KDM4C Promotes Proliferation and Migration of Multiple Myeloma Cells by Up-Regulating JAG1 Gene Expression

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Abstract: Multiple myeloma is a frequent hematological malignancy. Although progress has been made in therapeutic strategies, the prognosis of multiple myeloma is far from satisfactory. Therefore, it is imperative to investigate the precise mechanism of multiple myeloma progression. Lysine Demethylase 4C (KDM4C) was demonstrated to be a vital regulator in cancers, while its action on multiple myeloma remains elusive. Thus, we aimed to investigate the effect of KDM4C on multiple myeloma proliferation and explored the precise mechanism of action. In this study, 70 multiple myeloma patients and 45 normal donors (volunteers) were enrolled. Results showed that KDM4C was highly expressed in plasma of 70 multiple myeloma patients and multiple myeloma cells. Knockdown of KDM4C suppressed proliferation and migration of multiple myeloma cells. Besides, JAG1 expression was enhanced in plasma of 70 myeloma patients and multiple myeloma cells. JAG1 expression was positively correlated with KDM4C expression. Furthermore, KDM4C knockdown suppressed Notch signaling proteins Notch-1, NICD-1, and Hes-1 in multiple myeloma. Moreover, KDM4C knockdown suppressed the proliferation and migration of multiple myeloma cells through down-regulating JAG1 expression. Collectively, KDM4C promotes the proliferation and migration of multiple myeloma cells by up-regulating JAG1 gene expression. KDM4C may be a promising target for multiple myeloma therapy.

Key words: JAG1, KDM4C, Multiple myeloma, Migration, Proliferation

Introduction

Multiple myeloma is a frequent malignancy of plasma cells and accounts for 10% of hematological malignancy\textsuperscript{2}. The main characteristic of multiple myeloma is deregulated proliferation of clonal plasma cells, and it usually accompanied by the secretion of defective monoclonal immunoglobulin\textsuperscript{7}. At present, proteasome inhibitors and immunomodulatory drugs are the primary choices to improve the prognosis of multiple myeloma patients, while multiple myeloma patients' survival rate remains poor\textsuperscript{9}. Thus, it is imperative to investigate the precise mechanism of multiple myeloma development and search the valuable therapeutic strategies.

Lysine Demethylase 4C (KDM4C), a histone demethylase, has been extensively described in cancers\textsuperscript{4-7}. Accumulating evidence revealed that KDM4C exhibited deregulation in cancers, and it exerted a regulatory role in some cancers such as prostate cancer, ovarian cancer, and colon cancer\textsuperscript{8,9}. For example, highly expressed KDM4C potentiated the proliferation ability of prostate cancer cells through activating AKT and c-Myc\textsuperscript{8}. In ovarian cancer, KDM4C was overexpressed in the cancer stem cell population, and it was essential for the maintenance of ovarian cancer stem cell characteristics\textsuperscript{9}. In colon cancer, abnormal expressed KDM4C modulated sphere formation ability in colon cancer cells through mediating the crosstalk between Wnt and Notch pathways\textsuperscript{9}.

Besides, KDM4C was elevated in bone marrow samples of multiple myeloma patients\textsuperscript{10}. However, the role of KDM4C in multiple myeloma remains elusive currently.

Jagged1 (JAG1) is one of the ligands of the Notch pathway receptor, and it has been proved to participate in the regulation of cancer initiation and progression\textsuperscript{11}. For instance, enhancing JAG1 in lung cancer tissue was related to poor survival by promoting lung cancer metastasis\textsuperscript{12}. Highly expressed JAG1 in pancreatic cancer modulated aggressive behaviors of cancer cells and is related to the adverse prognosis of pancreatic cancer patients\textsuperscript{13}. Besides, the action of JAG1 on multiple myeloma has been discovered\textsuperscript{13-15}. Previous studies that JAG1 mediated activation of the Notch signaling pathway contributed to the proliferation of multiple myeloma and the acquisition of bortezomib resistance in myeloma cells\textsuperscript{13, 14}. Jia et al. discovered that miR-26b-5p inhibited multiple myeloma cell proliferation and induced apoptosis through binding to JAG1\textsuperscript{15}. Furthermore, Yamamoto et al. showed that JAG1 was the target of KDM4C in colon cancer\textsuperscript{9}. Therefore, we inferred that KDM4C might involve in the regulatory process of JAG1 on multiple myeloma.

Therefore, this study aimed to investigate the effect of KDM4C on multiple myeloma progression and explored the role of KDM4C in the regulation of JAG1 on multiple myeloma.

Materials and Methods

Patients’ samples and cell culture

The peripheral blood samples of 70 multiple myeloma patients and 45 normal donors (volunteers) were collected from Wuhan No.1 Hospital. All participants signed informed consents. The Ethics Committee of
Wuhan No.1 Hospital permitted the study (Approval No. 2016-0711).

Human nucleus pulposus cell line NPC was acquired from ScienCell Research Laboratories. Human multiple myeloma cell lines U-266 and RPMI-8226 were acquired from American Type Culture Collection (ATCC). Human multiple myeloma cell lines MOLP-8 and OPM2 were obtained from German Collection of Microorganisms and Cell Cultures (DSMZ). NPC cells were maintained in NP Cell Medium (ScienCell Research Laboratories, Carlsbad, CA, USA). U-266, RPMI-8226, MOLP-8 and OPM2 cells were maintained in RPMI-1640 Medium (Thermo Fisher Scientific Inc., MA, USA) plus 10% Fetal Bovine Serum (Thermo Fisher Scientific Inc., MA, USA) at 37°C, 5% CO₂ atmosphere.

Written informed consent was obtained from a legally authorized representative(s) for anonymized patient information to be published in this article.

Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted utilizing TRIzol™ LS Reagent (Invitrogen Inc., Carlsbad, CA, USA). Reverse transcription reaction was conducted to produce cDNA using SuperScript™ III First-Strand Synthesis System (Invitrogen Inc., Carlsbad, CA, USA). Afterward, SYBR Green Real-Time PCR Master Mixes (Invitrogen Inc., Carlsbad, CA, USA) were employed to perform qPCR assay. The primers of KDM4C and JAD1 were: KDM4C: F, 5′-AGGCCTAAAGCTGATGGAGA-3′; R, 5′-TTGGCCATGAAAGCTCGGAT-3′; JAD1: F, 5′-CTCATCAGC-CTGTCTCAAC-3′; R, 5′-GGCACACACACTTAATCA-3′. GAPDH was serviced as the control gene. The 2^(-ΔΔCt) method was utilized to analyze the relative mRNA expression of KDM4C and JAD1.

Western blot

Total proteins were isolated utilizing RIPA Lysis Buffer (Sigma-Aldrich Co., St. Louis, MO, USA) and quantified using the Bicinchoninic Acid Kit for Protein Determination (Sigma-Aldrich Co., St. Louis, MO, USA). Isolated proteins were separated on SDS-PAGE gels and moved to the PVDF membranes. Subsequently, the membranes were blocked using 4% non-fat milk for 1 h and hybridized with anti-KDM4C (1:2,000; Abcam Inc., Cambridge, UK), JAG1 (1:500; Abcam Inc., Cambridge, UK), Notch-1 (1:1,000; Abcam Inc, Cambridge, UK), NICD-1 (1:500; Abcam Inc, Cambridge, UK), Hes-1 (1:2,000; Abcam Inc, Cambridge, UK), and GAPDH (1:2,500; Abcam Inc, Cambridge, UK) antibodies at 4°C for 12 h. After the washing step, the membranes were incubated with immunoglobulin G (IgG) H&L (HRP) (Abcam Inc., Cambridge, UK). The density of blots was measured using ECL Plus Western Blotting Substrate (Thermo Fisher Scientific Inc.,
MA, USA). GAPDH was served as the control protein.

**Cell transfection**

The siRNA of KDM4C and overexpression plasmid of JAG1 were compounded in Shanghai GenePharma Co., Ltd (GenePharma Co., Ltd, Shanghai, China). To perform transfection, RPMI-8226 cells were plated into 6-well plates and cultured for 24 h. Afterward, cells were transfected with siRNA of KDM4C or overexpression plasmid of JAG1 using Lipofectamine™ 3000 Transfection Reagent (Invitrogen Inc., Carlsbad, CA, USA). Forty-eight hours later, the transfected efficiency was verified using Western blot or qRT-PCR.

**Cell counting kit-8 (CCK-8) assay**

Transfected RPMI-8226 cells were plated into 96-well plates (1 × 10^3 cells/well). At indicated time points (0, 24, 48, and 72 h), cells were added to 10 μl Cell Counting Kit-8 (CCK-8; Sigma-Aldrich Co., St. Louis, MO, USA) and incubated for 2 h. Afterward, the optical density (OD) values of cells were recorded using the Thermo Multiskan FC microplate reader (Thermo Fisher Scientific Inc., MA, USA) on the absorbance at 450 nm.

**Colony formation assay**

Transfected RPMI-8226 cells were plated into 6-well plates with 200 cells in each well and maintained at 37 °C, 5 % CO2 incubator. After 2 weeks, cells were fixed by Methanol (Sigma-Aldrich Co., St. Louis, MO, USA) for 20 min and dyed with 0.1% Crystal Violet Solution (Sigma-Aldrich Co., St. Louis, MO, USA) for 20 min. Subsequently, the colonies were photographed and counted (>50 cells).

**Transwell migration assay**

Transfected RPMI-8226 cells (1×10^5) were inoculated into the upper chamber of Transwell and maintained in the serum-free medium. The
complete medium with 10% Fetal Bovine Serum (Thermo Fisher Scientific Inc., MA, USA) was added to the lower chamber of Transwell. After cells were cultured for 24 h, the migrated cells were fixed by 4% Paraformaldehyde and dyed with 0.1% Crystal Violet Solution (Sigma-Aldrich Co., St. Louis, MO, USA), following by counting migrated cell numbers under the ZEISS Axioskop 2 light microscope (Carl Zeiss Inc., Jena, Germany).

**Statistical analysis**

Data were described as mean ± standard deviation (SD), and statistical analysis was conducted by SPSS Statistics 22.0 (SPSS, Chicago, IL, USA). Student’s t-test or one-way ANOVA was employed to determine significant differences. The criterion of statistically significance was P values below 0.05.
Results

KDM4C was highly expressed in multiple myeloma

To elucidate the effect of KDM4C in multiple myeloma, the KD-M4C expression in plasma of 70 multiple myeloma patients and 45 normal donors was detected. It was observed that KDM4C was significantly elevated in multiple myeloma patients’ plasma (P < 0.001, Fig. 1A). Besides, the mRNA and protein levels of KDM4C were also highly expressed in multiple myeloma cell lines U-266, MOLP-8, OPM2, and RPMI-8226 compared to human nucleus pulposus cell line NPC (P< 0.05, Fig. 1B, C). Especially, KDM4C presented the highest expression in RPMI-8226 cells, thus RPMI-8226 cells were selected to conduct the subsequent experiments. Taken together, KDM4C was highly expressed in multiple myeloma.

Knockdown of KDM4C suppressed proliferation and migration of multiple myeloma cells

To further investigate the role of KDM4C in multiple myeloma, two siRNAs of KDM4C were first introduced into multiple myeloma cells. Western blot results found that two siRNAs of KDM4C were significantly decreased the KDM4C expression (P< 0.05, Fig. 2A). However, the knockout efficiency of si-KDM4C#1 was higher than si-KDM4C#2 (Fig. 2A). Therefore, si-KDM4C#1 was used for the follow-up experiments. Next, the effects of knockdown of KDM4C on multiple myeloma cell proliferation and migration were determined. Results revealed that knockdown of KDM4C suppressed cell viability and colony formation ability of RPMI-8226 cells (P < 0.01, Fig. 2B, C). Besides, the Transwell migration assay found that decreased KDM4C inhibited cell migration ability (P < 0.01, Fig. 2D). Therefore, down-regulation of KDM4C suppressed the proliferation and migration of multiple myeloma cells.

JAG1 was highly expressed in multiple myeloma and positively correlated with KDM4C expression

To interpret the effect of JAG1 in multiple myeloma, the JAG1 expression in plasma of 70 multiple myeloma patients and 45 normal donors was determined. Results showed that JAG1 was increased in multiple myeloma patients’ plasma (P < 0.01, Fig. 3A). Besides, the mRNA and protein levels of JAG1 were also up-regulated in multiple myeloma cell lines U-266, MOLP-8, OPM2, and RPMI-8226 (P < 0.05, Fig. 3B, C). Afterward, the relationship between KDM4C and JAG1 was analyzed using Pearson correlation analysis. It was observed that the JAG1 expression was positively associated with the KDM4C expression (P < 0.0001, Fig. 3D). Thus, these results suggested that JAG1 was highly expressed in multiple myeloma and positively correlated with KDM4C expression.

Knockdown of KDM4C suppressed Notch signaling pathway in multiple myeloma

The previous study reported that JAG1 mediated Notch signaling pathway activation promoted the proliferation of multiple myeloma cells. Therefore, we determined the effect of KDM4C on the Notch signaling pathway in multiple myeloma. Results showed that down-regulation of KDM4C suppressed the protein levels of Notch signaling pathway proteins Notch-1, NICD-1, and Hes-1 (P < 0.01, Fig. 4). Hence, KDM4C suppressed Notch signaling pathway in multiple myeloma.

Knockdown of KDM4C suppressed proliferation and migration of multiple myeloma cells through down-regulating JAG1 expression

To investigate whether JAG1 mediated the regulation of KDM4C on proliferation and migration ability of multiple myeloma cells, JAG1 overexpression plasmid was co-transfected with si-KDM4C into RPMI-8226 cells. The expression of JAG1 in transfected RPMI-8226 cells was determined. The mRNA and protein levels of JAG1 were decreased by si-KDM4C but were increased by JAG1 overexpression plasmid (P < 0.01, Fig. 5A, B). Besides, knockdown of KDM4C suppressed cell viability, which was abrogated by overexpressed JAG1 (P < 0.01, Fig. 5C). Furthermore, the decreased colony formation ability induced by down-regulation of KDM4C was reversed by overexpressed JAG1 (P < 0.01, Fig. 5D). Moreover, knockdown of KDM4C suppressed cell migration ability but was abrogated by overexpressed JAG1 (P< 0.01, Fig. 5E). Collectively, KDM4C regulated the proliferation and migration ability of multiple myeloma cells through modulating JAG1 expression.
Discussion

Multiple myeloma is a frequent hematological malignant tumor\(^6\). Currently, proteasome inhibitors and immunomodulatory drugs are the primary choices for multiple myeloma patients, but the prognosis of multiple myeloma patients is still unsatisfactory due to the extremely high rate of cancer metastasis and progression\(^3,18\). Therefore, it is essential to investigate the precise mechanism of multiple myeloma progression. KDM4C was demonstrated to be a vital regulator in cancers\(^6,7,8\), while little is known about its action on multiple myeloma. Thus, we studied the effect of KDM4C on multiple myeloma progression in this study.

To investigate the action of KDM4C on multiple myeloma, the expression of KDM4C in multiple myeloma was first determined. Results showed that KDM4C was elevated in the plasma of multiple myeloma patients and multiple myeloma cells. These results were consistent with the previous study\(^9\). Lv et al., revealed that KDM4C was elevated in bone marrow samples of multiple myeloma patients\(^9\). The high expression of KDM4C is also found in other cancers such as prostate cancer, ovarian cancer, colon cancer, non-small cell lung cancer (NSCLC), and glioblastoma\(^6,8,19,20\). This evidence implied that the expression of KDM4C was highly specific in cancers, suggesting that KDM4C had the potential to become a cancer therapeutic target.

Next, we determined the action of KDM4C on aggressive behaviors of multiple myeloma cells. We found that silencing KDM4C suppressed the proliferation and migration ability of multiple myeloma cells. These results were consistent with the previous studies\(^6,20,21\). Lee et al. found that down-regulation of KDM4C inhibited proliferation and tumorigenesis of glioblastoma cells\(^20\). Lin et al. revealed that KDM4C knockdown suppressed proliferation and soft agar colony formation ability of prostate cancer cells\(^6\). Garcia et al. proved that knockdown of KDM4C reduced cell proliferation ability and migration capacity of triple-negative breast cancer cells\(^21\). Collectively, these findings revealed that KDM4C was highly expressed in multiple myeloma and knockdown of KDM4C suppressed proliferation and migration ability of multiple myeloma cells, suggesting that KDM4C might be an effective therapeutic target for multiple myeloma.

The ligand of the Notch pathway receptor JAG1 was demonstrated to participate in the regulation of cancer progression\(^10\). Therefore, we...
also detected that the abnormal expression of JAG1 in multiple myeloma. Results found that JAG1 was overexpressed in plasma of multiple myeloma patients and multiple myeloma cells, which was in line with the previous studies\(^{15, 22}\). Jia et al. found that JAG1 expression was enhanced in multiple myeloma\(^{15}\). Wang et al. also reported that JAG1 was highly expressed in multiple myeloma bone marrow aspirates and cells\(^{22}\). Besides, we found that the expression of JAG1 was positively correlated with the KDM4C expression, and knockdown of KDM4C suppressed Notch signaling pathway in multiple myeloma. Similarly, Yamamoto et al. found that KDM4C modulated the expression of JAG1 and the Notch signaling pathway\(^{20}\). The relationship between JAG1 and KDM4C implied that JAG1 might mediate the regulation effect of KDM4C on multiple myeloma. To investigate the role of JAG1 in the regulation influence of KDM4C on multiple myeloma, the aggressive behaviors of multiple myeloma cells were studied after JAG1 overexpressed plasmid and siRNA against KDM4C were co-transfected into multiple myeloma cells. Results revealed that knockdown of KDM4C suppressed cell proliferation and migration ability but was abrogated by overexpressed JAG1. The effect of JAG1 on multiple myeloma cell proliferation and migration was in agreement with the previous studies\(^{15, 22}\). Collectively, KDM4C regulated the proliferation and migration ability of multiple myeloma cells by modulating JAG1 expression.

KDM4C promotes the proliferation and migration of multiple myeloma cells by up-regulating JAG1 gene expression. KDM4C may be a promising target for multiple myeloma therapy.

Acknowledgements

This work was supported by the Medical science research project of Wuhan Municipal Health Commission. (Grant No. WX18Z05).

Competing Interests

The authors state that there are no conflicts of interest to disclose.

References

1. Liu J, Du F, Chen C, Li D, Chen Y, Xiao X and Hou X. CircRNA ITCH increases bortezomib sensitivity through regulating the miR-615-3p/PRKCD axis in multiple myeloma. Life Sci 262: 118506, 2020
2. Röllig C, Knop S and Bornhäuser M. Multiple myeloma. Lancet 385: 2197-2208, 2015
3. Mohy M, Cavo M, Fink L and Gonzalez-McQuire S. Understanding mortality in multiple myeloma: Findings of a European retrospective chart review. Eur J Haematol 103: 107-115, 2019
4. Zhao E, Ding J, Xia Y, Liu M, Ye B, Choi JH, Yan C, Dong Z, Huang S, Zha Y, Yang L, Cui H and Ding HF. KDM4C and ATF4 cooperate in transcriptional control of amino acid metabolism. Cell Rep 14: 506-519, 2016
5. Agger K, Nishimura K, Miyagi S, Messling JE, Rasmussen KD and Helin K. The KDM4/JMD2D histone demethylases are required for hematopoietic stem cell maintenance. Blood 134: 1154-1158, 2019
6. Lin CY, Wang BJ, Chen BC, Tseng JC, Jiang SS, Tsai KK, Shen YY, Yuh CH, Sie ZL, Wang WC, Kung HJ and Chiu CP. Histone demethylase KDM4C stimulates the proliferation of prostate cancer cells via activation of AKT and c-Myc. Cancers 11: 1785, 2019
7. Chen GQ, Ye P, Ling RS, Zeng F, Zhu XS, Chen L, Huang Y, Xu L and Xie XY. Histone demethylase KDM4C is required for ovarian cancer stem cell maintenance. Stem Cells INT, 2020 https://doi.org/10.1155/2020/8860185
8. Yamamoto S, Tateishi K, Kudo Y, Yamamoto K, Isagawa T, Nagae G, Nakatsuka T, Asaoka Y, Ijichi H, Hirata Y, Otoku M, Ikemoue T, Aburutani H, Omata M and Koike K. Histone demethylase KDM4C regulates sphere formation by mediating the cross talk between Wnt and Notch pathways in colonic cancer cells. Carcinogenesis 34: 2380-2388, 2013
9. Lv M and Liu Q. JMJD2C triggers the growth of multiple myeloma cells via activation of β-catenin. Oncolo Rep 45: 1162-1170, 2021
10. Liu Z, Zhu Y, Li F and Xie Y. GATA1-regulated JAG1 promotes ovarian cancer progression by activating Notch signal pathway. Ptooplasma 257: 901-910, 2020
11. Chang WH, Ho BC, Hisao YJ, Chen JS, Yeh CH, Chen HY, Chang GC, Su KY and YU SL. JAG1 is associated with poor survival through inducing metastasis in lung cancer. Plos One 11: e0150355, 2016
12. Lee J, Lee J and Kim JH. Association of Jagged1 expression with malignancy and prognosis in human pancreatic cancer. Cell Oncol 43: 821-834, 2020
13. Colombo M, Thümmler K, Miranda L, Garavelli S, Todoerti K, Apicella L, Lazzari E, Lancellotti M, Platonova N, Akbar M, Chirivi-Internati M, Soutar R, Neri A, Gooyear CS and Chiaromonte R. Notch signaling drives multiple myeloma induced osteoclastogenesis. Oncotarget 5: 10393-10406, 2014
14. Muguruma Y, Yahata T, Warita T, Hozumi K, Nakamura Y, Suzuki R, Ito M and Ando K. Jagged1-induced Notch activation contributes to the acquisition of bortezomib resistance in myeloma cells. Blood Cancer J 7: 650, 2017
15. Jia CM, Tian YY, Quan LN, Jianguo L and Liu AC. miR-26b-5p suppresses proliferation and promotes apoptosis in multiple myeloma cells by targeting JAG1. Patho Res Pract 214: 1388-1394, 2018
16. Wu X, Li R, Song Q, Zhang C, Jia R, Han Z, Zhou L, Sui H, Liu X and Zhu H. JMJD2C promotes colorectal cancer metastasis via regulating histone methylation of MALAT1 promoter and enhancing β-catenin signaling pathway. J Exp Clin Canc Res 38: 1-13, 2019
17. Yi DY, Su Q, Zhang FC, Fu P, Zhang Q, Cen YC, Zhao HY and Xiang W. Effect of microRNA-128 on cisplatin resistance of glioma SHG-44 cells by targeting JAG1. J Cell Biochem 119: 3162-3173, 2018
18. Feng Y, Zhang L, Wu J, Khadka B, Fang Z, Gu J, Tang B, Xiao R, Pan G and Liu J. CircRNA circ_0000190 inhibits the progression of multiple myeloma through modulating miR-767-5p/MAPK4 pathway. J Exp Clin Canc Res 38: 54, 2019
19. Wu X, Deng Y, Zu Y and Yin J. Histone demethylase KDM4C activates HIF1α/VEGFA signaling through the costimulatory factor STAT3 in NSCLC. Am J Cancer Res 10: 491-506, 2020
20. Lee DH, Kim GW, Yoo J, Lee SW, Jeon YH, Kim SY, Kang HG, Kim DH and Chun KH. Histone demethylase KDM4C controls tumorigenesis of glioblastoma by epigenetically regulating p53 and c-Myc. Cell Death Dis 12: 89, 2021
21. Garcia J and Lizcano F. Kdm4c is recruited to mitotic chromosomes and is relevant for chromosomal stability, cell migration and invasion of triple negative breast cancer cells. Breast Cancer: Basic Clin. Res. 12: 117823418773075, 2018
22. Wang Y, Lin Q, Song C, Ma R and Li X. Depletion of circ_0007841 inhibits multiple myeloma development and BTZ resistance via miR-129-5p/JAG1 axis. Cell Cycle 19: 3289-3302, 2020
