR5 in CC tissues and the corresponding PCT. Results showed that compared to PCT, WDR5 was up-regulated CSCC tissues (Fig.1 A and B), but had no significant change in cancer tissues of cervical adenocarcinoma and cervical adenosquamous cell carcinoma (Supplementary Figure 2A and B). We next analyzed the correlation between WDR5 expression and the TNM stage of CSCC patients, and found that WDR5 expression was positively correlated with the TNM stage—patients in III-IV stages had markedly higher WDR5 levels compared to patients in I-II stages (Fig. 1A and B).

CXCL8 is a target of WDR5

To explore the function of WDR5, we performed ChIP-seq to find its target gene. Results of ChIP-seq showed that a large amount of DNA fragment of CXCL8 was immunoprecipitated by anti-WDR5 antibody (Fig. 2A), indicating that CXCL8 is a target gene of WDR5. Results of WB showed that overexpression WDR5 in hCECs significantly up-regulated the expression of CXCL8, while the knock-down of WDR5 in CAFs markedly inhibited CXCL8 expression (Fig. 2B). Results of ChIP analysis also indicated that WDR5 could be recruited to the promoter region of CXCL8 (Fig. 2C). These results all suggested that CXCL8 expression was regulated by WDR5. Consistent with the expression pattern of WDR5, analysis on human tissues showed that CXCL8 was up-regulated in CSCC tissues compared to that in PCT, and its expression level was positively correlated with the TNM stage of patients (Fig. 2D).

CXCL8 promotes epithelial-mesenchymal transition (EMT) and induces the formation of CAFs
Using hCECs from PCT, we next investigated the function of CXCL8 in the progression of CSCC. Results showed that overexpression of CXCL8 markedly changed the shape of hCECs, and made it present the shape of stromal cells (Fig. 3A). The markers of EMT such as E-cadherin, N-cadherin, β-catenin, and snail were all changed markedly (Fig. 3B). To identify the characteristic of these transformed cells, we analyzed the expression of FAP, a marker of CAFs [14]. Results showed that CXCL8 overexpression significantly up-regulated the expression of FAP (Fig. 3C), indicating that CXCL8 is an enhancer of CAFs formation. Besides, the secretions of cytokines such as GM-CSF, IL-6, CCL2, and TGFβ, which were proved to promote malignancy progression, were all up-regulated upon CXCL8 overexpression (Fig. 3D), and the invasive ability of CaSki cells was significantly enhanced when they were incubated with the culture medium from cells overexpressing CXCL8 (Fig. 3F).

**Loss of CXCL8 function makes CAFs transformed into epithelial cells**

Using CAFs from CSCC tissues, we then performed knockout experiments to verify the role of CXCL8. Results showed that knock-down of CXCL8 made CAFs present the shape of epithelial cells (Fig. 4A), and changed the expressions of EMT markers (Fig. 4B). Besides, the knock-down of CXCL8 suppressed the expression of FAP (Fig. 4C), attenuated the secretions of cytokines (Fig. 4D). Additionally, compared to the culture medium from CAFs treated with si-NC, the culture medium from hCSCs with CXCL8 knockout significantly attenuated the invasive ability of CaSki cells (Fig. 4E). These results all proved that CXCL8 was involved in CSCC progression by enhancing the transformation of CAFs from epithelial cells.

**CXCL8 is regulated by the interaction between WDR5 and MLL1**

In mammals, WDR5 interacts with MLL1 to form the HMT complex and catalyzes the H3K4me3 in gene promoters [8]. We next investigated the regulation pathway of CXCL8 by using MM102, a molecule that can specifically block the interaction between WDR5 and MLL1. Results showed that in hCSCs from CSCC tissues, the expression of CXCL8 is down-regulated after WDR5 knockout, while the knockout of WDR5 had no impact on CXCL8 expression when MM102 was used, and MM102 markedly inhibited CXCL8 expression (Fig. 5A and C). Additionally, in hCECs from PCT, overexpression of WDR5 markedly induced CXCL8 expression, whereas WDR5 overexpression had no impact on CXCL8 expression when MM102 was used (Fig. 5B and D). ChIP analysis revealed that the distributions of WDR5, MLL1, and H3K4me3 in the CXCL8 promoter region were similar, and the recruitments of WDR5 and MLL1 in CXCL8 promoter were significantly higher in hCSCs from CSCC tissues than that in hCECs from PCT, as well as the H3K4me3 level of CXCL8 promoter (Fig. 5E and F).

**Blocking the interaction between WDR5 and MLL1 suppresses CC development**

We then performed in vivo study to explore the therapeutic value for CSCC by targeting the interaction between WDR5 and MLL1. Results showed that using MM102 could significantly inhibit tumor growth in mice model (Fig. 6A). Analysis on tumor body showed that MM102 could markedly attenuate the expressions of CXCL8 and FAP (Fig. 6B and C), and the markers of EMT (Fig. 6D), as well as the cytokines involved in tumor growth (Fig. 6E).
Overall, all the above results indicate that WDR5 plays a key role in the development of CC. Highly expressed WDR5 promotes the transformation of CAFs from epithelial cells, and enhanced the secretions of cytokines such as GM-CSF, VEGF, PDGF, and TGFβ, thus driving the progression of CC (Fig. 7).

**Discussion**

Tumor recurrence, metastasis, and the intractable TME remain the three key unsolved issues that hamper effective cancer treatment in clinical practice [15]. TME creates a protective niche that is beneficial for tumor cells proliferation and invasion, and protects tumor cells from conventional interventions, leading to therapeutic failure [16]. It is well known that the TME is a multicellular system with complex tumor-stromal interactions [16]. Stromal support is essential for multi-step cancer progression including tumor growth, metastatic dissemination, and ectopic colonization [17]. However, normal fibroblasts have been indicated to function as an inhibitor of cell proliferation and tumorigenesis [18]. Therefore, reprogrammed fibroblasts, also known as CFAs, are proposed to explain the pro-tumorigenic ability of fibroblasts in tumor stroma.

As the most abundant components in the tumor stroma, CAFs play a key role in the regulation of TME [15]. The role of CAFs has been extensively studied in vitro. Compared to normal fibroblasts, CAFs express different proteins and usually exhibit enhanced proliferative and migratory properties [19]. The most unique feature of CAFs is their ability to remodel the TME by their high capacity for cytokine secretions, including GM-CSF, TGFβ, IL-6, and CCL2 [20]. These cytokines can further recruit immunosuppressive cells into the tumor stroma, and lead to immune evasion [20]. One of the most important sources of CAFs is epithelial or endothelial cells adjacent to cancer cells undergoing EMT to form stromal cells, and further, transform into CAFs [21]. During the development of CSCC, normal epithelial cells gradually disappeared and are replaced by cancer cells and tumor stromal cells. Do these epithelial cells undergo apoptosis in the process of tumor dilatation? Or do they differentiate into another kind of cells to regulate CSCC progression? Previous studies have indicated that EMT occurs during CSCC oncogenesis and progression [22, 23]. So, can these normal epithelial cells be transformed into CAFs via EMT and further be involved in CSCC progression? No answer has been given in previous reports.

As a key regulator of HMT, WDR5 is crucial for H3K4me3, chromatin remodeling, and transcriptional activation of target genes [8]. It was also proven to function as an oncogenic protein and might serve as a novel epigenetic target in cancer treatment [9]. The role of WDR5 has been investigated in pancreatic cancer [24], breast cancer [25], prostate cancer [26], etc. However, its role in CC has not been explored yet. In this study, we proved that WDR5 expression was up-regulated in CSCC, and its level in cancer tissue is positively correlated with the TNM stage of patients, suggesting that WDR5 is an oncogenic protein that promotes the development of CSCC. A recent study reveals that WDR5 facilitates EMT and metastasis of cholangiocarcinoma by changing chromatin opening and target gene expression [27], however, no study has investigated the role of WDR5 in EMT in CSCC and its relationship with CFAs formation. Again, our results revealed that highly expressed WDR5 facilitates EMT of normal cervical epithelial cells, and
promotes the transformation of CAFs from epithelial cells, so as to drive the progression of CSCC. More importantly, we found a way to block the function of WDR5 by hindering the interaction between WDR5 and MLL1, and proved that loss of WDR5 function suppressed CSCC growth *in vivo*, and this may provide us a novel approach for CSCC treatment.

In conclusion, for the first time, this study investigated the role of WDR5 in CSCC development from the perspective of CAFs, and proved that highly expressed WDR5 induces EMT, promotes the formation of CAFs from epithelial cells, and enhanced the secretions of cytokines, thus driving the progression of CSCC.

**Declarations**

**Acknowledgements**

Not applicable.

**Author contributions**

Conception: Honglin Song and Fangli Sun; Interpretation or analysis of data: Fangli Sun, Nengxian Wu, Ying Lan, Qiuping Lu, and Linmei Mo; Preparation of the manuscript: Fangli Sun and Linmei Mo; Revision for important intellectual content: Honglin Song and Fangli Sun; Supervision: Honglin Song

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**Data availability**

Raw data and images can be obtained from the authors

**Code availability**

Not applicable.

**Conflict of interest**

All authors in this study declare that they have no conflict of interests.

**Ethical approval**
The usage of human tissues was approved by the Ethics Committee of the Guangxi Medical University Cancer Hospital (No.: H2019018.v03). Animal experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC). All animal treatments were approved by the Ethics Committee of the Guangxi Medical University Cancer Hospital (No.: KA190114.v02).

Consent to participate

All authors stated consent for participation.

Consent for Publication

For all authors the final version of the manuscript was available and consent for publication was stated.

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Tables

Table 1 Sequence of siRNAs and primers
|CXCL8 promoter 3'-5'| Forward/Sense (5'-3') | Reverse/Antisense (5'-3') |
|---|---|---|
|207-323| TCCTTTCTCACTAGGTGGC| TCATACCTGACGCTGTGCTA|
|411-532| TGGCTATGTTGAAATT CCT| GCACATGCCAGTAATCCCC|
|632-776| TAACCTTCTGCTCAAGGAC| AGCCAAAATAGAGAGACGCAA|
|925-1050| GGTCCTAGTCTCGCAC CCT| CTGCCCCACCCCTTTTCG GG|
|1074-1223| GGCCTTCCCAGTGACCTCT| ACCACACTTCGAGGATCAC GTC|
|1308-1433| GCCATCTGTGCGCCACTAT CTTT| GAGCACCTCGAACCGACTCC|
|1456-1602| AGGCACACTCTCCCCTCTAC CCTT| CCGCCTGCTCTTCCGCCCCT G|
|1623-1804| TGCTGGCTTTTTGGACACCC ACTT| CCGCCGCCACCTTGTGAGGA|

siRNAs

|Gene| Forward/Sense (5'-3') | Reverse/Antisense (5'-3') |
|---|---|---|
|si-NC| GAGGGGCUCACCGACACUCdTdT| GGCGAMGMGAUAGUAGUCAdTd T|
|si-WDR5| AGGGAGAUAUMGAACACAAdTd T| UMGMGUUCAAUUUCUCCC UdTd T|
|si-CXCL8| CCAGCAAMGUCAMGUAUAdTd T| AUACAGAMGMGAUAACMGdGd |
|Gene| Forward/Sense (5'-3') | Reverse/Antisense (5'-3') |
|---|---|---|
|WDR5| ATGCGACAGAGACCATCATAG| CGTGAGGATATGGGATG TGA A|
|GAPDH| ACAGTCAGCCGCGATCTTCTT | GTTAAAACGAGCCCTGGGTGA |

Abbreviation: CXCL8: C-X-C motif chemokine ligand 8; WDR5: WD repeat domain 5

**Figures**

**Figure 1**

WDR5 is up-regulated in CSCC tissues compared to that in PCT. Results of (A) immunohistochemistry and (B) RT-qPCR revealed that compared to PCT, WDR5 was up-regulated significantly in CSCC tissues, and its level is positively correlated with the TNM stage of CSCC. Data were shown as mean ± SEM. ***P<0.001. **
Figure 2

CXCL8 is a target of WDR5. (A) Results of ChIP-seq showed that a large number of DNA fragments of CXCL8 were immunoprecipitated by antibody anti-WDR5. (B) Results of WB revealed that CXCL8 expression is regulated by WDR5. (C) ChIP results showed that WDR5 can be recruited to the promoter region of CXCL8. (D) Immunohistochemistry shows that CXCL8 expression is positively correlated with the TNM stage of CSCC. Data were shown as mean ± SEM. ** \( P<0.01 \), *** \( P<0.001 \).
Figure 3

CXCL8 induces the formation of CAFs. (A) Overexpression of CXCL8 promoted EMT of hCECs, and made the hCECs present the shape of stromal cells. (B) Markers of EMT in hCECs all changed significantly after CXCL8 overexpression. (C) FAP (green) expression was significantly up-regulated in hCECs after CXCL8 overexpression. (D) Secretions of cytokines including GM-CSF, IL-6, CCL2, and TGFβ were all enhanced in hCECs after CXCL8 overexpression. (E) The invasive ability of CaSki cells was enhanced markedly after
being incubated with the culture medium from hCECs overexpressing CXCL8. Data were shown as mean ± SEM. ** $P<0.01$, *** $P<0.001$.

Figure 4

Loss of CXCL8 function makes CAFs transformed into epithelial cells. (A) Knock-down of CXCL8 reversed EMT of CAFs, and made the CAFs present the shape of epithelial cells. (B) Markers of EMT in CAFs all changed significantly after the CXCL8 knock-down. (C) FAP (green) expression was significantly down-regulated in CAFs after CXCL8 knock-down. (D) Secretions of cytokines including GM-CSF, IL-6, CCL2, and
TGFβ were all attenuated after CXCL8 knock-down. (E) The invasive ability of CaSki cells was attenuated markedly after being incubated with the culture medium from CAFs with CXCL8 knock-down. Data were shown as mean ± SEM. ** $P<0.01$, ***$P<0.001$. 

Figure 5
CXCL8 is regulated by the interaction between WDR5 and MLL1. (A and C) Expression of CXCL8 in CAFs was down-regulated after WDR5 knock-down, while the knock-down of WDR5 had no impact on CXCL8 expression when MM102 was used, and MM102 markedly inhibited CXCL8 expression in CAFs. (B and D) Expression of CXCL8 in hCECs was up-regulated after WDR5 overexpression, while the overexpression of WDR5 had no impact on CXCL8 expression when MM102 was used (E and F) ChIP analysis revealed that the distributions of WDR5, MLL1, and H3K4me3 in CXCL8 promoter were overlapping, and the recruitments of WDR5 and MLL1 in CXCL8 promoter were significantly higher in CAFs than that in hCECs, as well as the H3K4me3 level in this region. Data were shown as mean ± SEM. ** $P<0.01$, ***$P<0.001$. 
Figure 6

Blocking the interaction between WDR5 and MLL1 suppresses CC development. **A** Using MM102 significantly suppressed tumor growth. **B and C** Using MM102 (10 ug/g) markedly down-regulated the expressions of CXCL8 and FAP in tumor lesions. **D** Using MM102 (10 ug/g) markedly changed the expression levels of EMT markers. **E** Using MM102 (10 ug/g) significantly down-regulated cytokines secretions in tumor lesions. Data were shown as mean ± SEM. **P<0.01, ***P<0.001.
Overview of the mechanism of WDR5 promoting CSCC development. WDR5 promotes the EMT process and enhances the transformation of CAFs from normal epithelial cells. CAFs secret cytokines such as GM-CSF, IL-6, CCL2, and TGFβ, and further drives CSCC progression.
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