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ORIGINAL RESEARCH

DSG2 and c-MYC Interact to Regulate the Expression of ADAM17 and Promote the Development of Cervical Cancer

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Purpose: To explore the effect of DSG2 on the growth of cervical cancer cells and its possible regulatory mechanism.

Methods: The expression levels and survival prognosis of DSG2 and ADAM17 in cervical squamous cell carcinoma tissues and adjacent normal tissues were analyzed by bioinformatics. CCK-8 assay, colony formation assay and Transwell assay were used to detect the effects of DSG2 on the proliferative activity, colony formation ability and migration ability of SiHa and Hela cells. The effect of DSG 2 on the level of ADAM17 transcription and translation was detected by qPCR and Western blot experiments. The interaction between DSG2 and c-MYC was detected by immunocoprecipitation. c-MYC inhibitors were used in HeLa cells over-expressing DSG2 to analyze the effects of DSG2 and c-MYC on proliferation, colony formation and migration of Hela cells, as well as the regulation of ADAM17 expression.

Results: DSG2 was highly expressed in cervical squamous cell carcinoma compared with normal tissues ($P<0.05$), and high DSG2 expression suggested poor overall survival ($P<0.05$). After DSG2 knockdown, the proliferative activity, colony formation and migration ability of SiHa and Hela cells were significantly decreased ($P<0.05$). Compared with adjacent normal tissues, ADAM17 was highly expressed in cervical squamous cell carcinoma ($P<0.05$), and high ADAM17 expression suggested poor overall survival in cervical cancer patients ($P<0.05$). The results of immunocoprecipitation showed the interaction between DSG2 and c-MYC. Compared with DSG2 overexpression group, DSG2 overexpression combined with c-MYC inhibition group significantly decreased cell proliferation, migration and ADAM17 expression ($P < 0.05$).

Conclusion: DSG2 is highly expressed in cervical cancer, and inhibition of DSG2 expression can reduce the proliferation and migration ability of cervical cancer cells, which may be related to the regulation of ADAM17 expression through c-MYC interaction.

Keywords: cervical cancer, proliferation, migration, DSG2, c-MYC, ADAM17

Introduction

Cervical cancer is one of the most common malignant tumors in women, the histopathological type of cervical cancer is mainly squamous carcinoma and a few are adenocarcinoma. In 2020, there were about 604,000 new cases of cervical cancer and about 342,000 cervical cancer deaths worldwide.[1] Although the means of diagnosis and treatment of cervical cancer are constantly improving, the incidence of cervical cancer continues to rise and shows a trend of younger age. At present, the 5-year survival rate of patients with advanced cervical cancer is still very low, and the pathogenesis of cervical cancer is not very clear, which is one of the important causes of death of patients. Therefore, it is of great significance for clinical treatment of cervical cancer to conduct in-depth research on the potential molecular mechanisms that promote the progression of cervical cancer and provide effective therapeutic targets.

Desmoglein-2 (DSG2) is one of the most widely expressed transmembrane proteins in tissues, and the gene is located on chromosome 18q12.1. As a member of the classical cadherin family, DSG2 regulates intercellular connectivity and promotes desmosome assembly. Desmosomes are related to cell adhesion junctions, which play an important role in the

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genesis and development of tumors, especially in the migration and invasion of cancer cells. A large number of studies have confirmed that DSG2 can participate in the occurrence and development of various tumors as an oncogene. Chen et al found that DSG2 could confer stem cells with malignancy through wnt/β-catenin signaling pathway.[2] DSG2 could regulate lung cancer progression through EGFR/Scr/PAK1 pathway, and its high expression increased tumor resistance to osimertinib.[3,4] DSG2 was involved in the development of ovarian cancer and could be used as a biomarker for the prognosis of ovarian cancer.[5,6] Moreover, studies also showed that DSG2 could promote the proliferation of breast cancer cells by regulating the ERK signaling pathway and ALDH1A1 expression.[7] However, the role of DSG2 in cervical cancer has been poorly reported, and the mechanism of DSG2 involvement in the process of cervical cancer remains unclear. The purpose of this study was to investigate the role of DSG2 in cervical cancer and its regulatory mechanism, and to provide a theoretical basis for the targeted therapy of cervical cancer.

Materials and Methods

Cell Lines and Cell Culture

Human cervical cancer cells (HeLa and SiHa) were obtained from the Shanghai Institute of Cell Biology, Chinese Academy of Sciences (Shanghai, China). The cells were maintained at 37°C in a 5% CO_2 incubator in DMEM medium supplemented with 10% FBS (Life Technologies) and 1% Penicillin/Streptomycin. The cell lines were authenticated using short tandem repeat (STR) profiling by Genetic Testing Biotechnology Corporation (Suzhou, China) and routinely tested to exclude mycoplasma contamination.

Quantitative Real-Time PCR (RT-qPCR)

Total RNA was isolated from cells using a Trizol (Invitrogen) standard protocol. Synthesis of complementary DNAs (cDNAs) was completed using a HiScript 1st Strand cDNA Synthesis Kit (Vazyme Biotech, China). Real-time quantitative PCR reactions were then performed on a StepOnePlus™ Real-Time PCR System (Thermo Scientific) using AceQ qPCR SYBR Green Master Mix (Vazyme Biotech, China). Relative mRNA levels of target genes were analyzed using comparative Ct methods where Ct was the cycle threshold number normalized to GAPDH. Each reaction was performed in triplicate. The primers are shown in Table S1.

Western Blot (WB) and Co-Immunoprecipitation (Co-IP) Assay

Total protein was extracted using cell lysis buffer for Western blots (P0013, Beyotime) containing PMSF (100:1) at 4°C according to the manufacturer's guidelines. The concentration and quality of total protein was detected by Enhanced BCA Protein Assay Kit (P0009, Beyotime). All proteins were separated by SDS-PAGE, followed by semidry electroblotting onto PVDF membranes (Roche). Primary antibodies against DSG2 (21,880-1-AP, 1:1000, Proteintech), ADAM17 (29,948-1-AP, 1:2000, Proteintech) and c-MYC (67,447-1-Ig, 1:5000, Proteintech) were used. HRP-conjugated goat anti-rabbit IgG (AS014, 1:10,000, Abclonal) was used as the secondary antibodies. Protein bands were shown by enhanced chemiluminescence (ECL) method.

Co-IP assay was used to detect the interaction of target proteins in cells. Protein sample was immunoprecipitated by anti-DSG2 (21,880-1-AP, Proteintech), anti-c-MYC (67,447-1-Ig, Proteintech) and IgG (30,000-0-AP, Proteintech) antibodies at 4°C overnight with rotation, followed by the incubation with protein A/G Sepharose beads at 4°C for 2 h. After centrifuge and washing for three times, samples were incubated in IP lysate and loading buffer at 100°C for 5 min. Protein was separated by SDS-page and subjected to Western blotting with antibodies to DSG2 and c-MYC.

CCK-8 Assay

For CCK-8 assay, cells were seeded into 96-well plates at a concentration of 3000 cells/well. At different time points (0, 24, 48, 72 and 96 h) following incubation at 37°C, the CCK-8 assay was carried out by adding 10 μL CCK-8 reagent (Vazyme Biotech) to each well. Cell proliferation was determined by measuring the absorbance at 450 nm using a microplate reader (Safire, TECAN).

Colony Formation Assay

Cells were seeded in triplicate at a density of 1000 cells per well in six-well plates and continuing cultured 1–2 weeks for colony forming. The medium was changed every three days, and the colonies were fixed with methanol and stained with 0.1% crystal violet (Sangon Biotech, China).

Transwell Assay

Transwell assay was performed with a pore size of 8 μm (Corning, USA) in the Transwell chamber. Cells were digested by trypsin, resuspended into cell suspension (50,000 cells/well) and seeded into the upper chamber, and medium supplemented with 20% FBS was added to the bottom chamber. After incubation 24 h at 37°C, the migratory cells on the lower surface of Polycarbonate membrane were fixed in 100% methanol for 10 min, stained with 0.1% crystal violet for 15 min, and washed with PBS. Values for migration were evaluated by counting five fields per membrane under a microscope (OLYMPUS) at ×100 magnification.

Bioinformatics Analysis

Bioinformatic analysis was performed based on a combination of R, command line, and web-based bioinformatics tools. The following public databases were searched: the Cancer Genome Atlas (TCGA) database (https://gdac.broadinstitute.org/), Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/), and Cbioportal database (https://www.cbioportal.org/). BioGRID (http://thebiogrid.org/) and IntAct (https://www.ebi.ac.uk/intact/home) for protein interaction prediction.

Statistical Analysis

All experiments were performed in triplicate and data were shown as mean ± standard deviation unless otherwise indicated. Statistical analyses and graphs were performed by GraphPad Prism 7.0 (GraphPad Software, San Diego, CA, USA) and P value <0.05 as statistically significant. The significance difference between two groups was determined using the two-tailed Student's *T*–test or One-way ANOVA analysis.

Results

DSG2 is Up-Regulated in Human Cervical Cancer Tissues and is Associated with a Poor Prognosis in Cervical Cancer Patients

By analyzing the published mRNA expression profiles in the data obtained from The Cancer Genome Atlas (TCGA) database, we discovered that the DSG2 mRNA was noticeably upregulated in cervical cancer tissues compared with that in normal tissues (Figure 1A). Furthermore, analysis of datasets from TCGA database showed that high DSG2 expression in cervical cancer patients resulted in a shorter survival time (Figure 1B).

Knockdown of DSG2 Inhibites Proliferation and Migration of Cervical Cancer Cells

In order to further clarify the role of DSG2 in human cervical cancer, we conducted a series of cell function assays in HeLa and SiHa. As shown in Figure 2A, the si-DSG2-2 sequences with the highest knockdown efficiency of DSG2 in cervical cancer cell-line HeLa ($P < 0.01$). The qPCR Results showed that the knockdown rate of DSG2 in si-DSG2 group was 79.9% ($P < 0.05$) and 84.8% ($P < 0.01$) compared with si-NC in HeLa and SiHa cells (Figure 2B). Protein expression of DSG2 in si-DSG2 group was downregulated compared to si-NC group (Figure 2C). Thus, DSG2 in HeLa and SiHa cells was knocked down.

Based on the results of CCK-8 assay, we found that HeLa and SiHa cells in si-DSG2 group exhibited slower proliferation rate than that in si-NC group ($P < 0.01$) (Figure 3A). Consistently, the knockdown of DSG2 in HeLa and SiHa produced smaller and fewer cell clones ($P < 0.05$) (Figure 3B). Furthermore, we conducted Transwell assay to observe the effect of DSG2 on the migration ability of cervical cancer cells, and the results showed that the migration ability of HeLa and SiHa cells in si-DSG2 group was significantly reduced compared with those in si-NC group ($P < 0.001$) (Figure 3C).

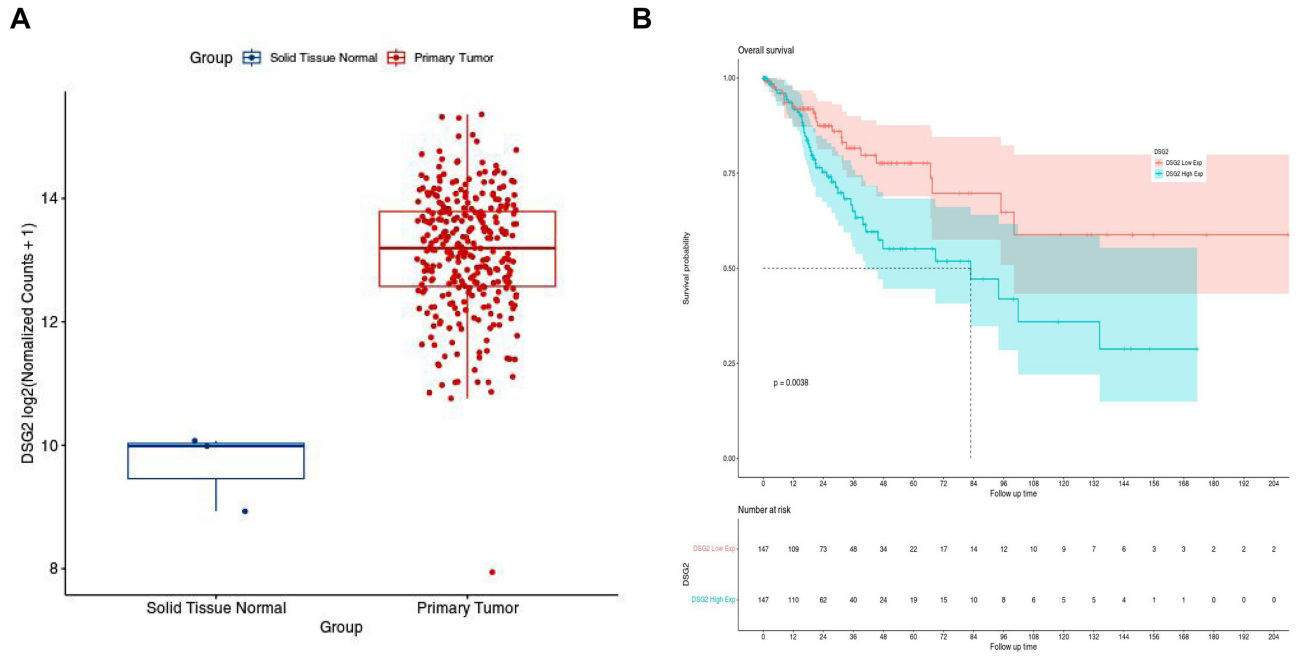

Figure 1 DSG2 is up-regulated in human cervical cancer and is associated with a poor prognosis. (**A**) Higher expression of DSG2 was found in cervical cancer samples than the normal tissues (based on TCGA database). (**B**) Kaplan–Meier plots of overall survival for cervical cancer samples with high/low DSG2 expression from the TCGA database.

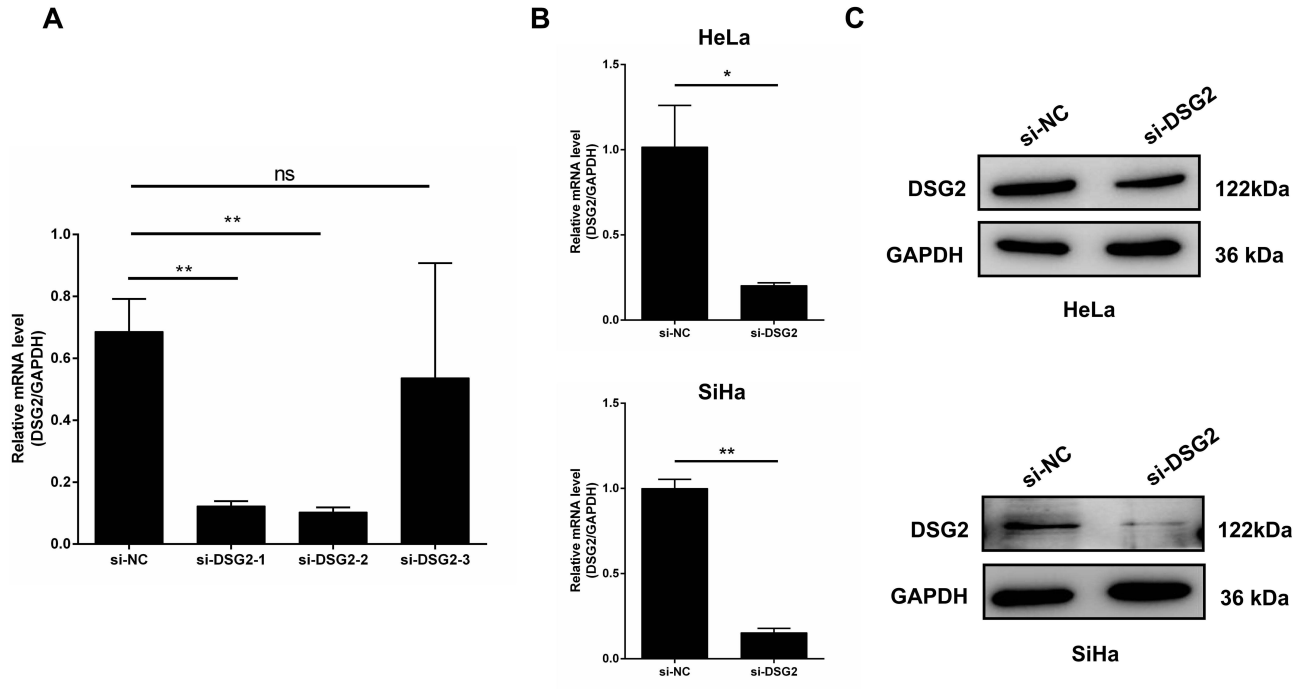

Figure 2 Knockdown of DSG2 in cervical cancer cells. (**A**) The knockdown efficiency of the three groups of siRNA in HeLa cells was 84%, 86% and 28%, respectively. (**B** and **C**) The specificity and validity of the siRNA knockdown of DSG2 expression in HeLa and SiHa cells was verified by qPCR (**B**) and WB (**C**). *P < 0.05, **P < 0.01.

DSG2 Regulates the Expression of ADAM17 in Cervical Cancer

In order to explore the potential downstream effector of DSG2 in cervical cancer, coexpedia online tool was used for identifying genes with co-expression profile with DSG2. ADAM17, which is well known as a strong regulator in tumor development, was focused. As indicated by the bioinformatics analysis of TCGA-CHOL dataset, ADAM17 expression showed high consistency with that of DSG2 (Figure 4A). We further to analyze ADAM17 expression and survival prognosis differences in cervical cancer

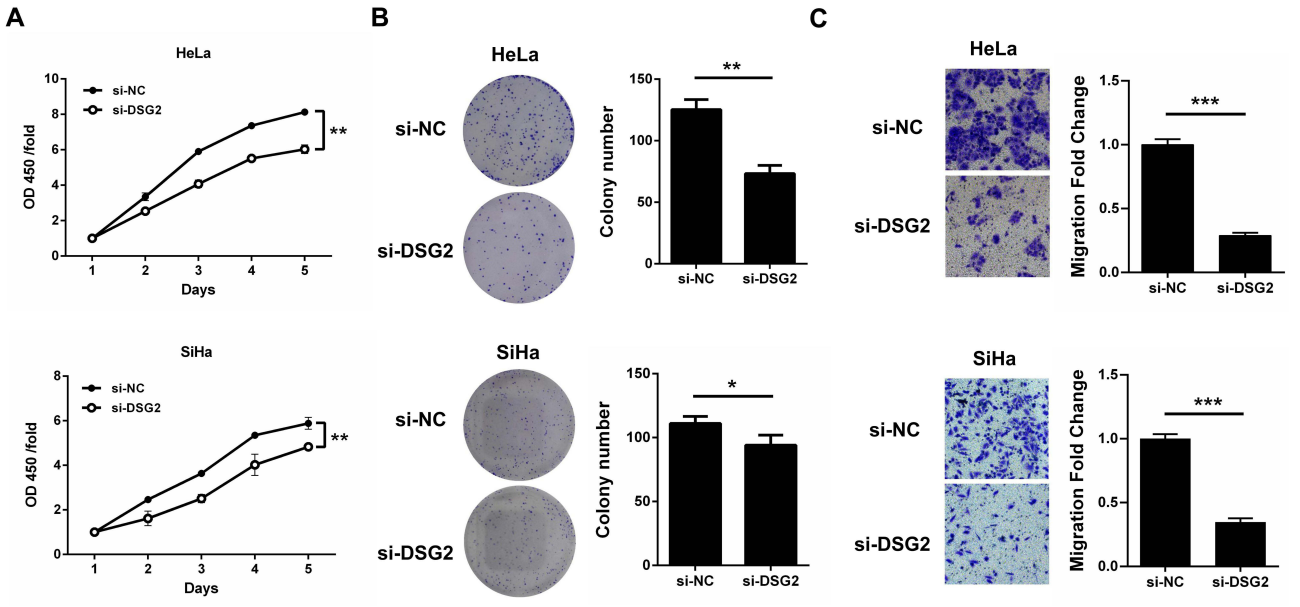

Figure 3 Knockdown of DSG2 inhibited proliferation and migration of cervical cancer cells. (**A**) The proliferation of HeLa and SiHa cells after knockdown of DSG2 was measured using CCK-8 assay. (**B**) The effect of DSG2 knockdown on cervical cancer cell clone formation. (**C**) The migration ability of HeLa and SiHa cells after knockdown of DSG2 was measured using Transwell assay. *P < 0.05, **P < 0.01, ***P < 0.001.

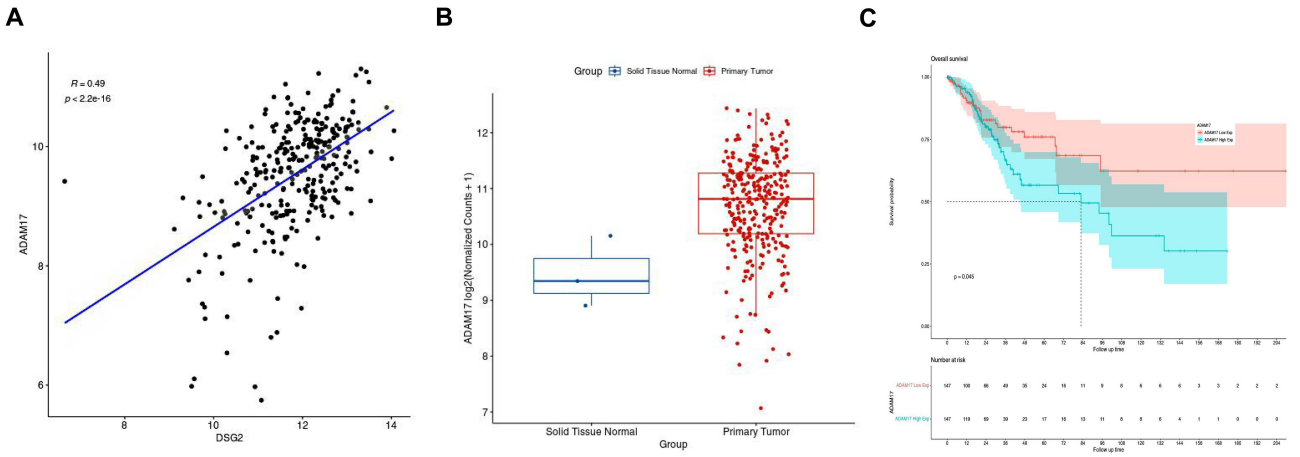

Figure 4 ADAM17 is up-regulated in human cervical cancer and is associated with a poor prognosis. (**A**) The bioinformatics analysis of TCGA-CHOL dataset showed the positive expression correlation between DSG2 and ADAM17. (**B**) Higher expression of ADAM17 was found in cervical cancer samples than the normal tissues (based on TCGA database). (**C**) Kaplan–Meier plots of overall survival for cervical cancer samples with high/low ADAM17 expression from the TCGA database.

tissues. By analyzing the published mRNA expression profiles in the data obtained from TCGA database, we discovered that ADAM17 was significantly higher expressed in cervical cancer tissues than normal tissues (Figure 4B), and high ADAM17 expression in cervical cancer patients resulted in a shorter survival time (Figure 4C).

We further observed the effect of DSG2 on ADAM17 expression in cervical cancer cells. The results showed that the mRNA (Figure 5A) and protein (Figure 5B) expression levels of ADAM17 were significantly decreased after DSG2 knockdown in HeLa and SiHa cells. The above results reminded us that the regulation of ADAM17 expression may be a critical route for DSG2 to regulate cervical cancer development. In addition, our previous predictive analysis found a strong interaction between DSG2 and c-MYC, and we further confirmed the interaction in HeLa cells. The results were detected by Co-IP assay in Figure 5C, DSG2 and c-MYC interacted in cervical cancer cells. The protein–protein interaction between DSG2 and c-MYC may be the reason why DSG2 could mediate the expression of ADAM17.

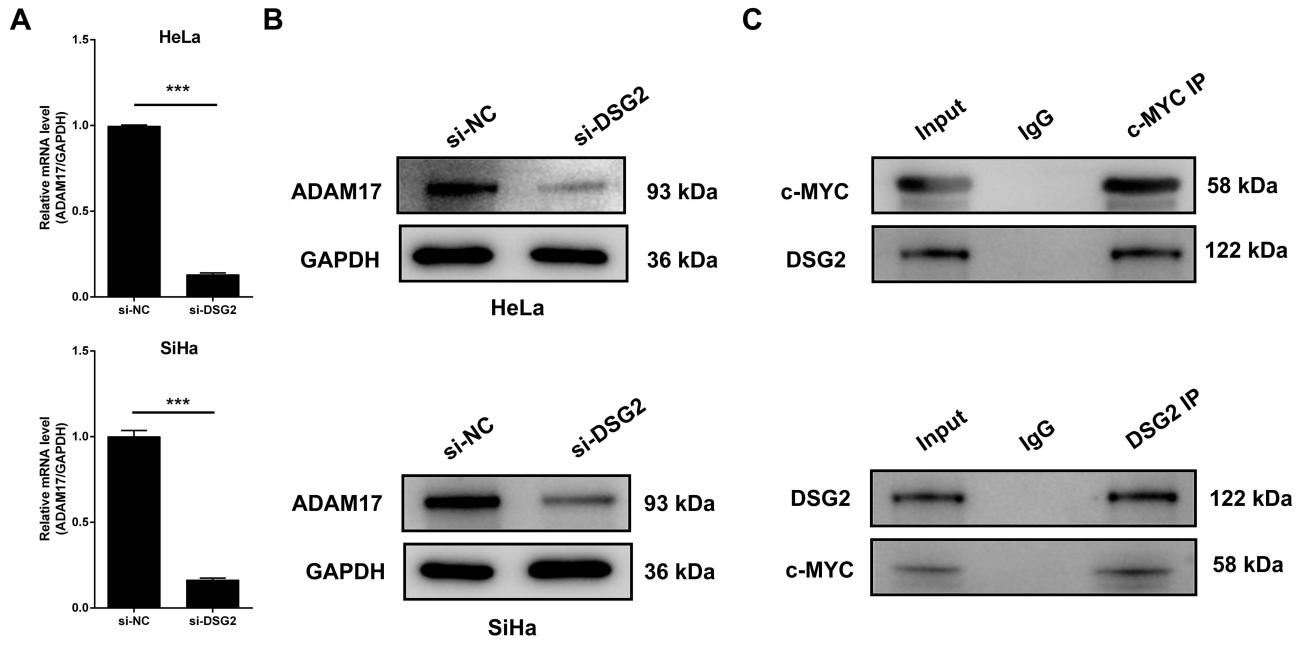

Figure 5 DSG2 regulates the expression of ADAM17 in cervical cancer. (**A**) The effect of DSG2 knockdown on ADAM17mRNA expression was detected by qPCR. (**B**) The effect of DSG2 knockdown on ADAM17 protein expression was detected by WB. (**C**) The interaction between DSG2 and c-MYC was detected by Co-IP assay. ***P < 0.001.

DSG2 Regulates Cervical Cancer Development by Interacting with c-MYC

We further observed the effects of DSG2 and c-MYC interaction on the proliferative activity and migration ability of cervical cancer cells. We transfected pcDNA (3.1)-DSG2 overexpression plasmid into HeLa cells, and detected the overexpression efficiency by qPCR and WB. The results showed that DSG2 mRNA (Figure 6A) and protein (Figure 6B) levels were significantly increased after transfection of DGS2 plasmid. DSG2 overexpressed HeLa cells were treated with c-MYC inhibitor (10,058-F4, 50 μM) to detect the effects of DSG2 and c-MYC on cell proliferation and migration. As shown in Figure 6C and 6D, DSG2 overexpression could significantly enhance cell proliferation activity and migration ability, and adding c-MYC inhibitor partially reversed the promoting effect of DSG2 overexpression on the proliferation (Figure 6C) and migration (Figure 6D) of HeLa cells. In addition, we examined the effects of DSG2 and c-MYC on the expression of ADAM17 protein. The results showed that c-MYC inhibitor could partially reverse the promoting effect of DSG2 on the expression of ADAM17 (Figure 6E). Accordingly, the interaction between DSG2 and c-MYC mediates the expression of ADAM17, which may be the reason why DSG2 affects the development of cervical cancer.

Discussion

Cervical cancer is one of the most common gynecological tumors, and its molecular mechanism is still not well understood. With the continuous exploration of the relationship between DSG2 and tumorigenesis, it has been found that DSG2 can play different or even opposite roles in different tumors through multiple transduction pathways. DSG2 is an important protein in the maintenance of intercellular adhesion, which can regulate cell apoptosis through enzyme-substrate action and cell proliferation through signal transduction.[8,9] Studies have found that DSG2 is overexpressed in human cervical cancer cells, and affects the malignant behavior of cervical cancer cells by mediating the activation of MAPK pathway, and the knockout of DSG2 can inhibit the proliferation, migration and invasion of cervical cancer cells.[10] However, the effect of DSG2 on the development of cervical cancer and its specific molecular mechanism still need to be further studied.

Bioinformatics analysis showed that DSG2 and ADAM17 were highly expressed in cervical cancer tissues, and their high expression suggested poor overall survival in cervical cancer patients, which is consistent with the findings that DSG2 and ADAM17 expression are associated with poor prognosis and contribute to the development of cervical cancer.[11,12] ADAM17 is a matrix metalloproteinase, which can hydrolyze MICA and MICB on the surface of tumor cells to prevent them from binding to the activated receptor NKG2D on the surface of NK cells, so that tumor cells can escape the immune killing of NK

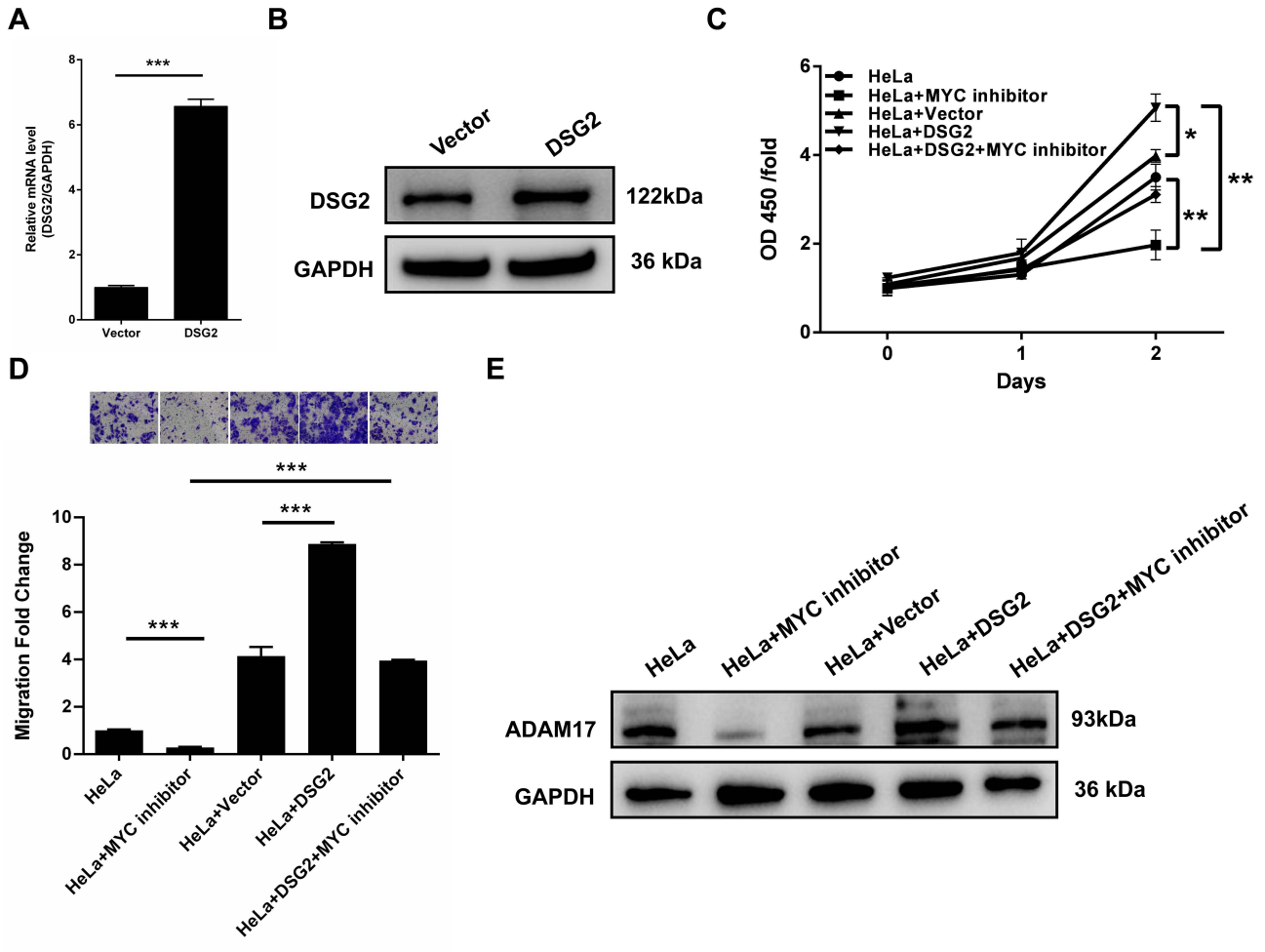

Figure 6 DSG2 regulates cervical cancer development by interacting with c-MYC. (**A**) HeLa cells were transfected with pcDNA(3.1)-DSG2 overexpression plasmid, and the mRNA level of DSG2 was detected by qPCR. (**B**) HeLa cells were transfected with pcDNA(3.1) overexpression plasmid, and the protein level of DSG2 was detected by WB. (**C** and **D**) DSG2 overexpressed HeLa cells were treated with a C-MYC inhibitor (10,058-F4, 50 μM), and the proliferative activity and migration ability were detected by CCK-8 (**C**) and clonal formation assay (**D**). (**E**) DSG2 overexpressed HeLa cells were treated with a C-MYC inhibitor, and ADAM17 protein expression was detected by WB.*P < 0.05, **P < 0.01, ***P < 0.001.

cells and promote tumor progression.[13] Previous studies have shown that ADAM17 is low expressed in normal tissues and cells, but highly expressed in various malignant tumors, which promotes the occurrence and development of the cancers.[14,15] Our study found that down-regulation of DSG2 expression could significantly reduce the proliferative activity, colony formation ability and migration ability of cervical cancer cells, and inhibit the expression level of ADAM17. Conversely, up-regulated DSG2 expression significantly enhanced the proliferative activity, colony formation ability and migration ability of cervical cancer cells, and significantly increased the expression level of ADAM17.

The proto-oncogene c-MYC, localized on chromosome 8q24, has three exons and two introns. c-MYC is mainly activated by amplification and rearrangement, which are mostly found between chromosomes 8 and 14 and plays a key role in the regulation of cell proliferation, differentiation and apoptosis.[16,17] In this study, we preliminarily found that DSG2 may interact with c-MYC to co-regulate ADAM17 expression in cervical cancer through bioinformatics analysis. Further experimental studies showed that compared with cervical cancer cells overexpressing DGS2, their proliferative activity and migration ability were significantly reduced after c-MYC inhibition, which may be related to the down-regulation of ADAM17 expression.

Conclusions

In our study, we showed that DSG2 and c-MYC interact to regulate ADAM17 expression in cervical cancer cells, thereby affecting the malignant biological behavior of cervical cancer cells. The above results provide an experimental basis for the treatment of cervical cancer.

Ethics Approval

Ethics approval was received from the Medical Ethics Committee of Tongji Medical College of Huazhong University of Science and Technology.

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Disclosure

The authors declare no conflicts of interest in this work.

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