

# Histone demethylase KDM4C is required for ovarian cancer stem cell maintenance

Guoqing Chen (✉ [chenguoqing@163.net](mailto:chenguoqing@163.net))

Southern Medical University Nanfang Hospital

Ping Ye

First Affiliated Hospital Of Gannan Medical College

Fa Zeng

Southern Medical University

Xionshan Zhu

Southern Medical University

Lu Chen

Southern Medical University

Yan Huang

Southern Medical University

Ling Xu

First Affiliated Hospital Of Gannan Medical College

Xiaoying Xie

First Affiliated Hospital Of Gannan Medical College

---

## Research

**Keywords:** KDM4C, ovarian cancer, OCT-4, cancer stem cells

**Posted Date:** December 18th, 2019

**DOI:** <https://doi.org/10.21203/rs.2.19188/v1>

**License:**  This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

---

**Version of Record:** A version of this preprint was published at Stem Cells International on August 29th, 2020. See the published version at <https://doi.org/10.1155/2020/8860185>.

# Abstract

Ovarian cancer is a highly deadly disease, which is often diagnosed at a late stage with metastases. However, most ovarian cancers relapse after surgery combined with platinum based chemotherapy. Cancer stem cells (CSCs) are stem-like cells that possess high tumorigenic capability and display higher resistant capability against current therapies. However, our knowledge of ovarian CSCs and their molecular mechanism remain sparse. In current study, we found that KDM4C, a histone demethylase, was required for ovarian cancer stem cells (CSCs) maintenance. Depletion of KDM4C significantly reduced the CSCs population and sphere formation in vitro. Moreover, we found that KDM4C can regulate the expression of stem cell factor OCT-4 via binding to its promoter. These data indicate that KDM4C is relevant for ovarian CSCs maintenance and underscore its importance as a potential therapeutic target.

## Introduction

Ovarian cancer has the highest mortality rate of any gynecological malignancies around the world [1]. Ovarian cancer has an asymptomatic onset and the majority of cases diagnosed are in the late stages with metastasis. Currently, surgery and chemotherapy are the main treatments for ovarian cancer, whereas a large majority of patients with advanced ovarian cancer relapse due to therapy resistance [2]. To overcome this dilemma in ovarian cancer, novel strategies such as targeted therapy and immunotherapy have been developed but have shown limited effects so far [3]. The recently proposed hypothesis of cancer stem cells (CSCs), also known as tumor-initiating cells, may provide a more effective approach for the treatment of ovarian cancer. CSCs are stem-like cells that possess the tumorigenic capability to self-renew and differentiate into multiple cell types [4]. Moreover, CSCs display higher resistant capability against current therapies and are believed to be responsible for tumor metastasis [5, 6]. Hence, understanding of the molecular regulation mechanism of CSCs might provide new targets for treatment of ovarian cancer.

In recent years, histone modifications, such as histone methylation and acetylation, are emerging as critical mechanisms to regulate gene expression [7]. It plays an important role for coordination and organization of the chromatin structure during a variety of biological processes such as DNA replication, repair and transcription [7]. In many tumors, changes of histone demethylases expression have been identified as a key characteristic during cancer initiation and progression, suggesting these genes might be functional important for cancer development [8]. Indeed, histone demethylases, such as KDM3A and KDM6A, have been shown to be essential for maintenance of CSCs in several types of cancers [9–12].

Deregulation of KDM4C, a H3K9me3 and H3K9me2 demethylase [13], has been identified in several solid tumors, such as esophageal squamous carcinoma, lung cancer, pancreas cancer, breast cancer [14–17]. In this study, we used sphere culture approach to enrich CSCs population of ovarian cancer and found that KDM4C is upregulated in the tumorspheres. Furthermore, we found that depletion of KDM4C inhibits the migration, invasion and CSCs properties of ovarian cancer cells. Finally, our data revealed that

KDM4C can regulate the expression of stem cell factor OCT-4 via binding to its promoter. In summary, we identify a crucial role of KDM4C in the maintenance of CSCs in ovarian cancer.

## Results

### 1. KDM4C is upregulated in the CSCs population in ovarian cancer cell lines

Cancer stem cells display greater ability to form spheres when placed in low attachment conditions in defined serum-free media (Figure 1A). To investigate the epigenetic regulation in ovarian cancer cells, we first performed qRT-PCR assay to detect the transcription level of 21 histone demethylases in SK-OV-3 ovarian cancer cells grown under adherent or sphere-forming conditions (Figure 1A, B). Our results demonstrated that KDM4C and KDM5C were significantly increased in SK-OV-3 spheres, while the rest of the histone demethylases showed comparable mRNA expression levels (Figure 1B). We observed a similar elevation of KDM4C mRNA expression and a significant decrease of KDM3C expression when comparing adherent and sphere culture of HO-8910 ovarian cancer cells (Figure 1C). Together, our data suggested that KDM4C might play a role in ovarian CSCs maintenance. In addition, Western Blot results showed that KDM4C protein level was increased in SK-OV-3 and HO-8910 sphere when compared with monolayer culture (Figure 1D). Consistent with previous reports, stem cells factor OCT4 was also enriched in these CSCs (Figure 1D) [18].

### 2. Downregulation of KDM4C inhibits cell migration and invasion of ovarian cancer cells

Next, we sought to investigate the function of KDM4C in the ovarian CSCs by generation of two stably transfected KDM4C shRNA in SK-OV-3 and HO-8910 cells. Both cell lines transfected with shRNA showed dramatic inhibition of KDM4C mRNA and protein level compared with the cells transfected with scramble control shRNA (Figure 2A, B). Transwell assay results showed that KDM4C downregulation significantly reduced the invasiveness of SK-OV-3 and HO-8910 cells (Figure 2C-E). In addition, depletion of KDM4C led to inhibition of migration ability of SK-OV-3 and HO-8910 cells (Figure 2F-H).

### 3. Downregulation of KDM4C inhibits CSCs properties of ovarian cancer cells

Sphere formation and colony formation assay were performed to examine the effect of KDM4C on CSCs properties of SK-OV-3 and HO-8910 cells. Our results revealed that sphere forming capacity was markedly diminished after depletion of KDM4C in SK-OV-3 and HO-8910 cells (Figure 3A-C). In addition, cell colonies were significantly reduced after KDM4C knockdown in SK-OV-3 and HO-8910 cells (Figure 3D-F). Furthermore, we evaluated the aldehyde dehydrogenase (ALDH) activity, a known stem cell feature, of ovarian cancer cells after KDM4C knockdown using flow cytometry. The results showed that the percentage of ALDL<sup>high</sup> population was reduced in the KDM4C knockdown cells compared with control (Figure 3G). Altogether, these results indicated that KDM4C is required for maintenance of CSCs characteristics in ovarian cancer cells.

### 4. KDM4C regulates OCT-4 gene expression via binding to its promoter.

KDM4C-mediated H3K9me3 histone di- and tri-methylation can regulate gene transcription. To study the role of KDM4C in OCT-4 gene expression, we performed chromatin immunoprecipitation (ChIP) assays to examine whether KDM4C can directly binds to the promoter region of OCT-4 using SK-OV-3 sphere. Indeed, our results showed that KDM4C bound to the promoter regions of OCT-4 (Figure 4A). In addition, our data revealed that depletion of KDM4C led to a dramatically increase in levels of di-, and trimethylated H3K9 histones on OCT-4 promoter (Figure 4B-C), consistent with decreased OCT-4 expression in these spheres (Figure 4D).

## Discussion

KDM4C, an oncogene that is frequently amplified in esophageal squamous cell carcinomas, is able to demethylate tri- and dimethylated lysine 9 on histone H3 and activates subsequent oncogenic pathways [13, 17]. Furthermore, the important role of KDM4C in CSCs maintenance has made this epigenetic factor a promising target for cancer intervention [19–21].

In this study, we used sphere culture assay to enrich the CSCs population in ovarian cancer cells. We screened 21 histone demethylases and identified KDM4C is upregulated in both SK-OV-3 and HO-8910 cells, suggesting a role of KDM4C in regulating “stemness” of ovarian cancer cells. We found that silencing of KDM4C led to repressed cell migration and invasion. Consistently, previously study has shown that KDM4C can increase cell migration and invasion via CUL4A in lung cancer [15]. In addition, KDM4C can interact with chromosomes during mitosis to regulate the breast cell proliferation, migration and invasion [16], suggesting a common oncogenic role of KDM4C in different cancer types.

Our results demonstrated that KDM4C is required for CSCs properties. Depletion of KDM4C reduced the sphere-forming, colony-forming ability and the proportion of ALDH<sup>high</sup> population in ovarian cancer cells. It has been reported that stem cell-like chromatin features in human glioblastoma CSCs is linked to a loss of H3K9me3 mark [21]. Genetic knockout mice model results also demonstrated that KDM4 activity is required for hematopoietic stem cell maintenance via accumulation of H3K9me3 on transcription start sites of stem cells-related genes [22]. In addition, our data revealed that KDM4C directly binds to the promoter region of pluripotency factor OCT-4 and regulates its expression. In conclusion, our data provide a novel epigenetic mechanism of CSCs regulation in ovarian cancer.

## Materials And Methods

### 1. Cell Culture

The SK-OV-3 and HO-8910 human ovarian cancer cell line was purchased from China National Infrastructure of Cell Line Resource. For monolayer culture, cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM, Invitrogen, China), containing 10% fetal bovine serum (FBS, Gibco, China), 1% penicillin/streptomycin (Invitrogen, China) at 37 °C under an atmosphere of 5% carbon dioxide and 95%

air. For sphere-forming assay, cells were seeded into 6-well ultra-low attachment plates (Corning, China) and cultured under 1:1 DMEM/F12 medium containing N2 supplement (100x, Invitrogen, China), B27 supplement (50x, Invitrogen, China), basic fibroblast growth factor (bFGF; 10 ng/ml, PeproTech, China) and human recombinant epidermal growth factor (EGF; 10 ng/ml, PeproTech, China).

## **2. shRNA transfection**

Lentiviral particles containing shRNA against human KDM4C and scrambled lentiviral particles were purchased from Genescript (China). In summary, SK-OV-3 and HO-8910 were cultured up to 70% confluence and were then treated with polybrene (Solarbio, China) and lentiviral particles containing shRNA against KDM4C or scrambled particles. Transfected cells were then selected using puromycin (MCE, China), and knockdown efficiency of KDM4C was assessed by qPCR and Western Blot.

## **3. Detection of gene expression by qRT-PCR and Western Blot**

Quantitative reverse-transcription polymerase chain reaction (qRT-PCR) was used to determine the mRNA expression levels of genes. Briefly, total RNA was extracted from cells by using the TRIzol reagent (Invitrogen, China) according to the manufacturer's instructions. RNA was reverse transcribed to cDNA by using a Reverse Transcription Kit (Takara, China). Real-time PCR analyses were performed with Power SYBR Green (Takara, China) and the primers were synthesized at Invitrogen, China. The primers for each gene were based on previous report [23]. Results were normalized to the expression of the human  $\beta$ -actin gene.

For Western blot analysis, the total protein was lysed in Radioimmunoprecipitation assay buffer (RIPA buffer, Solarbio, China). Samples were prepared under reducing conditions by using SDS-PAGE gels before being blotted and detected using anti-KDM4C antibody (Abcam, China), OCT-4 (Abcam, China) and  $\alpha$ -tubulin (Abcam, China).

## **4. Analysis of ALDH high Cell Subsets**

Aldefluor assay kit (Stem Cell) was used to measure the aldehyde dehydrogenase enzymatic activity (ALDH) following the manufacturer's instructions. In summary, 1,000,000 cells were stained in buffer containing ALDH substrate with or without DEAB and incubated at 37°C for 30 min. Cells were rinsed in PBS, and the fluorescence intensity was analyzed using a BD FACS Calibur flow cytometer.

## **5. Wound-healing assay and transwell assay**

Cell migration capability was evaluated by wound-healing assay.  $3 \times 10^5$  cells were plated in 6-well plates for 24 h. Monolayer cells were scraped using a sterile 200  $\mu$ L tip, washed with cold sterile PBS to remove cell debris and then replenished with fresh culture medium. Representative images were obtained at 0 h and 24 h at 20  $\times$  magnification using a light microscope. For transwell assay,  $2 \times 10^5$  cells were resuspended in serum-free DMEM medium and placed in the chamber of the Transwell (Corning, China), while 600  $\mu$ L of 1% serum DMEM medium being added to the lower chamber. After incubation for 24 h,

cells on the upper surface of the Transwell chamber were removed with cotton swap. The chamber was washed with PBS, fixed in pre-cooled methanol for 5 min and stained with 0.1% crystal violet solution for 10 min for image acquisition.

## 6. ChIP assays

For each ChIP reaction,  $2 \times 10^6$  spheroid cells were fixed in formaldehyde for 15 min at 37°C. Cross-linked chromatin was sonicated to obtain 200–500 bp fragments and immunoprecipitated using anti-KDM4C, anti-H3K27me2 or anti-H3K27me3 antibody (Abcam, China). Normal human IgG was used as a negative control.

## 7. Statistical analysis

Statistical analysis in all the experiments is based on at least three biological replicates and the error bars are drawn with the standard deviation (SD). The p value is calculated by using student's T test.

# Declarations

## Disclosure of Conflicts of Interest

No conflicts of interest were disclosed.

## Acknowledgements

This work was supported by the National Science Foundation of China (81260385 and 81360383), Clinical Research Project of Shenzhen Municipal Health Commission (SZLY2017017), Doctoral Project of Shenzhen Maternal and Child Health Hospital (FYA2018005) and Medical Science and Technology Research Fund Project of Guangdong (A2019363).

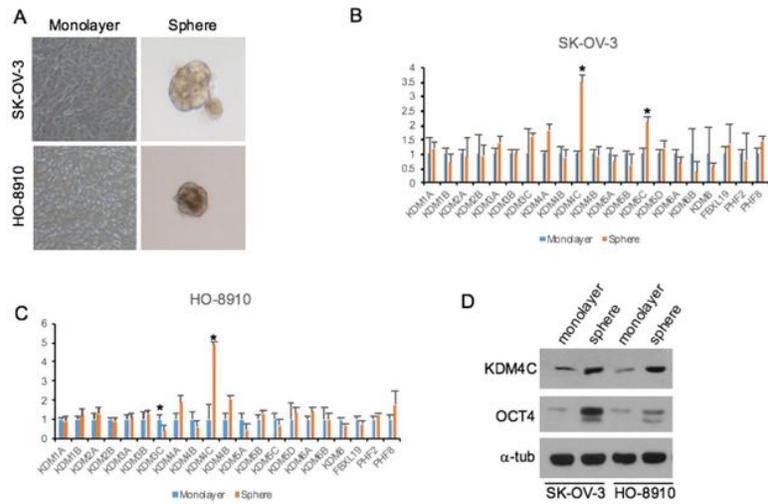
# References

- [1] S. Vaughan, J.I. Coward, R.C. Bast, Jr., A. Berchuck, J.S. Berek, J.D. Brenton, G. Coukos, C.C. Crum, R. Drapkin, D. Etemadmoghadam, M. Friedlander, H. Gabra, S.B. Kaye, C.J. Lord, E. Lengyel, D.A. Levine, I.A. McNeish, U. Menon, G.B. Mills, K.P. Nephew, A.M. Oza, A.K. Sood, E.A. Stronach, H. Walczak, D.D. Bowtell, F.R. Balkwill, Rethinking ovarian cancer: recommendations for improving outcomes, *Nat Rev Cancer* 11(10) (2011) 719-25.
- [2] E.A. Eisenhauer, Real-world evidence in the treatment of ovarian cancer, *Ann Oncol* 28(suppl\_8) (2017) viii61-viii65.
- [3] I. Tsibulak, A.G. Zeimet, C. Marth, Hopes and failures in front-line ovarian cancer therapy, *Crit Rev Oncol Hematol* 143 (2019) 14-19.

- [4] M.F. Clarke, Clinical and Therapeutic Implications of Cancer Stem Cells. Reply, *N Engl J Med* 381(10) (2019) e19.
- [5] F. Reim, Y. Dombrowski, C. Ritter, M. Buttman, S. Hausler, M. Ossadnik, M. Krockenberger, D. Beier, C.P. Beier, J. Dietl, J.C. Becker, A. Honig, J. Wischhusen, Immunoselection of breast and ovarian cancer cells with trastuzumab and natural killer cells: selective escape of CD44<sup>high</sup>/CD24<sup>low</sup>/HER2<sup>low</sup> breast cancer stem cells, *Cancer Res* 69(20) (2009) 8058-66.
- [6] Y. Miao, H. Yang, J. Levorse, S. Yuan, L. Polak, M. Sribour, B. Singh, M.D. Rosenblum, E. Fuchs, Adaptive Immune Resistance Emerges from Tumor-Initiating Stem Cells, *Cell* 177(5) (2019) 1172-1186 e14.
- [7] M. Lawrence, S. Daujat, R. Schneider, Lateral Thinking: How Histone Modifications Regulate Gene Expression, *Trends Genet* 32(1) (2016) 42-56.
- [8] M.T. McCabe, H.P. Mohammad, O. Barbash, R.G. Kruger, Targeting Histone Methylation in Cancer, *Cancer J* 23(5) (2017) 292-301.
- [9] Z. Meng, Y. Liu, J. Wang, H. Fan, H. Fang, S. Li, L. Yuan, C. Liu, Y. Peng, W. Zhao, L. Wang, J. Li, J. Feng, Histone demethylase KDM7A is required for stem cell maintenance and apoptosis inhibition in breast cancer, *J Cell Physiol* (2019).
- [10] S.M. Stief, A.L. Hanneforth, S. Weser, R. Mattes, M. Carlet, W.H. Liu, M.D. Bartoschek, H. Dominguez Moreno, M. Oettle, J. Kempf, B. Vick, B. Ksienzyk, B. Tizazu, M. Rothenberg-Thurley, H. Quentmeier, W. Hiddemann, S. Vosberg, P.A. Greif, K.H. Metzeler, G. Schotta, S. Bultmann, I. Jeremias, H. Leonhardt, K. Spiekermann, Loss of KDM6A confers drug resistance in acute myeloid leukemia, *Leukemia* (2019).
- [11] S. Ramadoss, S. Sen, I. Ramachandran, S. Roy, G. Chaudhuri, R. Farias-Eisner, Lysine-specific demethylase KDM3A regulates ovarian cancer stemness and chemoresistance, *Oncogene* 36(11) (2017) 1537-1545.
- [12] H. Sakaki, M. Okada, K. Kuramoto, H. Takeda, H. Watarai, S. Suzuki, S. Seino, M. Seino, T. Ohta, S. Nagase, H. Kurachi, C. Kitanaka, GSKJ4, A Selective Jumonji H3K27 Demethylase Inhibitor, Effectively Targets Ovarian Cancer Stem Cells, *Anticancer Res* 35(12) (2015) 6607-14.
- [13] P.A. Cloos, J. Christensen, K. Agger, A. Maiolica, J. Rappsilber, T. Antal, K.H. Hansen, K. Helin, The putative oncogene GASC1 demethylates tri- and dimethylated lysine 9 on histone H3, *Nature* 442(7100) (2006) 307-11.
- [14] Y. An, H. Cai, Y. Zhang, S. Liu, Y. Duan, D. Sun, X. Chen, X. He, circZMYM2 Competed Endogenously with miR-335-5p to Regulate JMJD2C in Pancreatic Cancer, *Cell Physiol Biochem* 51(5) (2018) 2224-2236.

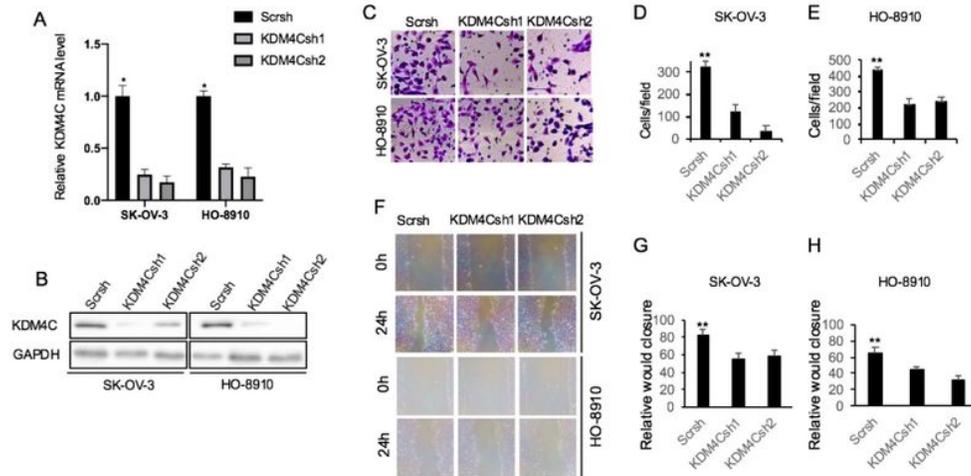
- [15] N. Li, D. Jiang, Jumonji domain containing 2C promotes cell migration and invasion through modulating CUL4A expression in lung cancer, *Biomed Pharmacother* 89 (2017) 305-315.
- [16] J. Garcia, F. Lizcano, KDM4C Activity Modulates Cell Proliferation and Chromosome Segregation in Triple-Negative Breast Cancer, *Breast Cancer (Auckl)* 10 (2016) 169-175.
- [17] Z.Q. Yang, I. Imoto, Y. Fukuda, A. Pimkhaokham, Y. Shimada, M. Imamura, S. Sugano, Y. Nakamura, J. Inazawa, Identification of a novel gene, GASC1, within an amplicon at 9p23-24 frequently detected in esophageal cancer cell lines, *Cancer Res* 60(17) (2000) 4735-9.
- [18] S.C. Parte, S.K. Batra, S.S. Kakar, Characterization of stem cell and cancer stem cell populations in ovary and ovarian tumors, *J Ovarian Res* 11(1) (2018) 69.
- [19] G. Liu, A. Bollig-Fischer, B. Kreike, M.J. van de Vijver, J. Abrams, S.P. Ethier, Z.Q. Yang, Genomic amplification and oncogenic properties of the GASC1 histone demethylase gene in breast cancer, *Oncogene* 28(50) (2009) 4491-500.
- [20] X. Yuan, J. Kong, Z. Ma, N. Li, R. Jia, Y. Liu, F. Zhou, Q. Zhan, G. Liu, S. Gao, KDM4C, a H3K9me3 Histone Demethylase, is Involved in the Maintenance of Human ESCC-Initiating Cells by Epigenetically Enhancing SOX2 Expression, *Neoplasia* 18(10) (2016) 594-609.
- [21] J.P. Mallm, P. Windisch, A. Biran, Z. Gal, S. Schumacher, R. Glass, C. Herold-Mende, E. Meshorer, M. Barbus, K. Rippe, Glioblastoma initiating cells are sensitive to histone demethylase inhibition due to epigenetic deregulation, *Int J Cancer* (2019).
- [22] K. Agger, K. Nishimura, S. Miyagi, J.E. Messling, K.D. Rasmussen, K. Helin, The KDM4/JMJD2 histone demethylases are required for hematopoietic stem cell maintenance, *Blood* (2019).
- [23] X. Wang, A. Spandidos, H. Wang, B. Seed, PrimerBank: a PCR primer database for quantitative gene expression analysis, 2012 update, *Nucleic Acids Res* 40(Database issue) (2012) D1144-9.

## Figures



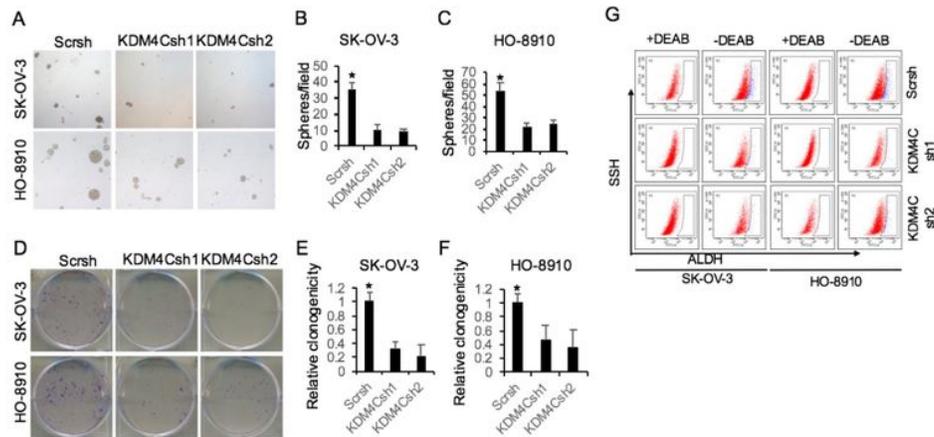
**Figure 1**

KDM4C is elevated in tumosphere of ovarian cancer cell lines. A. Representative images of monolayer and spheres of SK-OV-3 and HO-8910 cells. B. The mRNA expression of histone demethylases in monolayer and spheres of SK-OV-3 cells was assessed by Real-time RT-PCR. C. The mRNA expression of histone demethylases in monolayer and spheres of HO-8910 cells was assessed by Real-time RT-PCR. D. Western Blot analysis of monolayer or spheres of SK-OV-3 and HO-8910 cells for KDM4C and OCT-4 expression.  $\alpha$ -tubulin is used as control. \*  $P < 0.05$ .



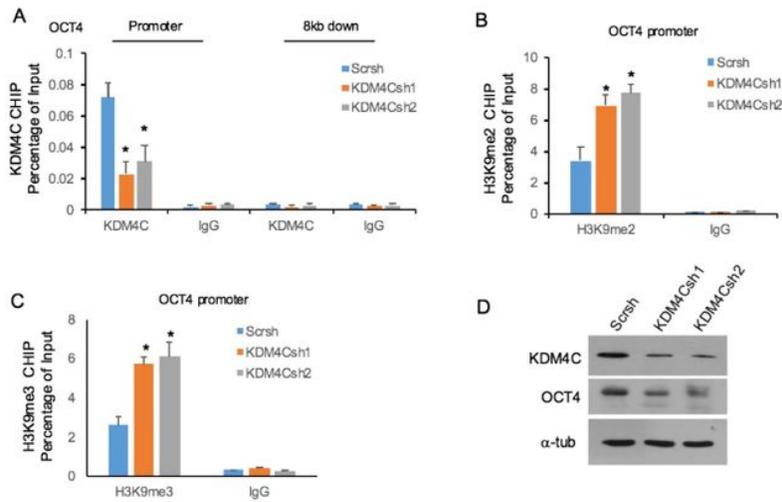
**Figure 2**

KDM4C is required for the invasion and migration ability of ovarian cancer cells. (A-B) The knock-down of KDM4C by shRNA. KDM4C expression was examined by qRT-PCR and Western Blot analysis. (C-D) Cell invasive ability of SK-OV-3 and HO-8910 cells determined by transwell assays was decreased after stable knockdown of KDM4C. (E-F) Migratory potential of SK-OV-3 and HO-8910 cells determined by wound-healing assays was decreased after stable knockdown of KDM4C. \* P<0.05. \*\* P<0.01.



**Figure 3**

KDM4C is required for stem cell properties of ovarian cancer cells. (A-C) Sphere formation ability of SK-OV-3 and HO-8910 cells was inhibited after KDM4C depletion. (D-F) Colony-forming ability was decreased after knockdown of KDM4C in SK-OV-3 and HO-8910 cells. G. The percentage of ALDH<sup>high</sup> population in SK-OV-3 and HO-8910 cells was reduced after knockdown of KDM4C. \* P<0.05.



**Figure 4**

Increased KDM4C occupancy and H3K9 methylation levels at the OCT-4 gene promoter. A. ChIP analysis of KDM4C occupancy at the OCT-4 promoter after KDM4C inhibition as quantified by real-time PCR. B. ChIP analysis of H3K9 di-methylation levels at the OCT-4 promoter. C. ChIP analysis of H3K9 tri-methylation levels at the OCT-4 promoter as quantified by real-time PCR. D. Western Blot analysis of SK-OV-3 spheres for KDM4C and OCT-4 expression.  $\alpha$ -tubulin is used as control. \*  $P < 0.05$ .