Pathology - Research and Practice 215 (2019) 532–538

Contents lists available at ScienceDirect

Pathology - Research and Practice

journal homepage: www.elsevier.com/locate/prp

Significant association between *KDM1A* promoter hypomethylation and colorectal cancer in Han Chinese

Jie Zhong[a,1], Ranran Pan[a,1], Xiuru Ying[a], Boyi Wu[a], Cong Zhou[a], Dongping Wu[b], Jieer Ying[c], Shiwei Duan[a,*]

[a] Medical Genetics Center, School of Medicine, Ningbo University, Ningbo, Zhejiang, 315211, China
[b] Department of Medical Oncology, Shaoxing People's Hospital, Shaoxing Hospital of Zhejiang University, Zhejiang, 312000, China
[c] Department of Medical Oncology, Zhejiang Cancer Hospital, Hangzhou, Zhejiang, 310022, China

ARTICLE INFO

Keywords:
Colorectal cancer
KDM1A
Hypomethylation
qMSP
Promoter

ABSTRACT

Lysine-specific histone demethylase 1A gene (*KDM1A*) promotes tumorigenesis. The aim of this study was to investigate the association between *KDM1A* methylation and colorectal cancer (CRC). Currently, we collected 37 paired CRC tissues and adjacent non-tumor tissues from Jiangsu province and 75 paired CRC tissues and adjacent non-tumor tissues from Zhejiang province to conduct a two-stage experiment to study the association between *KDM1A* methylation and CRC. We used qMSP to measure the *KDM1A* promoter methylation, and the percentage of methylation reference (PMR) to quantify the *KDM1A* promoter methylation level. To investigate the effect of the selected *KDM1A* fragment on gene expression regulation, we also performed a dual luciferase reporter gene assay. In the stage I study, the *KDM1A* promoter methylation level in CRC tumor tissues was significantly lower than that in adjacent non-tumor tissues (median PMR: 6.93% vs 10.25%, $P = 0.033$). The results of the stage II study were similar to those of the stage I study (mean PMR: 12.94% versus 17.42%, $P = 0.016$). In addition, a clinical pathology subgroup analysis found that *KDM1A* hypomethylation was associated with CRC only in patients with well-differentiated CRC (stage I: $P = 0.047$; stage II: $P = 0.040$). The dual luciferase reporter assay showed that the transcriptional activity of the recombinant pGL3-KDM1A plasmid was significantly higher (fold change = 2, $P = 0.0009$). In conclusion, our results suggest that *KDM1A* hypomethylation is significantly associated with CRC.

1. Introduction

CRC is a malignant lesion of the normal colonic epithelium that is converted to glandular epithelium [1]. CRC ranks third among the most common tumors in the world [2]. The annual global incidence rate is about 1.3 million [3]. In China, about 15.9 million people die of CRC each year, and the death rate of CRC ranks the fifth cause of death [2]. The 5-year survival rate of patients with early CRC is about 90%, but most patients with CRC have distant metastasis at the time of diagnosis [4], which makes the average 5-year survival rate of CRC less than 10% [5]. Therefore, it has been found that diagnostic biomarkers in patients with early CRC have important implications for the prognosis of patients with CRC.

The occurrence of CRC may be due to the accumulation of genetic and epigenetic changes in the genome [6]. As an important epigenetic mechanism, DNA methylation plays an important role in CRC [7]. Abnormal methylation of tumor suppressor genes is associated with cancer development [6], progression [8], invasion [9], and metastasis [10].

The lysine-specific histone demethylase 1 A gene (*KDM1A*), located at 1p36.12, encodes a nuclear protein that is highly expressed in a variety of cancers, including CRC [11], lung cancer [12], prostate cancer [13], oral cancer [14], neuroblastoma [15], and medulloblastoma [16]. The *KDM1A* promotes cancer through a variety of mechanisms [13].

Colonoscopy is the gold standard for the diagnosis of CRC, but its popularity is limited by its high cost and invasiveness [17]. DNA methylation biomarkers have broad prospects for early detection and

Abbreviations: CRC, colorectal cancer; *KDM1A*, the lysine (K)-specific histone demethylase 1A gene; qMSP, quantitative methylation specific polymerase chain reaction; PMR, percentage of methylated reference; CpG, cytosine-phosphate-guanine; TCGA, the data from Cancer Genome Atlas; GEO, Gene Expression Omnibus

* Corresponding author.
E-mail address: duanshiwei@nbu.edu.cn (S. Duan).

[1] JZ and RP are the co-first authors of this work.

https://doi.org/10.1016/j.prp.2018.12.005
Received 27 August 2018; Received in revised form 15 November 2018; Accepted 5 December 2018
0344-0338/ © 2018 Published by Elsevier GmbH.

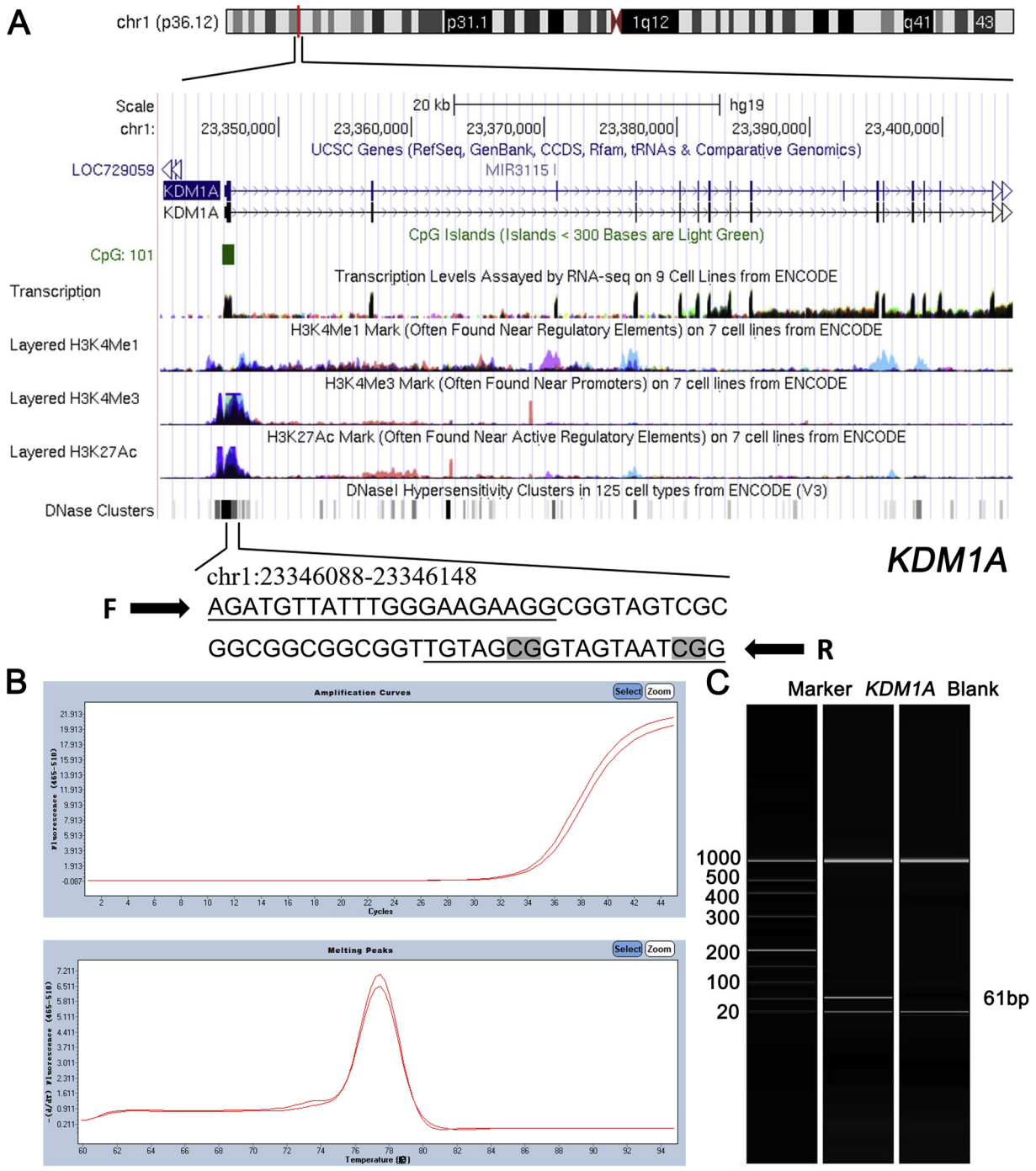

Fig. 1. Target sequence on *KDM1A* CpG island region. (A) The genomic position and functional annotation of fragment were based on human 2009 (GRCh37/hg19) assembly. The qMSP primers were underlined and two CpG sites on primers were in grey. F represented forward primer; R represented reverse primer. **(B)** The images were the qMSP results of primers (The amplification curve was above and the melting curve was below). **(C)** The image was the capillary electrophoresis result of a qMSP product.

prognosis of cancer [18,19]. Therefore, we designed a two-stage study to explore the association between *KDM1A* methylation level and CRC.

2. Materials and methods

2.1. Subjects

In the stage I study, we collected samples of cancer tissues and adjacent tissues from 37 CRC patients at the Third Affiliated Hospital of Nanjing University of Traditional Chinese Medicine (Jiangsu Province, China). In the stage II study, we collected samples of cancerous and paracancerous tissues from 75 CRC patients from Zhejiang Cancer Hospital (Zhejiang, China) and Shaoxing First People's Hospital (Zhejiang, China). All patients were diagnosed by pathology, and no radiotherapy or chemotherapy was performed before surgical resection. All clinical information was derived from the patient's medical history (including gender, age, tumor location, tumor size, tumor differentiation, lymph node metastasis, and TNM staging). Written permission from the Ethics Committee of Ningbo University Medical College has been obtained. Each patient participating in the study has signed a

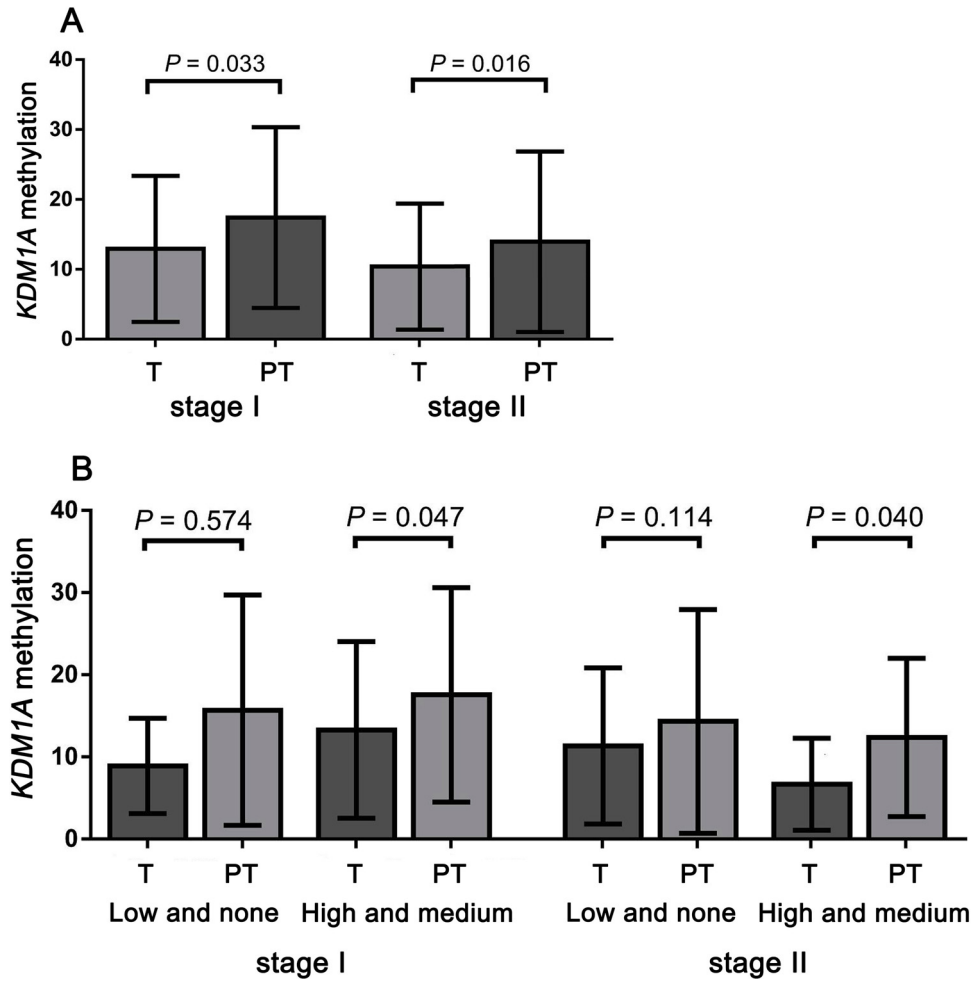

Fig. 2. Comparison of *KDM1A* methylation in tumor and para-tumor tissues from CRC patients. (A) *KDM1A* hypomethylation was found to be associated with CRC based on the comparison between tumor tissues and para-tumor tissues of 39 and 75 CRC patients in the stage I and stage II studies. **(B)** The association of *KDM1A* hypomethylation was specific to CRC patients with tumors of high and median differentiation. T represented tumor, and PT represented para-tumor. "Low and none" and "High and medium" denoted tumors with low/none and high/median differentiation, respectively.

written informed consent form.

2.2. DNA extraction, bisulfite conversion and quantitative methylation-specific polymerase chain reaction (qMSP)

The extraction of DNA from tissue samples and subsequent bisulfite conversion experiments was detailed in our previous work [20]. The *KDM1A* gene sequence used in our study is based on the UCSC website (chr1:23346088-23346148, http://genome.ucsc.edu/, Human GRCh37/hg19). In this study, we used the qMSP method to detect the methylation level of the *KDM1A* promoter in tumor tissues and the adjacent non-tumor tissues. The qMSP experiments were performed in DNA samples that had been subjected to bisulfite conversion to determine the methylation level of the *KDM1A* promoter region. In the qMSP experiment, only DNA molecules that contain the two methylated CG sites on the downstream primer can be amplified by the *KDM1A* primer set [21,22]. The methylation level of the two CG sites can be calculated according to the previous study [23]. The forward primer sequence of the *KDM1A* is 5′-AGATGTTATTTGGGAAGAAGG-3′, and the reverse primer sequence is 5′-CCGATTACTACCGCTACA-3′. The forward primer sequence of the ACTB gene is 5′-TGGTGATGGAGGAG GTTTAGTAAGT-3′, and the reverse primer sequence is 5′-AACCAATA AAACCTACTCCTCCCTTAA-3′. The percentage of methylation reference (PMR) was used to quantify the level of methylation. PMR is described as $2^{-\Delta\Delta CT} \times 100\%$, and $\Delta\Delta CT = \Delta CT$ (target gene) - ΔCT (ACTB) = [CT (target gene) - CT (ACTB)] (sample DNA) - [CT (target gene) - CT (ACTB)] (fully methylated DNA) [23]. Details of the qMSP experiment can be obtained from our previous article [20].

2.3. Dual luciferase reporter gene assay

The HEK293 T cell line was used for the dual luciferase reporter gene experiment. A fragment of *KDM1A* CpG island (-200 bp to +261 bp) digested with XhoI and KpnI (New England Biolabs, Ipswich, MA) was ligated to the pGL3-Basic vector using a DNA ligation kit (TaKaRa, Japan). The negative control was an empty pGL3-Basic vector (Promega, Madison city, WI, USA) and the positive control was a pGL3-Basic vector containing the upstream of the SV40 promoter. Details of the dual luciferase reporter gene experiment can be found in our previous article [20].

2.4. TCGA and GEO data analysis

The sample expression data of the Cancer Genome Atlas (TCGA) database we used was from the University of North Carolina, and the sample source was not indicated. Therefore, future study is needed to verify the relevant findings in the Chinese population. At the same time, the expression data of the Gene Expression Omnibus (GEO) database was derived from the mouse C2C12 mesenchymal progenitor cell line (GSE30192), showing a negative correlation between *KDM1A*

methylation and expression.

2.5. Statistical analysis

Data that conforms to a normal distribution was expressed as mean ± standard deviation, and data of a skewed distribution was expressed as a median (interquartile range). Nonparametric tests were used to calculate differences in *KDM1A* methylation between tumor and para-tumor tissues. A two-sided *P* value less than 0.05 was considered as statistically significant.

3. Results

We conducted a two-stage study to investigate the differences in *KDM1A* promoter methylation between CRC tumor tissues and adjacent non-tumor tissues. Two CG sites in the target fragment were used to indicate *KDM1A* promoter methylation (Fig. 1A). The amplification and melting curves showed that the qMSP product was homogeneous, and capillary electrophoresis results showed that the length of the qMSP product was 61 bp as expected (Fig. 1B).

In the stage I study, we performed qMSP experiments in 37 cancer tissues and 37 matched paracancerous tissues from patients in Jiangsu Province. The results showed that the methylation level of the promoter region of *KDM1A* in tumor tissues was significantly lower than that in adjacent non-tumor tissues (median PMR: 6.93% vs 10.25%, $P = 0.033$, Fig. 2A). To further validate our results, a stage II study was performed in another 75 pairs of cancerous and paracancerous tissues of CRC patients in Zhejiang Province. We obtained similarly significant results (mean PMR: 12.94% vs 17.42) %, $P = 0.016$, Fig. 2A). Clinical pathology subgroup analysis showed that the association of *KDM1A* hypomethylation with CRC was only shown in patients with highly differentiated CRC (mean PMR: 8.90% vs 17.57%, $P = 0.047$, Fig. 2B, stage I; Mean PMR: 6.68% vs 12.38%, $P = 0.040$, Fig. 2B, stage II). Meanwhile, we also compared *KDM1A* methylation of cancer tissues between subgroups by clinical characteristics (Table 1).

Then we analyzed the relationship between methylation and expression of *KDM1A* and CRC based on the data of TCGA database, and evaluated its diagnostic value. We searched a total of 9 cg sites located on the *KDM1A* sequence, as shown in Fig. 3A. The statistical results showed that the methylation levels of cg25977026, cg03967533 and cg23271558 were statistically significant between CRC tissues and adjacent tissues (Fig. 3B). In addition, the methylation levels of cg25977026 ($r = -0.320$, $P = 7E-11$) and cg23271558 ($r = -0.186$, $P = 2E-4$) were inversely correlated with *KDM1A* expression, while the methylation levels of cg06958034($r = 0.189$, $P = 2E-4$)and cg03967533 ($r = 0.158$, $P = 0.002$) were positively correlated with *KDM1A* expression (Fig. 3C).

We also used the receiver operating characteristic curve (ROC curve) to evaluate the diagnostic value of the methylation level of the promoter region of *KDM1A* gene, and the area under the curve (AUC) was used as the criterion for the diagnostic value. As shown in Fig. 4, the methylation of four CG sites on the *KDM1A* (cg14015920, cg25977026, cg03967533, and cg23271558) could well predict the risk of CRC with a mean AUC of 0.738, a sensitivity of 82.8%, and a specificity of 42.2% ($P = 2E-7$).

GEO analysis indicated that *KDM1A* expression was significantly upregulated in myoblast cell lines treated with 5′-AZA-deoxycytidine (fold change = 1.45, $P = 0.004$, Fig. 5A). To examine whether the *KDM1A* fragment is capable of regulating gene expression, we performed a dual luciferase reporter gene assay. The results showed that the transcriptional activity of the recombinant pGL3-KDM1A plasmid was significantly higher compared to the pGL3-Basic control (fold change = 2, $P = 0.0009$, Fig. 5B).

4. Discussion

Our two-stage study showed that the methylation level of the *KDM1A* promoter region in CRC tumor tissues was significantly lower than that of the matched non-tumor tissues, and that *KDM1A* methylation was associated with CRC differentiation. The information retrieved from the GEO database showed an inverse correlation between *KDM1A* methylation and expression of the *KDM1A*. Our dual luciferase reporter assay showed that the *KDM1A* fragment promoted gene expression. Therefore, we hypothesized that hypomethylation of *KDM1A* may lead to the risk of CRC by regulating gene expression.

Up-regulation of *KDM1A* expression is thought to be associated with proliferation of cancer cells [24], and it has been shown to be upregulated in a variety of cancers [25–27], including CRC [11]. *KDM1A* is a histone methylation-modified demethylase, and *KDM1A* overexpression in CRC cells is associated with *LGR5* expression and inhibition of the Wnt/β-catenin pathway [11]. In CRC, mutations in β-catenin activate the Wnt/β-catenin pathway gene LGR5, which may ultimately lead to CRC [28]. In this study, we reported for the first time that methylation of the *KDM1A* may be involved in the development of CRC.

The clinical characteristics of patients (including age, tumor size, tumor differentiation, lymph node metastasis, and TNM stage) are commonly used to assess the prognosis of cancer [29]. In contrast, molecular biomarkers have the advantage of non-invasive convenience in assessing prognosis. High expression of *KDM1A* is not only associated with poor prognosis in tongue cancer [30], but is also associated with lymph node metastasis and distant metastasis in colon cancer [31]. Our

Table 1
Comparisons of *KDM1A* methylation of cancer tissues between subgroups by clinical characteristics.

Clinical characteristics	Variable	Stage I experiment			Stage II experiment		
		Number	PMR	P value	Number	PMR	P value
Gender	Male	25	11.18 ± 6.35	0.588	48	10.79 ± 9.01	0.558
	Female	12	16.60 ± 1.57		27	9.74 ± 9.17	
Age (years)	≤ 65	20	12.94 ± 1.23	0.611	55	11.78 ± 9.05	0.012
	> 65	17	12.93 ± 8.12		20	6.65 ± 7.98	
Tumor size	≤ 6 cm	34	13.69 ± 1.05	0.057	63	7.85(3.92, 15.90)	0.483
	> 6 cm	3	4.40 ± 3.14		12	9.04 ± 9.45	
Differentiation	Low and none	3	11.33 ± 1.07	0.444	60	11.34 ± 9.49	0.111
	High and medium	34	8.90 ± 5.80		15	6.68 ± 5.60	
Lymph node metastasis	Positive	18	13.98 ± 8.60	0.206	42	10.82 ± 8.60	0.430
	Negative	19	11.95 ± 1.21		33	9.89 ± 9.63	
TNM stage	I + II	–	–	–	37	10.18 ± 9.11	0.757
	III + IV	–	–		38	10.63 ± 9.04	

PMRs were the percentages of methylation reference of tumor tissues. The data of normal distribution were expressed as mean ± SD, otherwise they were expressed as median (interquartile range). *P* value was calculated by Spearman rank test.

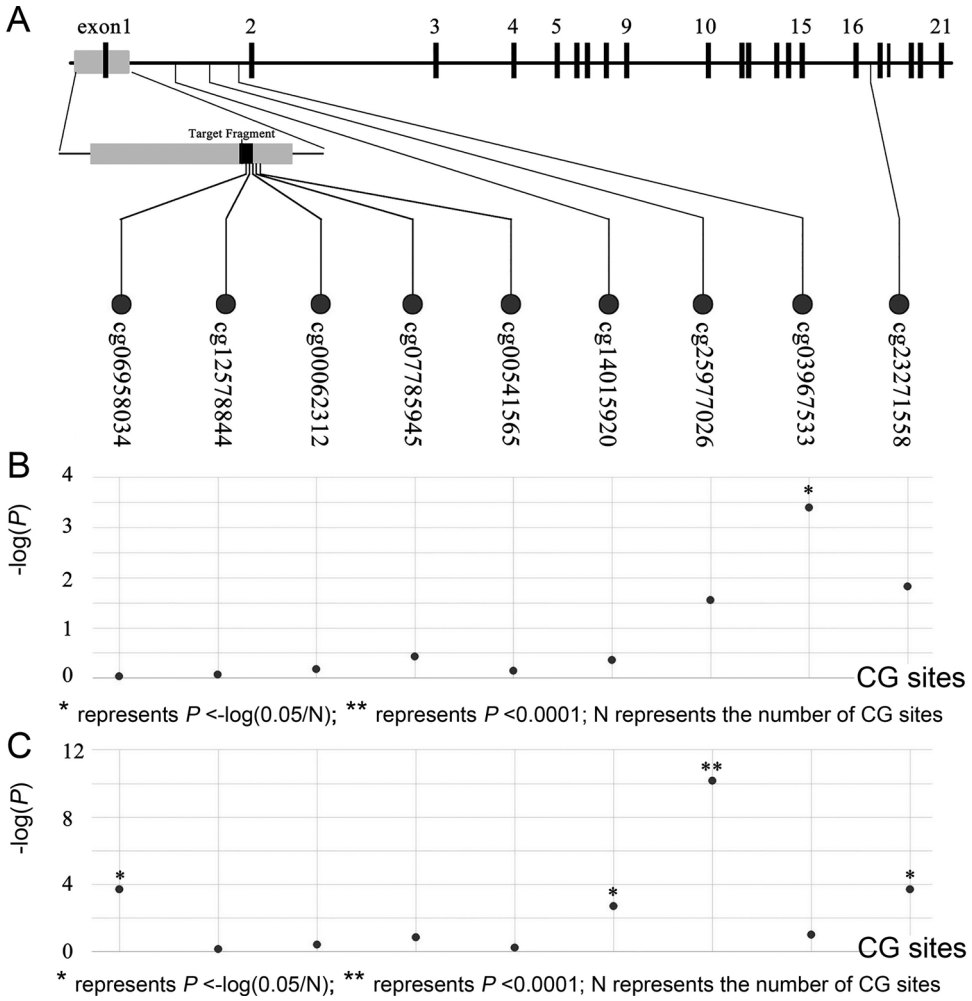

Fig. 3. 9 CG sites from TCGA datasets. (A) Genomic map (B) Differences of *KDM1A* methylation levels of CG sites between case and control group (C) Correlation between *KDM1A* methylation level and mRNA expression level of each CG sites.

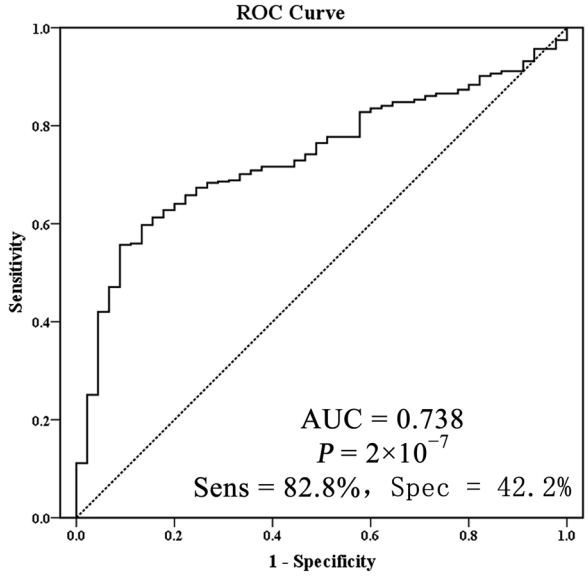

Fig. 4. Diagnostic value of KDM1A gene body methylation combined with 4 CG sites. A model constructed by KDM1A gene body methylation combined with 4 CG sites was used to screen for CRC. The involved CG sites were cg14015920, cg25977026, cg03967533, and cg23271558.

results suggested that hypomethylation of the *KDM1A* might be a biomarker associated with well-differentiated CRC.

DNA methylation does not alter gene sequence and it can silence gene expression [32]. Our study showed that the *KDM1A* was hypomethylated in CRC tumor tissues. And accumulating evidence showed that the *KDM1A* was highly expressed in tumor tissues. We used TCGA database data to analyze the methylation level and gene expression of each CG locus on *KDM1A*, and found that there were differences in methylation level of each CG locus and *KDM1A* expression. Among them, the three CG sites in the TCGA database showed significant hypomethylation in cancer tissues, which was consistent with the hypomethylation results of the CpG island in the *KDM1A* promoter region in the our work, indicating the methylation of the fragment in the qMSP experiment can represent the methylation of the *KDM1A*.

In addition, data from the TCGA database showed that two CG sites in the gene body of the *KDM1A* were negatively correlated with their expression, while a CG site in gene body and one CG site on the CpG island was positively correlated with *KDM1A* expression. In addition, TCGA data analysis found that the combination of four CG sites in the gene region of *KDM1A* might be a diagnostic marker for CRC. The above results illustrate the complexity of the function of DNA methylation modification of *KDM1A*. Future research needs to be done to elaborate the role of CpG sites in different regions of the *KDM1A* in the molecular pathogenesis of CRC.

In this study, we used two batches of tissue samples from different regions to study the association of *KDM1A* methylation with the risk of

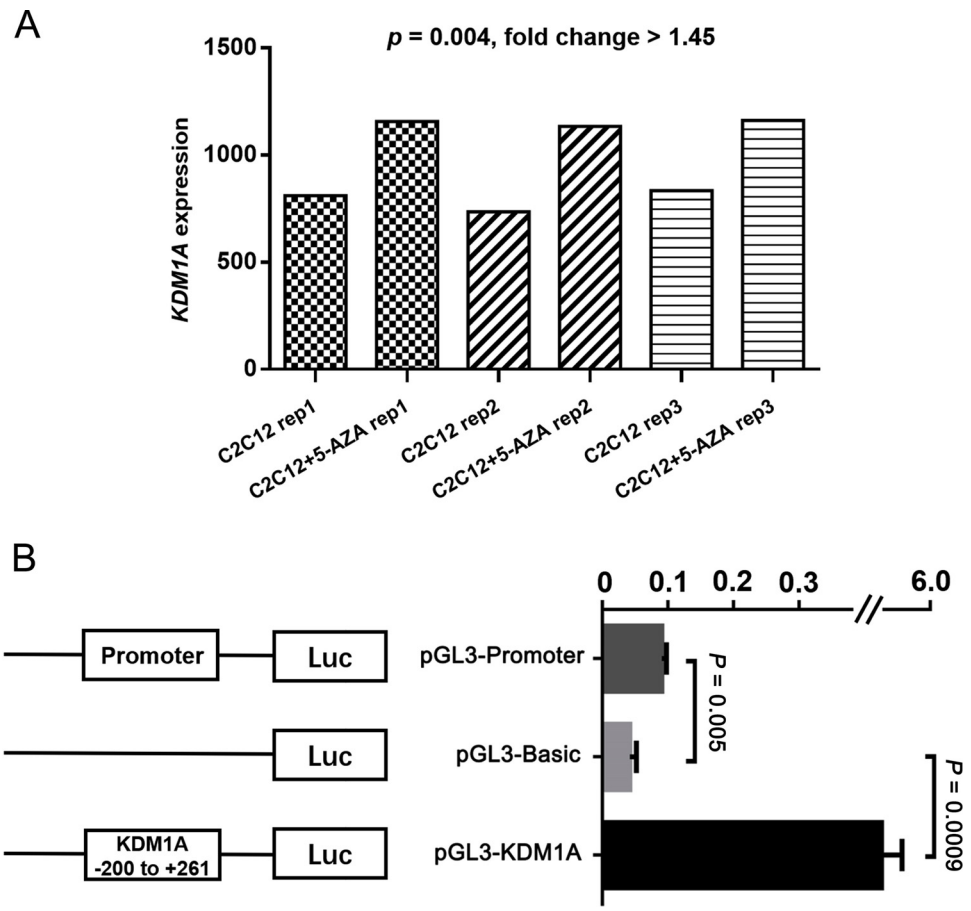

Fig. 5. *KDM1A* hypomethylation was correlated with higher *KDM1 A* expression. (A) *KDM1A* expression in C2C12 cell line before and after the treatment of demethylation agent 5′-AZA-deoxycytidine. Data were from GEO database (GSE30192). **(B)** Dual-luciferase reporter gene assay in HEK-293 T cell line. The pGL3-Basic vector was used as a negative control, and pGL3-Promoter vector was used as positive control. The pGL3-KDM1A stood for the recombinant *KDM1A* fragment ligated to pGL3-Basic vector. And relative luciferase activity was performed in quadruplicate.

CRC. In both experiments, the hypomethylation of the *KDM1A* promoter was found to be associated with CRC risk. The qMSP method was used in this study to detect *KDM1A* promoter methylation levels. The qMSP method is a method for quantitatively examining methylation levels. qMSP is more sensitive than the MSP method in methylation assays [33] and has a lower false positive rate [34]. However, our research still has some shortcomings. First, due to the lack of sufficient tissue samples, we were unable to perform *KDM1A* expression experiments in 112 pairs of samples, but bioinformatics analysis of TCGA and GEO data sets indicated that *KDM1A* methylation may regulate the expression of *KDM1A*. However, since the population involved in the TCGA and GEO data is unknown, future study is needed to verify the relevant findings in the Chinese population. Moreover, our samples were limited to Jiangsu and Zhejiang provinces. The effects of dietary habits on gene methylation were not fully considered. Finally, our sample size is small, and we still need further large sample experiments to verify our findings in the future.

In conclusion, our study found that *KDM1A* hypomethylation was closely related to CRC, providing a new idea for molecular biology research on the development of CRC in the future.

Conflict of interest statement

The authors declare no conflict of interest.

Acknowledgements

This research is supported by K. C. Wong Magna Fund in Ningbo University. We thank TCGA and GEO databases for their open access.

References

[1] E. Sakai, A. Nakajima, A. Kaneda, Accumulation of aberrant DNA methylation during colorectal cancer development, World J. Gastroenterol. 20 (4) (2014) 978–987.
[2] J. Huang, et al., DNA hypermethylated status and gene expression of PAX1/SOX1 in patients with colorectal carcinoma, Oncol. Ther. 10 (2017) 4739–4751.
[3] S.L. Rasmussen, et al., Hypermethylated DNA, a circulating biomarker for colorectal cancer detection, PLoS One 12 (7) (2017) e0180809.
[4] W.C. Ting, et al., Common genetic variants in Wnt signaling pathway genes as potential prognostic biomarkers for colorectal cancer, PLoS One 8 (2) (2013) e56196.
[5] T. Oh, et al., Genome-wide identification and validation of a novel methylation biomarker, SDC2, for blood-based detection of colorectal cancer, J. Mol. Diagn. 15 (4) (2013) 498–507.
[6] Y. Ma, Y. Chen, I. Petersen, Expression and promoter DNA methylation of MLH1 in colorectal cancer and lung cancer, Pathol. Res. Pract. 213 (4) (2017) 333–338.
[7] N. Rezvani, et al., Detection of SPG20 gene promoter-methylated DNA, as a novel epigenetic biomarker, in plasma for colorectal cancer diagnosis using the MethyLight method, Oncol. Lett. 13 (5) (2017) 3277–3284.
[8] M. Kanda, Y. Kodera, Recent advances in the molecular diagnostics of gastric cancer, World J. Gastroenterol. 21 (34) (2015) 9838–9852.
[9] F. Coppede, et al., Genetic and epigenetic biomarkers for diagnosis, prognosis and treatment of colorectal cancer, World J. Gastroenterol. 20 (4) (2014) 943–956.
[10] F. Coppede, The role of epigenetics in colorectal cancer, Expert Rev. Gastroenterol. Hepatol. 8 (8) (2014) 935–948.
[11] H.C. Hsu, et al., CBB1003, a lysine-specific demethylase 1 inhibitor, suppresses colorectal cancer cells growth through down-regulation of leucine-rich repeat-containing G-protein-coupled receptor 5 expression, J. Cancer Res. Clin. Oncol. 141 (1) (2015) 11–21.
[12] L. Kong, et al., KDM1A promotes tumor cell invasion by silencing TIMP3 in non-small cell lung cancer cells, Oncotarget 7 (19) (2016) 27959–27974.
[13] V. Kashyap, et al., The lysine specific demethylase-1 (LSD1/KDM1A) regulates VEGF-A expression in prostate cancer, Mol. Oncol. 7 (3) (2013) 555–566.
[14] D. Hanrahan, et al., Linguistic and cultural challenges in communication and translation in US-Sponsored HIV prevention research in emerging economies, PLoS One 10 (7) (2015) e0133394.
[15] J.H. Schulte, et al., Lysine-specific demethylase 1 is strongly expressed in poorly differentiated neuroblastoma: implications for therapy, Cancer Res. 69 (5) (2009) 2065–2071.
[16] K.W. Pajtler, et al., The KDM1A histone demethylase is a promising new target for

the epigenetic therapy of medulloblastoma, Acta Neuropathol. Commun. 1 (2013) 19.
[17] K. Lam, et al., DNA methylation based biomarkers in colorectal cancer: a systematic review, Biochim. Biophys. Acta 1866 (1) (2016) 106–120.
[18] Y. Dong, et al., DNA methylation as an early diagnostic marker of cancer (Review), Biomed. Rep. 2 (3) (2014) 326–330.
[19] M. Boccardi, et al., Atypical nucleus accumbens morphology in psychopathy: another limbic piece in the puzzle, Int. J. Law Psychiatry 36 (2) (2013) 157–167.
[20] R. Chen, et al., AGTR1 promoter hypermethylation in lung squamous cell carcinoma but not in lung adenocarcinoma, Oncol. Lett. 14 (4) (2017) 4989–4994.
[21] M. Tajadini, M. Panjehpour, S.H. Javanmard, Comparison of SYBR Green and TaqMan methods in quantitative real-time polymerase chain reaction analysis of four adenosine receptor subtypes, Adv. Biomed. Res. 3 (2014) 85.
[22] H. Thomassin, C. Kress, T. Grange, MethylQuant: a sensitive method for quantifying methylation of specific cytosines within the genome, Nucleic Acids Res. 32 (21) (2004) e168.
[23] L.S. Kristensen, et al., Sensitive melting analysis after real time- methylation specific PCR (SMART-MSP): high-throughput and probe-free quantitative DNA methylation detection, Nucleic Acids Res. 36 (7) (2008) e42.
[24] S.P. Narayanan, et al., Integrated genomic analyses identify KDM1A's role in cell proliferation via modulating E2F signaling activity and associate with poor clinical outcome in oral cancer, Cancer Lett. 367 (2) (2015) 162–172.
[25] S.X. Lian, et al., Lysine-specific demethylase 1 promotes tumorigenesis and predicts prognosis in gallbladder cancer, Oncotarget 6 (32) (2015) 33065–33076.
[26] S. Nagasawa, et al., LSD1 overexpression is associated with poor prognosis in basal-like breast cancer, and sensitivity to PARP inhibition, PLoS One 10 (2) (2015) e0118002.
[27] Z.K. Zhao, et al., Overexpression of LSD1 in hepatocellular carcinoma: a latent target for the diagnosis and therapy of hepatoma, Tumour Biol. 34 (1) (2013) 173–180.
[28] H. Clevers, R. Nusse, Wnt/beta-catenin signaling and disease, Cell 149 (6) (2012) 1192–1205.
[29] L.L. Han, et al., Aberrant NDRG1 methylation associated with its decreased expression and clinicopathological significance in breast cancer, J. Biomed. Sci. 20 (2013) 52.
[30] C. Yuan, et al., High expression of the histone demethylase LSD1 associates with cancer cell proliferation and unfavorable prognosis in tongue cancer, J. Oral Pathol. Med. 44 (2) (2015) 159–165.
[31] J. Ding, et al., LSD1-mediated epigenetic modification contributes to proliferation and metastasis of colon cancer, Br. J. Cancer 109 (4) (2013) 994–1003.
[32] L.D. Moore, T. Le, G. Fan, DNA methylation and its basic function, Neuropsychopharmacology 38 (1) (2013) 23–38.
[33] G. Malpeli, et al., Methylation-associated down-regulation of RASSF1A and up-regulation of RASSF1C in pancreatic endocrine tumors, BMC Cancer 11 (2011) 351.
[34] L.S. Kristensen, et al., Assessment of quantitative and allelic MGMT methylation patterns as a prognostic marker in Glioblastoma, J. Neuropathol. Exp. Neurol. 75 (3) (2016) 246–255.