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Effect of histone demethylase KDM5A on the odontogenic differentiation of human dental pulp cells

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ABSTRACT

Human dental pulp cells (hDPCs) possess the capacity to differentiate into odontoblast-like cells in response to exogenous stimuli. Histone methylation is one of the most robust epigenetic marks and is essential for the regulation of multiple cellular processes. Previous studies have shown that histone methyltransferases (HMTs) and histone demethylases (HDMs) are crucial for the osteogenic differentiation of human bone marrow, adipose tissue, and tooth tissue. However, little is known about the role of histone methylation in hDPC differentiation. Here, the expression levels of HMTs and HDMs were profiled in hDPCs undergoing odontogenic induction. Among several differentially expressed enzymes, HDM KDM5A demonstrated significantly enhanced expression during cytodifferentiation. Furthermore, KDM5A expression increased during early passages and in a time-dependent manner during odontogenic induction. Using a shRNA-expressing lentivirus, KDM5A was knocked down in hDPCs. KDM5A depletion resulted in greater alkaline phosphatase activity and more mineral deposition formation. Meanwhile, the expression levels of the odontogenic markers DMP1, DSPP, OSX, and OCN were increased by KDM5A knockdown. As a histone demethylase specific for tri- and dimethylated histone H3 at lysine 4 (H3K4me3/ me2), KDM5A deficiency led to a significant increment in total H3K4me3 levels, whereas no significant difference was found for H3K4 me2. H3K4me3 levels on the promoters of the odontogenic markers increased after KDM5A knockdown in hDPCs. These results demonstrated that KDM5A is present in hDPCs and inhibits the odontogenic differentiation potentiality of hDPCs by removing H3K4me3 from specific gene promoters, suggesting that KDM5A-dependent histone demethylation may play an important role in reparative dentinogenesis.



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KEYWORDS

histone methylation; KDM5A; human dental pulp cells; odontogenic differentiation; H3K4me3



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Introduction

Human dental pulp cells (hDPCs) isolated from the postnatal human dental pulp comprise a heterogeneous mesenchymal cell population with the capacity to differentiate into odontoblastlike cells and to generate reparative dentine in response to exogenous stimuli or injury [1-3]. hDPCs can be a useful cell resource for developing biological treatment strategies for dental pulp repair and regeneration, and their therapeutic applications hinge on an understanding of the molecular mechanisms that induce odontogenic differentiation[4]. While growing evidence has demonstrated that signaling pathways, growth factors, and epigenetic regulators are involved in the odontogenic differentiation process of hDPCs [5-7]. Especially the epigenetic regulatory mechanisms underlying hDPC differentiation have attracted increasing attention. Recent evidence has illustrated that epigenetic modifications, such as histone acetylation and DNA (de)methylation, are involved in reparative dentinogenesis [8–14].

Histone methylation is a robust epigenetic mark that modifies the lysine and arginine residues of histone N-terminal tails and controls the accessibility of DNA at a particular locus by altering its chromatin compaction[15]. Histone methylation can signal either activation or repression, depending on the sites of methylation. Methylation at arginine residues, and di-/tri- Methylation at the H3K4, H3K36, and H3K79 are normally characterized as active marks, while methylation at the H3K9, H3K27, and H4K20 residues remain controversial [16,17]. The establishment and maintenance of histone methylation are dynamically regulated by site-specific histone methyltransferases (HMTs) and histone demethylases (HDMs), which play important roles in multiple cellular processes, including cell proliferation, differentiation, senescence, DNA damage response, oncogenesis and individual development [18-21]. During murine tooth germ development, covalent histone modifications characterized by H3K4me3 and H3K27me3 can be detected in the first molars of mouse embryos. In addition, several HMTs and HDMs are expressed in the mesenchyme and the epithelium of the tooth germ [22,23]. During the odontogenic differentiation of human dental pulp

stem cells (hDPSCs), histone methylation levels in the promoters of mineralization markers, such as RUNX2 (RUNX family transcription factor 2, also known as CBFA1), MSX2, and DLX5, are altered, followed by the upregulation of gene expression levels[24]. This evidence suggests that histone methylation may be relevant to the odontogenic differentiation of dental-derived mesenchymal stem cells. However, little is known about how histone methylation controls the odontogenic differentiation of hDPCs.

In the present study, we investigated differentially expressed HMTs and HDMs during the odontogenic differentiation of hDPCs. The HDM KDM5A, also known as JARID1A/RBP2, was found to be highly expressed in the differentiated cells [25,26]. To determine the existence and function of KDM5A in the odontogenic differentiation of hDPCs, KDM5A expression was profiled during cell passaging and odontogenic induction. shRNA was then used to knock down KDM5A expression levels in hDPCs, and the role of KDM5A depletion in the odontogenic differentiation potentiality of hDPCs was estimated.

Materials and methods

Ethics committee approval and patient consent

This study was approved by the Ethics Committee of the Affiliate Stomatology Hospital of Sun Yatsen University. Informed written consent was obtained from the donors. The investigation conformed to the principles outlined in the Declaration of Helsinki.

Cell isolation and culture

To obtain hDPCs, healthy human impacted third molars or premolars were collected from young adults aged 18 to 25 years in the Department of Oral and Maxillofacial Surgery at the Affiliate Stomatology Hospital of Sun Yat-sen University after informed patient consent was obtained according to ethical guidelines. hDPCs were isolated and cultivated as previously described[27]. Briefly, minced pulpal tissues were digested for 20 minutes at 37°C in a solution with 3 mg/mL type I collagenase and 4 mg/mL dispase (Gibco, CA, USA) and were then cultured in Dulbecco's Modified Eagle Medium (DMEM) (Gibco) plus 10% fetal bovine serum (FBS) (Gibco), 100 units/ mL penicillin and 100 mg/mL streptomycin (Gibco) at 37°C in a humidified atmosphere of 95% O_2 and 5% CO_2 . Cells that migrated out of pulp pieces after 2 weeks were trypsinized, harvested and passaged serially. Cells collected at passage 2 or 3 were then used for further study.

Induction of odontogenic differentiation

For odontogenic differentiation, hDPCs were seeded into 24-well plates (2×10^4 cells per well) and 6-well plates (10^5 cells per well) and expanded in DMEM containing 10% FBS until they reached 80% confluence. The cells were then exposed to mineralization medium (MM), which contained DMEM supplemented with 5% FBS, 50 µg/L ascorbic acid, 10 mM βglycerophosphate and 10^- [7] M dexamethasone (Sigma-Aldrich, St. Louis, MO, USA). Cells treated with growth medium (GM) were used as the control group. The culture medium was changed every 3 days.

Real-time qPCR

Total RNA was extracted from the hDPCs using TRIzol reagent (Life Technologies Corporation, CA, USA) following the manufacturer's protocol. Reverse transcription was performed using a Transcriptor First Strand cDNA Synthesis Kit (Roche, Basel, Switzerland). Real-time qPCR was performed using a LightCycler 480 with SYBR Green I Master (Roche). Specific primers were designed using Primer Express v3.0 software and were synthesized by Invitrogen (Life Technologies Corporation). The primer sequences are shown in Table 1. The housekeeping gene β -actin was used as a reference. Relative gene expression was calculated using the comparative $2^{-\Delta\Delta Ct}$ method. Each experiment was performed in triplicate.

Western blot analysis

Total proteins were harvested using RIPA lysis buffer with phenylmethylsulfonyl fluoride (PMSF, Beyotime Biotechnology, Haimen, China), while nucleoproteins were extracted with nuclear and cytoplasmic extraction reagents (Thermo Scientific, Rockford, IL, USA). Then, the concentrations of total proteins or nucleoproteins were measured with a bicinchoninic acid (BCA) protein assay (Beyotime Biotechnology). Equal amounts of protein were electrophoresed in a 4% ~ 20% Tris-Gly Mini Precast PAGE Gel (Hegao Biotechnology, Shanghai, China) and were then blotted onto polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). The membranes were incubated with the following primary antibodies overnight at 4°C: anti-KDM5A (Abcam, Cambridge, UK; 1:500), anti-DSPP (Santa Cruz Biotechnology, Santa Cruz, CA, USA; 1:200), anti-DMP1 (Abcam; 1:400), anti-OCN (Abcam; 1:1000), anti-OSX (Abcam; 1:1000), anti-H3K4me2 (Abcam; 1:500), anti-H3K4me3 (Abcam; 1:500), anti-H3 (Abcam;1:2000), and anti- β -actin (Bioworld, MN, USA; 1:5000). Then, the membranes were steeped in horseradish peroxidase (HRP)conjugated anti-rabbit (Abcam; 1:2000) or antimouse (Abcam; 1:2000) secondary antibody for 1 hour at room temperature. Protein bands were visualized with an enhanced chemiluminescence system (Millipore ECL Western Blotting Detection System, Millipore), and band densities were obtained and normalized to β -actin using ImageJ 1.47 software (National Institutes of Health, MD, USA).

Alizarin red S staining

To assess mineralization in vitro, transfected cells seeded in 6-well plates were allowed to undergo odontogenic differentiation for 14 days. After culture, the cells were rinsed three times with PBS, fixed with 4% paraformaldehyde solution for 30 minutes and rinsed in distilled water. Mineral depositions were stained with 1% alizarin red S (GL Biochem, Shanghai, China) solution for 10 minutes at room temperature and were rinsed several times in distilled water. The mineralized nodules were then photographed. For quantitative analysis, the stains were dissolved with 10% cetylpyridinium chloride (CPC, Sigma-Aldrich) for 40 minutes, and the absorbance of the solution was measured at a wavelength of 562 nm using an automated microplate reader (Sunrise, Tecan, Salzburg, Switzerland). Quantitative measurements were calculated according to the generated standard curve.

Table 1. The primer sequences for real-time qPCR analysis.

ReverseTACACCACTONGAGACTONASH1 LForwardGATONTCACGCATAATCCCCDOT1ForwardGATONTCACGCATAATCCCCDOT1ReverseCCCTGGAGGTAGCTGGGAACCSETSEDD1)ForwardGATOSTACACTGCAGCATCACGCAGACCG9AForwardGGTCGTACAGCGGCATCACGCMLL1ForwardCCGGAATCATGGAGGCACMLL1ForwardCCGGAATCATGGAGGCACMLL1ReverseCCGGAACCTGTCTCTCCCMLL2ForwardGTTGTGGACACCTGTCTTCTCTMLL3ReverseCCGGAACCTGTCTCTCTCCML14ReverseCCGGAACCTGTCTCTCTCTML15ReverseCTGAATCGGAACCTGAACGCACML14ReverseCTGAATCGGAACCTGAACGCACML15ReverseCTGAATCGGAACCTGAATCGCACML14ReverseCTGGGAACCTGTAATCGCACCML15ReverseCGGCAACACACACCTCTCATCTCCACCTCACTCACCCCCC	β-actin	Forward	CTGCGGGTATCCATGAGA
ASH1 L Forward GATGATCTACGCTANGCCCC Beverse TAGTTTGACACCTACAGACCC DOT1 Forward MAGAGATGACACTTACCTCT DOT1 Forward GAGCGTACAGGTACTGGTACACC Beverse CGCTGGACAGCGTACTGGTACACC SESTESDB1) Forward GGTGTACAGGTACTGGTACAGC Beverse CGGGCATCTATGCAGTACACC GAA Forward GGTGGTACAGGTGCAGACTGGCACAGC Beverse CGGACATTACGCATTAAGG ML12 Forward GTGTGGGACAGGTGTGGAGGAC ML12 Forward GTGTGGGACAGTTGGAGGAC ML13 Forward GTGGGGCATTGGAGGACC ML14 Forward GGTGGGACGGACCGTGC ML15 Forward GGTGGCACGGACCGTGC ML16 Forward GGTGGCACGGACCGACC ML17 Forward GGTGGCACGGACCGACC ML18 Forward GGTGGCACGGACCGACC ML14 Forward GGTGGCACGGACCGACC ML15 Forward GGTGCGCACGACCGCACC NSD1 Forward GGGCGCACGACCGCACC PRMT1 Forward GGGCGCGCACCACACGCCC PRMT1 Forward GGCGCGCCCCCACCACC PRMT3 Forward GGCGCGCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC		Reverse	TACCACCACTGAGAACGATG
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ESETISDB1) Reverse GAAGCATCAGTCACCATCAGTCACC GOA Forward GGTCGTACAACGTCGTCGCC GOA Reverse CGGCGCTATAGCAGTACACCT MLL1 Forward CCACAGAATCACACTCATAGGA ML22 Forward GTGTGTGCGGGATTGTGGGGGCA ML22 Forward GTGTGTGCGGGATTGTGGGGCA ML23 Forward GTGTGTGCGCGGATTGTGGGGCA ML23 Forward GTGTGTGCGCGGATTGTGGGGCA ML23 Forward GTGTGTGCGCGGATTGTGGGGCA ML24 Forward GTGTGTGCGCGGATTGTGGGGCG ML25 Forward GTGTGTGCGCGGATTGTGGGGCG ML25 Forward GTGTGTGGGGGGATTGTGGGGCG ML25 Forward GTGCGGGATTGTGGGGCG ML25 Forward GTGCGGGATTGTGGGGCG ML25 Forward GTGCGGGATTGTGGGGCG ML26 Forward GTGCGGGATTGTGGGCGC ML26 Forward GTGCGGGATTGTGGGCGC ML26 Forward GTGCGGGATTGTGGGCGC ML27 Forward GTGCGGGATTGTGGGGCG ML26 Forward GTGCGGGGATTGTGGGGGGGGGGGGGGGGGGGGGGGGGG		Reverse	CGCTCGGAGGTAGCTGGTAGAAC
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MLL5 Forward ATGTICACCTTCACTTCACTTCACTGCAGTCACAGTCACAGTCACAGTCACAGTCACAGTCACAGTCAGT		Reverse	GATGGGGAACTTGTAGTCGTAGGT
ReverseTTGAGCAGGANTTACAGATCAGCTNSD1ForwardCCGCCAGCAGGAGCTGCTCATTReverseAGGTTATGAGATCATCACCAAGGPRMT1ReverseCCAGCCGCCTGCGCACCAGGGACACCGReverseCCAAGCTAGCGAGCGGGCGCGCAPRMT4ForwardCCAAGCGACGGGGCGCGCAReverseCAAGTCATAGCGGTCCCCACCTAATCGReverseGAACCTCTCCCACCTAATCGCReverseGGGACGTCTTCCACCTAATCGCPRDM4ForwardCCAAGGAGGGGTCGTCGCCACCTAATCGCReverseGGGAGGTGTTGTCAATCACACAReverseGGGAGGTGTTGTGTGAATACACAPRDM12ForwardGGGAGGTGTTGTGTGAReverseCCCCAGAGAGGTGTTGTGTGAReverseCCCCAGAACGACTCCCGCCGCPTIPForwardGGCGAACTGACCCCCAACGPTIPForwardCCCCGAACCTGCCCCAACGPTIPForwardCCCGCAACCTGCCCCCAACAReverseGGCAAACGACCCCCCAACAReverseGGCAAACGACCCCCCAACASETD1AForwardACCCGCGGTCCTCTGCAACAGGSETD2ForwardCCACCAACAGCACACACACACAGCAACGSETD3ForwardCCACCAACAGCACACACGCCCCCAACCReverseGGCAAACAGCCCCCCCCACTCGSETD3ForwardCCACCAACAGGACAACGGCAACCGCSMVD3ForwardCCACCAACAGGACAACGGCCCCACTCGSMVD3ForwardCCCCCACACAGGACACGCCCCACTCGSMVD3ForwardCCCCCACACAGGACACGCCCCACTCGSMVD3ForwardCCCCCACACAGGACACGCCCCACTCGSMV34ForwardCCCCCACACAGGCCCCCACTCGSMV34ForwardCCCCCACATACCCCCACTCGCCCCCACCCSMV34	MLL5	Forward	ATGTTCAGTCTTCACCTTCATCTCA
NSD1ForwardCCGCCAGACAGACIGCTCATTReverseAGGTCTATGAGACCATCACCACACAPRMT1ForwardGCAACCTCACTTACCGCAAACTReverseTCTAACTGATGACGTCTGACTACCGCAACGReverseCAAGGCAGGGGACACGCReverseCAAGGCATGAGCGGCACCGCReverseGAACCGTCCTCACTCACTGCCGCGAReverseGAACCGTCCTCACCTCAACTCACCCCCReverseGAACCGTCCTCCACCTAATGCCGCGGAATACACAReverseGAACCGTCCTCCACCTAATGCCGCGCAATACACAReverseGAACGGTGTTCATGCGGGAATACACAPRDM12ForwardReverseGGAAGGTGTTCATGGGGGATATCACAAPRDM16ForwardReverseGGAAGGTGTTCATGGGGPTIPForwardReverseGGCAAACTCCCGACGGATCAGGCPTIPForwardACCCGCACCTCGCCCCAAAReverseGGCAAACTCCCACCAGAReverseGGCAAACTCCCACCAGASETD1AForwardReverseGGCAAACGTCCCACAGAReverseGGCAAACGACCCCCCAAAReverseGGCAAACGACCCCCCAAAReverseGGCAAACGTCCCCCCAAAReverseGGCAAACGTCCCCCCAAAReverseGGCAAACGTCCCCCCAAACGGSETD2ForwardGREverseGCCAACGTCCCCCCCAAACGGSETD3ForwardReverseGGCAAACGTCCCCCCCTCGGCGTCCTCGSWTD2ForwardReverseGGCAAACGCCTCCCCCCCTCCCCCCCCCCCCCCCCCCCC		Reverse	TTGAGCAGGAATTACAGATCCAGGT
PRMT1FeverseAGGTCTATCAGCAACATPRMT4ForwardGCACCTCACTACGCCAACATPRMT4ForwardCCAAGGCAGGGGACACGCPRMT4ReverseCAAGTCATACGGCAACACTPRMT4ForwardGCAACCTCACCGCAREverseTAATGGCGGGCACCCCPRDM4ForwardGAAGTCATACGCGCCPRDM4ForwardCAAGTCATCGCCGCATACCCPRDM12ForwardCCAGGAAGGGTTCAATGAGGATPRDM12ForwardTGGGAGGTGTTCAATGAGGATPRDM16ForwardTTCCACCTAGCTGCCGCATACCGCPRDM16ForwardTTCCACCTAGCTGCCGCATACGCPTIPForwardAGTGCTCACGGCATTCGGTCAATGAGGTTAPTIPForwardAGTGCTCACGGCCTTCCGCCAAGSETD1AForwardACCCGGAACCTCTCCACCAACGSETD2ForwardACCTCGCGCACCCCCCAAASETD2ForwardCCCGGAACCTCCCAACGGCATTCGGSETD3ForwardCCCGGAACCTCCACAACCTGACCCCCCCAAReverseGGCAAACCAGCCCCACTCGSETD4ForwardCCCCGAGCCCCCCCGAACReverseGCCAAACCTCCCAAAGCAAACCTCAAAACTCCReverseGCCAAACCTCCCAAAGCAAACCTCAAAACTCCReverseCCCGCAACCTCCCAAACCTCCAAACCTSMVD3ForwardCCACCAGGACAGGCAAAGCAAAGCAAAGCCAAAGCCAACCTReverseGCGAAACCTCCCAACCTGCCCGAACCTSUV39H1ForwardCCCCAGGACGCGTCCTCTCACACAGCGAACCTReverseGCGAAACCGACCGCTCCTACAACCGGGGKDM3AForwardCCCCCAGGACCGCTCCTCAAAGCGGACCGTCCTCACAACCGAACCTReverseGCGCAACCGCCCCCCACCGAACCGTKDM3AForwardCCCCCAGGAC	NSD1	Forward	CCGCCAGACAGACTGCTCATT
PIN11ForwardGCCCCCCCCCCCCCACTAReverseCTAACTICIGCCTTGACCGATPRMT4ForwardCCAAGGCAGGCGACGCReverseCAAGGCAGGCGCCCCCCCCCCCCCCCCCCCCCCCCCCC		Reverse	AGGTCTATGAGATCCATCACCAAAG
PRMT4 Forward CCAAGGCAGGGGACACG PRMT5(IBP72) Forward GAACCGTCCTCCACCTAATGC Reverse CAAGTCATAGGCGGTCCGCA PRDM14 Forward GAACCGTCCTCCACCTAATGC Reverse TGATGGGGTCCTTTTCAAACACT PRDM14 Forward CAATACCATCTCCACCCGC Reverse AGGACTCATCCGTCAATACACA PRDM12 Forward TGGGAGGTGTTCAATGAGGAT Reverse CCCCAGGAAGGTGTTCAATGAGGAT PRDM16 Forward TTCCACCTAGACTCCGGACATCGG PTIP Forward TTCCACCTAGACTCCGGACATCGG PTIP Roward TTCCACCTAGACTCCGACGCTTCA Reverse CCGCAACCTCCACGCAG SETD1A Forward AGCCCACCTGCACCGCCAA Reverse GGCAAACAGCACCCCCCAAG SETD1A Forward ACCCAACCTGACCCCCCAAG Reverse AGCCAACCTGCACCCCCAAG SETD1A Forward ACCCAACCTGACCCCCCCAAA Reverse ACCCCAACCTGACCACCCCCAAG SETD1A Forward ACCCAACCTGACCCCCCCAAA Reverse ACCCCAACCTGACCACCCCCAAG SETD2 Forward ACCCAACCTGCCCACCCAAG SETD2 Forward ACCCAACCTGCCCCCCAAA Reverse ACCCCAGCAGCCAACTCCAACG SETD2 Forward CCAAACCACCCCCCCAAA Reverse ACCCCAGCAGCCAACTCCAACG SETD3 Forward CCAACCTGCCCCCCCAAA Reverse ACCCGCAACCCCCCCCAAA Reverse CGCGAAACCAAGCCCAACTCCAACG SETD3 Forward CCAACCCACCTCCCCAACA Reverse CGCGAACGCCAACTCCCAACAG SETD3 Forward CCCCAGGCAACCCCCCCCCAAA Reverse ACCCGAGACAGCCAACTCCCAACG Reverse ACCGGAACCCCCCCCCCAAA Reverse CCTGCACACCCCCCCCCCAAAACCCC Reverse ACCGGAACAGCCAACTCCCCCCCCCCCCCCCCCCCCCCC	PRMT1	Forward	GCACCCTCACTTACCGCAACT
PHM14ForwardCCAAGCLANGGGATCGCAReverseCAAGCLANGGCGATCGCAPRMT5(IBP72)ForwardReverseTGATGGGGTCCTCACCTTATCCReverseCAAATCACCTTCAACCGCCReverseCAAATACCATCTCACCGCCPRDM4ForwardCCCCAGGAAGGTGTTCAATCAGGATReverseCCCCAGGAAGGTGTTCAATCAGGATReverseCCCCAGGAAGGTGTTGTGTGGAPRDM16ForwardReverseAAATCGCTCCACGTCCAGATCAGGCPTIPForwardReverseCGCGAACCTGCCAGACCGCAGCPTIPForwardACCCAGCAGACGTCCCAGCCCCCAAAReverseCGCGAACCTGCCCCCCAAAReverseGGCAAACGAGCCCCCCAAGSETD1AForwardReverseGGCAAACGAGCCCCCCAAGReverseGCCCAACCTGACCCCCCCAAAReverseGGCAATCCGCCCCCCCCAACReverseGGCAATCAGCCCCCCCAACGCSETD7ForwardCCCCGACCTCCTCTGReverseSETD7ForwardReverseGGTCATAGCCAACCGCCCCCCAACReverseGGTCATAGCCAACGCACCCCCAACGCReverseGTCATAGCCAAGGAACGCCCCCAACGReverseCCCCCCAAGGACAACGCCCCCCAACGCReverseCCCCCCAAGGACAAGGCATCACACTSMYD3ForwardReverseCCGGGTCACCATCCCAACTCCAACCCCCCCCCCCCCCAACCCCCC		Reverse	ICIAACIIGIIGGCIIIGACGAI
NeverseCLARGICATIAGUCGICGCAPRMTS(IBP72)ForwardGAACCTATGCCPRDM4ForwardCAATACCATCTCAATGCCPRDM4ForwardCAATACCATCTCACCCGCCReverseAGAGACTATCCCCCGAATACACAPRDM12ForwardTGGGAGGGTGTTCAATGAGATReverseCCCCAGAGAGGTGTTCAATGAGGATReverseCCCCAGAGAGGTGTTCAATGAGGATReverseAATGCTCAACGGCATTCAGTCAGGCGTTAReverseAATGCTCAACGGCATCCGACGPTIPForwardACCCACCTTCACCGCACGSETD1AForwardACCCACCTGACAGCGCATCCGATGSETD2ForwardACCCAGCAACGCCCCCAAAReverseGGCAAACGACCCCCAAAATReverseGGCAAACGACCCCCAAAAATReverseGGCAAACGACCCCCAAAAAATReverseGGCAAACGACCCCCAAAAAACGCCSETD2ForwardCCCAGCAAGCACCCCCAAAAAACGCCSETD3ForwardCCCAGCAACGCCCAAAAACGCCSETD4ForwardCCACCAAGAACCTCCAAAAAACTCCReverseGGCAAACGTCCCAAAAAACTCCSWD2ForwardCCACCAAGGACCTCCAAAACACCCCCAAAGACGCSWD2ForwardCCACCAGGACGGTGTCACTTSWD3ForwardCCACCAGGAGGGACGGCSWD3ForwardCCACCAGGACGGTGTCACATCAACACCSWD3ForwardCCCACGAGGAGGACGCGSWD3ForwardCCCACGAGGAGGCGTCACATTSMD2ForwardCCCACGAGGAGGACGTReverseCGGAACGCTGTCACATACCACCGCGCGCGReverseGGCAAACGTTCACACAGCCCCGCGCGCGReverseGGCAACGTTCGCAGTTACCACAGGCCCGGCGGGGAGACGTReverseGGCAACGTTCGCAGTTACCC	PRM14	Forward	
PKMIS(IBP/2) Forward GAACCGICCICACCTAAIGC Reverse GAACCGICCICACCTAACACA PRDM4 Forward CAAATACCATCTCACCGGCC PRDM12 Forward TGGAGGGTGTTGATGAGGAT PRDM12 Forward TGGAGGGTGTTGATGAGGAT PRDM16 Forward TGCCAGGAGGTGTGTGATGA Reverse AAATGCTCCAGGCATCCAGC PTIP Forward ACGCACCGACGGCATCCAGC SETD1A Forward ACGCACCTGACCACCCCAAG SETD2 Forward ACCCCCCACAAG SETD2 Forward ACCCCCCACAAG SETD2 Forward ACCCCCCAAA Reverse AACTICCGGCGTCCTCTG SETD7 Forward ACCCAACCTGACCCCCAAAC Reverse ACCTICCGCCAACCGGCATCCGT SETD7 Forward ACCCAACCTGCTCCCCAAAC SETD8 Forward CCCCACCAACG SETD8 Forward CCCCCAACGCGACTCCATCC SETD8 Forward CCCCCCAACGCAACCCCCAACG SETD8 Forward CCCCCCAACGCAACCCC SWYD2 Forward CCCCCCAAGGCATCCG SWYD3 Forward CCCCCCAACGGCAACCCC SUV39H1 Forward CCCCACGAGGCATCCGACG SUV39H1 Forward CCCCGCAACGTGTGCCTG SWYD3 Forward CCCCGCAACGTGCAACGCGAACTTC SUV39H1 Forward CCCCGCAACGGCAACTTCAACT Reverse ACGGGAACGGCATCCCAACG SUV39H1 Forward CCCCGCACGGCGTCCCTG SWYD3 Forward CCCCGCACGGGCGTCCTCTG SWYD3 Forward CCCCGCACGGGCGCCTCCAACG SUV39H1 Forward CCCCGCGGCGTCCTTGCACCCG SUV39H1 FORWARG CCCGCGGCGTCCTGCACGG SUV39H1 FORWARG CCCGGGCGCTCCTGCACGG SUV39H1 FORWARG CCCGGGCGCGCGCATCCC SUV39H1 FORWARG CCCGGGGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCG		Reverse	CAAGICATAGGCGGICGGCA
NeverseTotAlogoti CLITICACARCEPRDM4ForwardCAANGCGUCLITICACARCCPRDM12ReverseAGGACTCATTCCGCGGAATACACAPRDM13ForwardTGGGAGGTGTCAATGAGGATReverseCCCCAGGAAGGTCTGGTGAAPRDM16ForwardTTCCACCTTAGATCTGAGGCTTTAReverseAAATGCTCCGACGCPTIPForwardAGTGCTCAGACTCCGACGSETD1AForwardACCCAACCGCACCCCCAAAReverseGGCAAACGAACCCCCCCCAAAReverseGGCAAACGAACCCCCCCCGTSETD2ForwardACCCGAAGCCCCCCCCAAAReverseGGCAAACGAACCCCCCCCTAACReverseGGCAAACGAACCCCCCCCCAAACReverseGGCAAACGACCCCCCCCCAAACReverseACCCGAGCAAGCAAACTCCCCCCAAACReverseGGCCATAGGCATAGGCAACGGSETD7ForwardCGACGAGACCTCCCAAACCReverseGGCATAGCCATAGGCATAGGCAACGGSETD8ForwardCCACCAAGGACAAGGACAAGGSMYD2ForwardCCCACCAAGGACAAGGCAAAGGCReverseACGGGACAAGGACAAGGCAAGGCReverseACGGGACAAGGACAAGGCCATAGCCSUV39H1ForwardCCCAGTAGCCAACGTTCCCCGACACTReverseGTGCAACAGCCCCGCGCCCGKDM2AForwardCCCCGGCACCCTGCTGCTCCAAACCTReverseGTGCACACGTTGCGCCCCGAACGTReverseGTGCACACGTTGCGCCCCGAACGTReverseGTGCACACGTTGCGCCCCGAACGTReverseGTGCACACGTTGCGCCCCGAACGTReverseGTGCACCCTGCACACGTCCCAAACGCCCCCAACACGTReverseGTGCACCCTGCCGCCTGCTGCTCCAAACGCGAACGGGCACCTReverseGTGCACCCTGCC	PRMT5(IBP72)	Forward	
PHDM4 Forward CAAATACCH CLACCGCC Reverse AGAGACTCATCCGTGAATACACA PRDM12 Forward TGCGAGGTGTTCAATGAGGAT Reverse CCCCAGGAAGGTGTTGTGTGGA Reverse CCCCAGGAAGGTGTTCGAGCGCTTAA Reverse CCCCAGGAACGCCACCGCAGG PTIP Forward AGTCGTCACACGGCATTCAGTC Reverse CGCAACCTCTCCAGACCCCCCAAA Reverse SETD1A Forward ACCCAGCAACCGACCACCTGT SETD2 Forward ACCCAGCAGCGCACTCGTCAAAC SETD2 Forward ACCCAGCAGCAGCACCCCCAAA SETD3 Forward ACCCAGCAGCAGCACCCCCAAA SETD4 Forward ACCCAGCAGCAGCACCCCCAAAC SETD5 Forward ACCCAGCAGCAACCTCCTAACCACACGG SETD7 Forward CCCCAGCAGCAGCATACCGCAACGG SETD8 Forward CCACCAGCAGCATACCACACGG SWPD2 Forward CCACCAAGGATAAGGCATACAC SWPD3 Forward CCCCCACTTCGACCACATCACAC Reverse ACGGAAACAGCCCCCGCATCACT SUV39H1 Forward CCCACAGGACGCCCCCGCATCACT Reverse CATCGAGACGCCCCCCTAA Reverse GGAAACAGCCCCCATACACT Reverse CCAGGAAACGCCCCCATCACT RCM29 Forward CCCCACAGACGCCCCCCTAA	22214	Reverse	
NeverseAdaGat/Tictor Incort Incor	PRDM4	Poverse	
PhD/m12 Pol/wald Pol/wald Pol/wald Reverse CCCCAGGAAGGTGTGTGTGA PRDM16 Forward TTCCACCTTAGATTCTGAGGCTTTA Reverse CAAATGCTCCAGACTCCGACG PTIP Forward AGTGGTACAGCGCATTCAGTC Reverse CGGAAACCAGCCCCAAG SETD1A Forward ACCCAGCAGCAGCCCCAAA Reverse GGCAAACAGACCCCCCCCAA Reverse GGCAAACAGACCCCCCAACG SETD2 Forward ACCCAGCAGCAGCAACCAGCCCCAAAC SETD3 Forward GCCAAATCACTCCTCGTCCTCG SETD4 Forward GCCAAATCACTCCTCCAAAC SETD5 Forward GCCAAATCACTCCTCCAAAC SETD8 Forward CCACCAAGGAACCTCCCAAAAACTCC Reverse GCTCCTTCCACTCCAATCAA GCAAATCACTCCTTCACTCCAATCAA SMYD2 Forward CCACCAAGGCACAGGATAAGGC SWYD3 Forward CCACTGGGACGCACGACTCCC SUV39H1 Forward CCCACTGGGACGCACCGACCTGG Reverse CCTGGTCATCTGGGAGCACGACCTGCCGAACCT Reverse CCTGGTCACACTTGAGGCACCGACCT Reverse CCTGGTCACACTGGACACCT SUV39H1 Forward CCACCACAGGCCCGGACCCGG Reverse CCTGGTCACCCTGACCCTGAACCT Reverse CCTGGTCACACGTTCCCCGGCATAGC <tr< td=""><td></td><td>Forward</td><td></td></tr<>		Forward	
PRDM16 Forward TCCACCTIAGATTCTGAGGCTTTA Reverse AAATGCTCCAGACTCCGACG PTIP Forward ACCCACCTGACCTCCGACG Reverse CCCGAACCTCTCCACCAAG SETD1A Forward ACCAACCGGCATCCAGA SETD2 Forward ACCCACCGCACAAAT Reverse GGCAAAACAGACCCCACTCG SETD2 Forward ACCCACCGCACAAAT Reverse GGCAAACCGCCTCCTCG SETD7 Forward CCCAGCACAGCAACCTCAAAAT Reverse GGCAAACCGCCCCCAAA Reverse GGCAAACCGCCCCCAAACG SETD8 Forward CCAGCAGACCTCCATAGGCACGG SETD8 Forward CAGCAGAACCTCCAAAACTCC Reverse GGCAAACCTCCAAAAACCGG SETD8 Forward CCACCAGAACAGGGCAACTCC SMYD2 Reverse ACGGAAACCTCCAAAAGGC SUV39H1 Forward CCACCAGGAACCTCCAAAGCC SUV39H1 Forward CCCACTGGGAGCAACTTCG SUV39H1 Forward CCACTGGGAGCAACTTCAACCG SUV39H1 Forward CCACTGGGAGCAACTTCAACCG SUV39H1 Forward CCACTGGAGCACACTCAACCCG SUV39H1 Forward CCACTGGAGCACACTCAACCCG SUV39H1 Forward CCACTGGGACGCATCACT Reverse ATGAAGTGGGACGCATCACT Reverse CCTGGTCATTCTAGCAACGCCG SUV39H1 Forward CCACTGGAGCGCATCACT Reverse CCTGGTCATTCTAGCACACT Reverse CCTGGTCAACGCCGCGCACCCT KDM2A Forward GTGACACTTCGGGCCCATCACT Reverse CACGAAACGCCGCGCGCCGCGCGCGCGCGCGCGCGCGCG	PRDIVITZ	Polivaru	
InternetForwardAATGCTCCAGACTCCGACGPTIPForwardAATGCTCCGACCTCCACAAGSETD1AForwardACCAACCTGCCACCAAGSETD2ForwardACCAACCTGCCAAAATReverseGGCAAACAGACCCCCACTGTSETD2ForwardACCCACCTGCCAAAATReverseGGCAAACAGACCCCCAAAATReverseGGCAAACAGACCCCAAACTSETD2ForwardGCAAATCACTCCTTG GSETD3ForwardCACAGCACAGCCACAGGSETD4ForwardCACAGCACAAGCAAACTCCAAAACTCCReverseGGTATAGCCATAGGCAACGGReverseSETD8ForwardCCACCAAGACAAGGATAAGGCSMYD2ForwardCCACCAAGGACAAGGATAAGGCReverseAATGCTCCTCCACTTCAATCAASMYD3ForwardCCCAGTATCCTGTGCATATCTGSUV39H1ForwardCCAGGAACGTGCAATCATReverseACGGAAACGTGCACATCAACTReverseCCTGGTGATTGTGGAGCAAACTTSUV39H1ForwardCCAGGAAGCCCTGCACTReverseACGGAAACGCCTGCCACCTReverseACGGAACGCTGCGACCGTReverseACGGAACGCTGCGACCGTReverseCGGTGCATCACCGTCCACCTAAReverseGGACAACCGCTTCCCACCTAAReverseGGGACACCGTGCCGACCGTReverseCTGCGGTCACACAGGCGGAGATReverseCTGGCGTCACACAGGCGGAGATReverseCTGCGGTCACACAGGCGGGAGATReverseCTGGGCACACAGCCGCGCGGGGATReverseCTGGCGTACACAGTACCAGAGCGGAGATReverseCTGGCGTCACACAGTACCAGAGCGGGGGAReverseTGGCGCTGCCAAGAGCGGAGATReverseCTGCGC	PRDM16	Forward	TTCCACCTTAGATTCTGAGGCTTTA
PTIPForwardAGTGCTACACGGCATTCAGTCReverseCGCAACCTGACACGGCATCAGTCSETD1AForwardACCACCTGACACGGCATCAGTCReverseGGCAAACCTCGTReverseSETD2ForwardACCACGGCATCCCCAAAReverseReverseGGCAAACAGCCCCCCTCGTSETD7ForwardGCAAATCACCCTTCACTCCAAAReverseGGTCATAGCCATAGGCAACGGSETD7ForwardCGCAACATCCTCAAAATCCCCAAAACTCCCAAAACTCCCAATAGGCAACGGSETD8ForwardCGACGAGAACCTCCCAAAAACTCCReverseGGTCATAGCCATAGGCAAAGGSMYD2ForwardCCACCAAGGACAAGGATAAGGCReverseReverseAGTGGTGCGTGCGTGCATATCTGSMYD3ForwardCCCAGTAGCTCCATTCAACAReverseACGGAAAACGTCCAATCAAACCCSUV39H1ForwardCCCAGTGGGTGGTGGTGCATCACTReverseCCTGGGTGGTGGTGGTGCACTTSUV39H1ForwardCCCAGTGGGTGGCGTGCCGCGReverseCCTGGGTGGTGGTGGCGCCCGKDM2AForwardCCCAGTGGGCCCCGGCGCGCCGCReverseGGAAACGTTCCGACTReverseGGACAACGCTGCCGCGCGCGCGCGCGCGCGCGCGCGCGCG	T NDWITO	Reverse	
ImForwardReverseCCGCAACCTCCACCAAGSETD1AForwardACCAACCTGACCACCCCAAAReverseGGCAAACGACACCAACCCAACGTSETD2ForwardACCCACGACAGCAAACTCAAAATReverseAACTTCCGGCGTTCCTCTGSETD7ForwardGCAAACAACCAAACTCAAAATReverseGGTCATAGCCATAGCCAACGGSETD8ForwardCAGCAGAACCTCCAAAACTCCReverseGGTCATAGCCATAGCCATAGCGAACGGSMYD2ForwardCCACCAAGGACAAGGATAAAGCCSMYD3ForwardCCCACCAAGGACAAGGATAAGGCSUV39H1ForwardCCCAGTAGCGAACGGCCACACGReverseACGGAAACGTCCACACTSUV39H1ForwardCCCGGAGGGGATACCTCReverseACGGAAACGCCTCACACTReverseCCTGGGATGGGGCATACCTReverseCCTGGGAGGGGCACCCTReverseCCTGGGAGGGGGCACCTReverseCCTGGGAGGGGCCCCGACCTReverseGGACAGCACCCCGCCCGReverseGGACAGCATCCCTGCCCCACAReverseGGACAGAGCCCTCCCCCCACAReverseGGACAGCACCCCCGCCCCGKDM2AForwardGTGACACCTCTCCACCAGGAGCCGTReverseReverseCTCGGTCACACAGTACCCCTAAReverseCTCGGTCACACAGTACCCTAAKDM3AForwardGTGACCCTTCCACAGGAGGGGReverseKDM4AForwardReverseCCACATAGCCATGCCTTACCACAGGGGGKDM4BForwardReverseCAGGACACGCTACACTACCCTACCCACGGGKDM4AForwardReverseCAGCTGACACAGGCCCTACCTReverseCAGCTGACACAGGCCCTACCT <td>ΡΤΙΡ</td> <td>Forward</td> <td>AGTCGCTACACGGCATTCAGTC</td>	ΡΤΙΡ	Forward	AGTCGCTACACGGCATTCAGTC
SETD1A Forward ACCAACCTGACCACCCAAA Reverse GGCAAACGACCCCACTCGT SETD2 Forward ACCCCGACAACGACCCCACTCGT Reverse AACTTCCGGCGTTCCTCTG SETD7 Forward GCAAATCACTCCATAGCGAACT Reverse GGTCATAGCCATAGCCAAACG Reverse GGTCATAGCCATAGCCATAGCG SETD8 Forward CCACCAAGGACAACGG SETD8 Forward CCACCAAGGACAAGGATAAGGC Reverse TCTTCCTTCCCACTTCCAATCCA Reverse ACGAAACGTCCCAAGACAAGGATAAGGC Reverse ACGGAAACGTCCATAGCGATATCTG SMYD2 Forward CCACCAAGGACAAGGATAAGGC SWYD3 Forward CCCACTAGGCAACGTCACATCG Reverse ACGGAAACAGTCACATTCG SUV39H1 Forward CCCACTGGGACACGTCACATTCG SUV39H1 Forward CCCACTGGGAGCACAGGATAAGC SUV39H1 Forward CCCACTTGGAGCCCTCAAACTT EZH2(KMT6A) Forward CCATGGAGAGCGACAACGTCCCTA Reverse ATAGATGTTTTTGGAGCCCCG KDM2A Forward CCACGAGGATACGT Reverse CTGGGTCACACGTCCCTAA Reverse CTGGGTCACACGTCCCTAA Reverse CTGGGTCACACGTCCCTAA Reverse CTGGGTCACACGTCCCTAA Reverse CTGGGTCACACGTCCCTAA Reverse CTGGGTCACACGTCCCTAA Reverse CTGGGTCACACAGTACCCTAA Reverse CTGGGTCACACAGTACCTACC Reverse CTGGGTCACACAGTACCCTAA Reverse CTGGGTCACACAGTACCCTAA Reverse CTGGGTCACACAGTACCCTAA Reverse CTGGGTCACACAGTACCACTACC Reverse CTGGGTCACACAGTACCCTAA Reverse CTGGGTCACACAGTACCCTAA Reverse CTGGGTCACACAGTACCCTAA Reverse CTGGGTCACACAGTACCCTAA Reverse CTGGGTCACACAGTACACGTATCC Reverse CCCGGTCACACAGTACACGTATCCCCACAGTACCCATAGGCGAGAT Reverse CCCGGTCACACAGTACACGTATCCCACAGTACCCATAGGCGAGAT Reverse CCCGGTCACACAGTACACAGTACCCTAA Reverse CCCGGTCACACAGTACACGTATCCC Reverse CCCGCACAAGTACACGTTAGCCATACACGTTAGCCATAGGCGAGAT Reverse CCCCGCATAGACCCAGTACACAGTACCCATAGCCTTACACATGACATGACGGGG KDM4B Forward GGCACCCTCATACCCTACACGGG KDM4B Forward GGCACCCTCATACGCGTACCCTACACGCGACGGG Reverse CATATTCTCAGCATTACCCCCTATACCCCTAACGCGACGGG KDM4B Forward AGCCTTCACCACGTCACACGCGACGGG Reverse CATATTCTCAGCATTACCCCCTATACCCCCTATACCCCCTATACCCCCTATACCCCCC		Reverse	CCGCAACCTCTCCACCAAG
ReverseGGCAAACAGACCCCACTCGTSETD2ForwardACCCAGACAAGCAAACTCAAAATReverseGGCAAACGAACCCCACTCGTReverseAACTTCCGGGGTTCCTCTGSETD7ForwardGCCAAATCACTCCTTACTCCCAAACReverseGGTCATAGCCATAGCCAAAGCGSETD8ForwardCAGCAGAAACCTCCAAAACTCCReverseGGTCATAGCCATAGGCAACGGSMYD2ForwardCCACCAAGGACAGGATAAGGCReverseAATGACGTGCGTGCATATCTGSMYD3ForwardCCCACTAGGCACAGGATAAGCCSUV39H1ForwardCCCACTGGGACGCATCACTReverseACTGGGACGCATCACTReverseCCTGGTCATTGTAGGCAAACCTSUV39H1ForwardCCATCTGGGACGCATCACTReverseCCTGGTCATTGTAGGCAAACCTReverseCCTGGTCATTGAGGCAAACCTReverseCCTGGTCATTGAGGCAAACCTReverseGACAGAACGCCTGGCCGGKDM2AForwardCCATCACGGTTCCCACCTAAReverseGACAGAACGCCTGCTGCTCAAReverseGACAGAACGCCTGCTGCTCAAReverseGACAGAACGCCTGCTGCTCAAReverseCTGGGTCACACAGGACCGTRomardGTGCACACTTACACAAGGCAGATTReverseTCCCCACATGACCATGACCTTAACAACGAGACCGTReverseTCCCCACATGACCATGACCTTACAReverseTCCCCACATGACCATGACCTTACAACAGGCGAGATReverseTCCCCACATGACCATGACCTTACAACAAGGCCAGGGKDM3AForwardReverseTAGGACCTTGCACCTTACCACGGGGKDM4AForwardReverseCATGTGGCACATTAACGAGGGGGGGGGGGAACCGCCTCCTACCCAGAACCCCTTCCKDM4 CForwardRoward<	SETD1A	Forward	ACCAACCTGACCACCCCAAA
SETD2ForwardACCCAGACAAGCAAACTCAAAAT ReverseSETD7ForwardGCAAATCCTCCTTCGSETD7ForwardGCAAATCACTCCTTCACTCACACAAC ReverseSETD8ForwardCAGCAGAACCTCCAAAGCCAAAGCGASMYD2ForwardCCACCAAGGACAAGGGTAAGGC ReverseSMYD3ForwardCCCACCAAGGACAAGGCAAAGCTSWYD3ForwardCCCAGTATCTCTTGCTCAATCAASUV39H1ForwardCCCAGTATCTCTTGGGACGCATACT ReverseSUV39H1ForwardCCCTGGTCATTGGGACGCATCACT ReverseSUV39H1ForwardCCCAGTATCTGGAGCGAACTSUV39H1ForwardCCTGGTCATTGTGGACGCATCACT ReverseSUV39H1ForwardCCTGGTCATTGTGAGCGAAACTTSUV39H1ForwardCCTGGTCATTGTGAGCAAACTTReverseCTGGGACGCATCACT ReverseReverseSUV39H1ForwardCCTGGTCATTGTGAGCAACTTReverseCTGGGTCACCTGACCTGACCT ReverseReverseSUV39AForwardCCTGGTCATTGTGAGCCACGT ReverseKDM2AForwardCTGGGTCACACGTGCTGACACAGTACC ReverseKDM3AForwardTGCCCCACTAGACACGTACCATGACATTAG ReverseKDM3AForwardTGCCCCACTGCACATGACATGACATTAG ReverseKDM4AForwardTGGACCTCTACACCAGCACTGACATACC ReverseKDM4AForwardGCACTCTACACCAGGACGCAACGGCAACG ReverseKDM4 CForwardGCACTCTACACCAGGACCCTACCTCACCACAGCCCTCACCCCCCCTACACCGCCCCCCCC		Reverse	GGCAAACAGACCCCACTCGT
ReverseAACTTCCGGCGTTCCTCGSETD7ForwardGCAAATCACTCCTTACACTCCAACGReverseGGTCATAGCCATAGGCAACGGSETD8ForwardCAGCAGAACCTCCAAAAACTCCReverseTCTTCCTTCCCACTTCAATCAASMYD2ForwardCCACCAAGGACAAGGCATAAGGCReverseAATGACGTGCGTGCATATCTGSMYD3ForwardCCCAGTATCTTTGGCAATACCASUV39H1ForwardCCATCTGGGACGACACTSUV39H1ForwardCCATCTGGGACGCATCACTReverseCCTGGTCATTGTGGAGCCCTGATTCSUV39H1ForwardCCTGGGACGCATCACTReverseCTGGGACGCATCACTReverseCCTGGTCATTGTGGGACCCTGACTReverseCCTGGTCATTGTGGGACCCTGACCTReverseCCGGGACACCGTGCCTGATCACAReverseGGACAGACGCTGCCTGACCTReverseGGGACAACGTTCCCACCTAAReverseGGGACAACGTTCCCACCTAACReverseGGGACAACGCTGCTGCTCAAReverseGGGACAACGCTGCTGCTCAAReverseGGGACACCACAGTACACGTACCTReverseCGGGTCACACAGTACACGTACCTReverseTGCCCAATAGACCATAGACGTACCARACTCTCTCTAACACATGACATTAGReverseRDM3AForwardTGCCCCAATAGACCAGTACACAGACGCGAGATREVERSeTAGGACCTTCAACACAGTACACAGTACACRACTCACCAAGACGCCAGTGCACTAGCCATTAGReverseRDM3AForwardTGCCCCAACGAGCCGACTGCACTAGCREVERSeTAGGACCTTCAACACAGTCACACAGGCGRACTCACCAAGACCGCAGGTReverseREVERSeCAGGACTGCACACTGCACTACACAGGCGREVERSeCAGGCGGCAACCGCAGCGGGGGGGGGGGGGGGGGGGGG	SETD2	Forward	ACCCAGACAAGCAAACTCAAAAT
SETD7ForwardGCAAATCACTCCTTCACTCCAAAC ReverseSETD8ForwardCAGCAGAACCTCCAAAAACTCC AGCCATAGCCATAGCCATAGCCATAGCCATAGCCATAGCCATAGCCATAGCCATAGCAA CAGCAGAACGCTTCCAATCAASMYD2ForwardCCACCAAGGACAAGGATAAGGC ReverseSMYD3ForwardCCACCAGTATCTGTGCGTGCATATCAC ReverseSUV39H1ForwardCCACTGGGACACATTCAAAGCSUV39H1ForwardCCATCTGGGACGCATCACT ReverseEZH2(KMT6A)ForwardCCTGGTGCATTGTGGGAGCACCTTCAAAGCKDM2AForwardCCGGGACAGCTCACT ReverseKDM2AForwardCCGGGTCACGTCGCCCGACCT ReverseKDM2AForwardCGGGTCACCGTCCACATCAAT ReverseKDM2AForwardGTGACACTTCTGGAGCCCTAA ReverseKDM3AForwardGTGACCATCACATACAAGGCGAGCATCATT ReverseKDM3AForwardTGCCCCACATAGACCATGCAATCAATCAA ReverseKDM3AForwardTGCCCCACATAGACCATGACATAGCAGT ReverseKDM4AForwardTGGACCTTCACACAGGCAGACCATGACCAGGGGGKDM44ForwardGTGACATCACAAGCGCAACCG ReverseKDM44 CForwardGTGACACTAGCCCTCACACGAACCG ReverseKDM4 CForwardGTGACACTAGCCCCTAACCAGAACCG ReverseKDM4 CForwardGTGACGTGACACAGGCGCAACG Reverse		Reverse	AACTTCCGGCGTTCCTCTG
ReverseGGTCATAGCCATAGCCATAGGCAACGGSETD8ForwardCAGCAGAACCTCCAAAAACTCCReverseCTCTCCTTCCACTTCCAATCAASMYD2ForwardCCACCAAGGACAAGGATAAGGCReverseAATGACGTTGCGTGCATATCTGSMYD3ForwardCCCAGTATCTCTTTGCTCAATCACReverseACGGAAACAGTCACATTCAAAGCSUV39H1ForwardCCCAGTATCGGGACACACTReverseCCTGGTCATTGTAGGCAAACTTEZH2(KMT6A)ForwardCCCGAGAGTGGACCCTGACCTReverseATAGATGCTTTTTGGAGACCCTGACCTReverseGGCACAACGGTGGCACCCGKDM2AForwardCGTGGTCACACGATCCCCGACCTReverseGGCACACAGCGCTGCTCCAAReverseGGCACACAGTGCCCGACGTReverseCTGGGTCACACAGTACCGTReverseCTGGGTCACACAGTACCGTReverseCTGGGTCACACAGTACACTTAGReverseCTGGGTCACACAGTACACTAGCAGTATCTKDM3AForwardReverseTAGACCTTGCCAGTTTGAGACACTGAKDM4AForwardReverseCATATTCTCACAGCATAACGGGGKDM44 CForwardACCForwardKDM4 CForwardACCCACAGAGCCCCTACTCCKDM4 CForwardACCForwardReverseCAGCTGACACAGTCCCTACTCKDM4 CForwardACCForwardReverseCAGCTGACACAGTCCCTACTCKDM4 CForwardReverseCAGCTGACACAGTCCCTACTCKDM4 CForwardACCForwardReverseCAGCTGACACAGTCCCTACTCKDM4 CForwa	SETD7	Forward	GCAAATCACTCCTTCACTCCAAAC
SETD8ForwardCAGCAGAACCTCCAAAAAACTCC ReverseSMYD2ForwardCCACCAAGGACAAGGATAAGGC ReverseSMYD3ForwardCCCACAAGGACAAGGATAAGGCSMYD3ForwardCCCAGTATCTGCTGCAATACAC ReverseSUV39H1ForwardCCCAGTAGTGGCACACTTCAAAGCSUV39H1ForwardCCGAGAGTGGCCCTGCTCAT ReverseEZH2(KMT6A)ForwardCCGGGCATTGAGCCTGACTT ReverseREVERSECCTGGTCATTGTAGGCAAACTTEZH2(KMT6A)ForwardCCTGGTCATTGGGAGCCCGACCT ReverseKDM2AForwardCATAACCAACGTCCCCGCGKDM2AForwardCATAACCAACGTCCCCAATAGACCTT ReverseKDM2BForwardGTGACATCTTTCTGGGAGACCGT ReverseKDM3AForwardTGCCCAATAGACCATGACATAGA ReverseKDM3BForwardTCCCCAATAGACCATGACATAGA ReverseKDM4AForwardTGGACCTTCACAGCATGACCAAGGCGGGG ReverseKDM4AForwardGTGACCTCTACAGAGACCGGAGGGGGGGGGGGGGGGGGG		Reverse	GGTCATAGCCATAGGCAACGG
ReverseTCTTCCTTCCCACTTTCAATCAASMYD2ForwardCCACCAAGGACAAGGATAAGGCReverseAATGACGTTGCGTGCATATCTGSMYD3ForwardCCCAGTATCTTTGCTCAATCACReverseACGGAAACAGTCACATTCAAAGCSUV39H1ForwardCCATCTGGGACGCATCACTReverseCCTGGTCATTGTAGGCAAACTTEZH2(KMT6A)ForwardCCGGAGAGTGTGACCCTGACCTReverseATAGATGCTTTTGGAGCCCCGKDM2AForwardCATAACCAACGGTTCCCACCTAAReverseGACAGAACGCCTGGTCCAAReverseGGCACACAGTACACGTTCCCACCTAAReverseCTGGGTCACACAGTACACGAGAGCGTGCTCAAKDM2BForwardGTGCACACTTTCTGGAGACCGTReverseCTGGGTCACACAGTACACAGGAGAGTKDM3AForwardTGCCCGAGTACACAGAGCCAGGCGAGATReverseTCCCCACATGACCATGACACTAGAGACAGTACACAATCAReverseTAGGACCTTGCCAGTTTGGGAGCCGCKDM3BForwardTGCCCCACATGACCATGACATTAGAKDM4AForwardTGGACCTTGCCAGATTAACCAGGGGKDM4BForwardGTCATCACCAAGAACCGCAACGKDM4 CForwardAAGACTAGCCCTCATCCKDM4 CForwardAAGACTAAGCCCCTCATCC	SETD8	Forward	CAGCAGAACCTCCAAAAACTCC
SMYD2ForwardCCACCAAGGACAAGGATAAGGCReverseAATGACGTTGCGTGCATATCTGSMYD3ForwardCCCAGTATCTCTTTGCTCAATCACReverseACGGAAACAGTCACATTCAAAGCSUV39H1ForwardCCATCTGGGACGCATCACTReverseCCTGGTCATTGTAGGCAAACTTEZH2(KMT6A)ForwardTCCGAGAGTGTGACCCTGACCTReverseATAGATGCTTTTGGAGACCCGACKDM2AForwardCATAACCAACCGTTCCCACCTAAReverseGACAGAACGCCTGCTGCTCAAKDM2BForwardGTGACATCTTCTGGGAGACCGTReverseCTGGGTCACACAGTACACTACTKDM3AForwardTGCCCACATGACACAGTACCACTACAReverseTCCCACATAGACCATGACATTAGKDM3BForwardTGCCCACATGACCATGACATTGAKDM4AForwardTGGACCTCTTACACCTCAACTACCACATGACCAKDM4ForwardGTCATCACCAAGAACCGCTCCTACCCACGAGGGGGACCKDM4 CForwardGTCATCACCAAGACCGCTCCTACCCACGAGACCCKDM4 CForwardAAGACTAAGCCCCTCATACCAAGGACCCKDM4 CForwardAAGACTAAGCCCCCCATACCCAAGACCGCACCCCACAGACCCCCAAGACCCCCCAAGACCCCCC		Reverse	TCTTCCTTCCCACTTTCAATCAA
ReverseAATGACGTTGCGTGCATATCTGSMYD3ForwardCCCAGTATCTCTTTGCTCAATCACReverseACGGAAACAGTCACATTCAAAGCSUV39H1ForwardCCATCTGGGACGCATCACTReverseCCTGGTCATTGTAGGCAAACTTEZH2(KMT6A)ForwardTCCGAGAGTGTGACCCTGACCTReverseATAGATGCTTTTGGAGCCACCTReverseATAGATGCTTTTGGAGCCCGKDM2AForwardCATAACCAACGCGTCCCACTAAReverseGACAGAACGCCTGCTCCAAReverseGACAGAACGCCTGCTCCAAKDM3AForwardGTGACATCTTTCTGGGAGACCGTReverseTCCCACATAGACCATGACATTAGKDM3BForwardTGCCCGAGTACACAGCAGTACACTAGACATTAGKDM4AForwardTGGACCTTGCCAGCATCACAATCACKDM4BForwardGTCATCACCAAGAACCGCACGGAGAKDM44 CForwardGTCATCACCAAGAACCGCAACGKDM4 CForwardGTCATCACCAAGAACCGCAACGKDM4 CForwardGTCATCACCAAGAACCCCTCATACCAAGA	SMYD2	Forward	CCACCAAGGACAAGGATAAGGC
SMYD3ForwardCCCAGTATCTCTTTGCTCAATCACReverseACGGAAACAGTCACATTCAAAGCSUV39H1ForwardCCATCTGGACGCATCACTReverseCCTGGTCATTGTAGGCAAACTTEZH2(KMT6A)ForwardTCCGAGAGTGTGACCCTGACCTReverseATAGATGCTTTTGGAGCCCGKDM2AForwardCATAACCAACCGTTCCCACCTAAReverseGACAGAACGCCTGCTGCTCCAAKDM2BForwardGTGACATCTTTCTGGGAGAGACCGTReverseCTGGGTCACACAGTACACGTATCTKDM3AForwardTGCCCGAGTACACAGTACACGTATCTKDM3BForwardTGCCCACATAGACCATGACATTAGKDM4AForwardATCTCTCTAACACTCATACAAATCAReverseTAGAACCTTGCACATTACAATCAReverseCATATTCTCTGGCAGATCACCAGTACCACGAGTACCACGATCACKDM4AForwardGTCATCACCAAGAACGCCACGGCAGAGKDM44ForwardGTCATCACCAAGAACCGCAACGAGCGCAACGKDM44ForwardGTCATCACCAAGAACCGCAACGAKDM44ForwardGTCATCACCAAGAACCGCAACGAKDM44ForwardGTCATCACCAAGAACCGCAACGAKDM44ForwardGTCATCACCAAGAACCGCAACGAKDM44ForwardGTCATCACCAAGAACCGCAACGAKDM42ForwardAAGACTAAGCCCCTACTCKDM42ForwardAAGACTAAGCCCCTACTCKDM42ForwardAAGACTAAGCCCCTACTCKDM42ForwardAAGACTAAGCCCCTACTCKDM42ForwardAAGACTAAGCCCCTACTCKDM42ForwardAAGACTAAGCCCCTACTCKDM42ForwardAAGACTAAGCCCCTCATACCAAGAKDM42ForwardAAGACTAAGCCCCTCATACCAACAGCCC		Reverse	AATGACGTTGCGTGCATATCTG
ReverseACGGAAACAGTCACATTCAAAGCSUV39H1ForwardCCATCTGGGACGCATCACTReverseCCTGGTCATTGTAGGCAAACTTEZH2(KMT6A)ForwardTCCGAGAGTGTGACCCTGACCTReverseATAGATGCTTTTGGAGCCCCGReverseKDM2AForwardCATAACCAACCGTTCCCACCTAAReverseGACAGAAGCCTGCTGCTGCAAReverseKDM2BForwardGTGACATCTTTCTGGAGAGCCCGTReverseCTGGGTCACACAGTACCGTATCTReverseKDM3AForwardTGCCCGAGTACACAGTACCGTATCTKDM3BForwardTCCCACATAGACCATGACATTAGKDM4AForwardTGGACCTTGCAGCAGTACACGTACCCKDM4BForwardTGCACCAGGACCAGGCGAAGCGKDM4 CForwardGTCATCACAAGACCCCTCATACCAGA	SMYD3	Forward	CCCAGTATCTCTTTGCTCAATCAC
SUV39H1ForwardCCATCTGGGACGCATCACTReverseCCTGGTCATTGTAGGCAAACTTEZH2(KMT6A)ForwardTCCGAGAGTGTGACCCTGACCTReverseATAGATGCTTTTTGGAGCCCCGKDM2AForwardCATAACCAACCGTTCCCACCTAAReverseGACAGAACGCTGCTGCTCAAKDM2BForwardGTGACATCTTTCTGGGAGACCGTReverseCTGGGTCACACAGTACACGTATCTKDM3AForwardTGCCCGAGTACACAGGCGAGATReverseCTGGGTCACACAGTGCACAGGAGACGTReverseTCCCACATAGACCATGACATAGKDM3BForwardTGCCCGAGTACACAGCATGACACAAGTCACACAGTACACAKDM4AForwardTGGACCTTGCCAGTTTGGATCATCACCACAGTACACCGGGGGGGG		Reverse	ACGGAAACAGTCACATTCAAAGC
ReverseCCTGGTCATTGTAGGCAAACTTEZH2(KMT6A)ForwardTCCGAGAGTGTGACCCTGACCTReverseATAGATGCTTTTTGGAGCCCCGKDM2AForwardCATAACCAACCGTTCCCACCTAAReverseGACAGAACGCTGCTGCTGAAKDM2BForwardGTGACATCTTTCTGGGAGACCGTReverseCTGGGTCACACAGTACACGTATCTKDM3AForwardTGCCCGAGTACACAGGCGAGATReverseCTCGGTCACACAGTACACGTATCTKDM3BForwardTGCCCACATAGACCATGACATAGReverseTCCCACATAGACCATGACATAGReverseTCCCACATAGACCATGACATAGAKDM4AForwardTGGACCTTCTCACAGCATTAACGGGGKDM4BForwardGTCATCACCAGAACCGCAACGReverseCATATTCTTCAGCATTAACGGGGKDM4 CForwardAGACTAAGCCCCTCATACCAGGA	SUV39H1	Forward	CCATCTGGGACGCATCACT
EZH2(KM16A)ForwardICCGAGAGIGIGACCCIGACCIReverseATAGATGCTTTTGGAGCCCCGKDM2AForwardCATAACCAACCGTTCCCACCTAAReverseGACAGAACGCTGCTGCTGCAAKDM2BForwardGTGACATCTTTCTGGGAGACCGTReverseCTGGGTCACACAGTACACGTATCTKDM3AForwardTGCCCGAGTACACAGGCGAGATReverseTCCCACATAGACCATGACATTAGKDM3BForwardATCTCTCTAACACCTGATACAAATCAReverseTAGGACCTTGCCAGTTTGGATTCATKDM4AForwardTGGCCCTACAGCATCACCGAGACReverseCATATTCTTCAGCATTAACGGGGKDM4BForwardGTCATCACCAGAACCGCCAACGReverseCAGCTGGACACAGTCCCTACTCKDM4 CForwardAAGACTAAGCCCCTCATACCAGA		Reverse	CCTGGTCATTGTAGGCAAACTT
KeverseATAGATGCTTTTGGAGCCCCGKDM2AForwardCATAACCAACCGTTCCCACCTAAReverseGACAGAACGCTGCTGCTGCTGAAKDM2BForwardGTGACATCTTTCTGGGAGACCGTReverseCTGGGTCACACAGTACACGTATCTKDM3AForwardTGCCCGAGTACACAAGGCGAGATReverseTCCCACATAGACCATGACATTAGKDM3BForwardATCTCTCTAACACCTGATACAAAATCAReverseTAGGACCTTGCCAGTTTGGATTCATKDM4AForwardTGGACCTCTACAGCATCAACTACCReverseCATATTCTTCAGCATTAACGGGGKDM4BForwardGTCATCACCAGAACCGCCAACGReverseCAGCTGGACACAGTCCCTACTCKDM4 CForwardAGACTAAGCCCCTCATACCAGA	EZH2(KM16A)	Forward	
KDM2AForwardCATAACCAACCGTTCCCACCTAAReverseGACAGAACGCCTGCTGCTGAAKDM2BForwardReverseCTGGGTCACACAGTACACGTATCTKDM3AForwardReverseTCCCCACATAGACCATGACATTAGKDM3BForwardATCTCTCTAACACCTGATACACAAGTCAReverseTAGGACCTTGCCAGTTCGATACACAAGTCAKDM4AForwardReverseCATATTCTTCAGCATTAACACTCATKDM4BForwardGTCATCACCAGAACCGCCAACGReverseCATATTCTTCAGCAATTAACGGGGKDM4AForwardGTCATCACCAGAACCGCCAACGReverseCATATTCTTCAGCAATTAACGGGGKDM4BForwardGTCATCACCAGAACCGCCAACGReverseCAGCTGGACACAGTCCCTACTCKDM4 CForwardAGACTAAGCCCCTCATACCAGAA	KDM2A	Keverse	
KeverseGACAGAACGCCTGCTGCTGCTGCTCAAKDM2BForwardGTGACATCTTTCTGGGAGACCGTReverseCTGGGTCACACAGTACACGTATCTKDM3AForwardTGCCCGAGTACACAAGGCGAGATReverseTCCCACATAGACCATGACATTAGKDM3BForwardATCTCTCTAACACTCATACAAATCAReverseTAGGACCTTGCCAGTTTGGATTCATKDM4AForwardTGGACCTCTACAGCATCAACTACCReverseCATATTTCTTCAGCATTAACGGGGKDM4BForwardGTCATCACCAGAACCGCAACGReverseCAGCTGGACACAGTCCCTACTCKDM4 CForwardAGACTAAGCCCCTACACAACGCAACGA	KDM2A	Forward	
KDM2BForwardGTGACATCTTTCTGGGAGACCGTReverseCTGGGTCACACAGTACACGTATCTKDM3AForwardReverseTCCCACATAGACCATGACATTAGKDM3BForwardATCTCTCTAACACTCATACAAATCAReverseTAGGACCTTGCCAGTTTGGATTCATKDM4AForwardForwardTGGACCTCTACAGCATCAACTACCReverseCATATTTCTTCAGCATTAACGGGGKDM4BForwardGTCATCACCAGAACCGCAACGReverseCAGCTGGACACAGACCGCAACGReverseCAGCTGGACACAGTCCCTACTCKDM4 CForwardKDM4 CForward	KDM2D	Reverse	GACAGAACGCCIGCIGCICAA
KEVERSECTGGGTCACACAGTACACGTATCTKDM3AForwardTGCCCGAGTACACAAGGCGAGATReverseTCCCACATAGACCATGACATTAGKDM3BForwardATCTCTCTAACACTCATACAAATCAReverseTAGGACCTTGCCAGTTTGGATTCATKDM4AForwardTGGACCTCTACAGCATCAACTACCReverseCATATTTCTTCAGCATTAACGGGGKDM4BForwardGTCATCACCAGAACCGCCAACGReverseCAGCTGGACACAGTCCCTACTCKDM4 CForwardAGACTAAGCCCCTCATACCAGA	KDIMZB	Forward	
NomeForwardForwardReverseTCCCACATAGACCATGACATTAGKDM3BForwardReverseTAGGACCTTGCCAGTTTGGATTCATKDM4AForwardForwardTGGACCTCTACAGCATCAACTACCReverseCATATTTCTTCAGCATTAACGGGGKDM4BForwardGTCATCACCAGGACACCGCAACGReverseCAGCTGGACACAGTCCCTACTCKDM4 CForwardKDM4 CForward		nevelse Forward	
KDM3B Forward ATCTCTCTAACACTCATACAAATCA Reverse TAGGACCTTGCCAGTTTGGATTCAT KDM4A Forward TGGACCTCTACAGCATCAACTACC Reverse CATATTTCTTCAGCATTAACGGGG KDM4B Forward GTCATCACCAAGAACCGCAACG Reverse CAGCTGGACACAGTCCCTACCGCAACG Reverse CAGCTGGACACAGTCCCTACTC KDM4 C Forward AGACTAAGCCCCTCATACCAGA	הכווויסא	Reverse	
KDM3D Forward ACCECTACACICATACAATCA Reverse TAGGACCTTGCCAGTTTGGATTCAT KDM4A Forward TGGACCTCTACAGCATCAACTACC Reverse CATATTTCTTCAGCATTAACGGGG KDM4B Forward GTCATCACCCAAGAACCGCAACG Reverse CAGCTGGACACAGTCCCTACTC KDM4 C Forward AGACTAAGCCCCTCATACCAGA	КЛМЗВ	Forward	
KDM4A Forward TGGACCTCTACAGCATCAACTACC Reverse CATATTTCTTCAGCATTAACGGGG KDM4B Forward GTCATCACCCAAGAACCGCAACG Reverse CAGCTGGACACAGTCCCTACTC KDM4 C Forward AAGACTAAGCCCCTCATACCAGA	עכווועא	Reverse	TAGGACCTTGCCAGTTGGATTCAT
Reverse CATATTTCTTCAGCATTAACGGGG KDM4B Forward GTCATCACCAAGAACCGCAACG Reverse CAGCTGGACACAGTCCCTACTC KDM4 C Forward AAGACTAAGCCCCTCATACCAGA	KDM4A	Forward	TGGACCTCTACAGCATCAACTACC
KDM4B Forward GTCATCACCAAGAACCGCAACG Reverse CAGCTGGACACAGTCCCTACTC KDM4 C Forward AAGACTAAGCCCCTCATACCAGA		Reverse	CATATTTCTTCAGCATTAACGGGG
Reverse CAGCTGGACACAGTCCCTACTC KDM4 C Forward AAGACTAAGCCCCTCATACCAGA	KDM4B	Forward	GTCATCACCAAGAACCGCAACG
KDM4 C Forward AAGACTAAGCCCCTCATACCAGA		Reverse	CAGCTGGACACAGTCCCTACTC
	KDM4 C	Forward	AAGACTAAGCCCCTCATACCAGA

(Continued)

	Reverse	CATAACAACTTGCATGAACCCGT
KDM4D	Forward	ATTCCCTTCAATCGCATAACTCA
	Reverse	CTTGTCCATCAAAGCCCCAT
KDM5A	Forward	TTACCAACAGGTCAGACGCAT
	Reverse	GGTTTGCTACATTCCTCGGCG
KDM5B	Forward	TTGTCCCCATTGTCGGAGGTC
	Reverse	TTACTTTCGGCTTGGTGCGTC
KDM5 C	Forward	GTGAGCGGAGGTTTCCTAATA
	Reverse	GGCGCAAGGCAGGTTGTT
KDM6A	Forward	GGACAAGTTGAATCCACCTACACC
	Reverse	AGAGCTCCAGCAAGGCCACGTATT
KDM6B	Forward	TACACCTTGAGCACAAACGGA
	Reverse	AGCCTCTCCTTCGCTTGCCTC
JMJD1 C	Forward	AAACCCCATAATAATGAGAAGGCAG
	Reverse	GAGTGTCAACAGACTTCCGCTTC
JMJD5	Forward	CCATCCTGGGGGGACATCCTTCTTA
	Reverse	ACCAAAAACTGCTCCCTGAAATGC
IM ID6	Forward	GTGGCATGTTGTCCTCAATCTC
	Reverse	TTTGGTCTCCCTCTTACCGTCT
LSD1	Forward	
	Reverse	ATGTGGCAACTCGTCCACCCAC
PHF2	Forward	CCCCGACGAGCCCTGGTGTGTGTTC
11112	Reverse	CTCACCGTCATCCGACACGTACT
PHE8	Forward	TCTCGGCGAACCAAGATAGCA
	Reverse	TTGGTCTTATTATCAGGGTCGCC
KIAA1718	Forward	AACTTCCCCATACCATTCCCGA
	Reverse	CATTIGICAGCAGTAATCGTAG
N066	Forward	TCTACCGCTGACCTGGATTCG
1000	Reverse	CCTGCGGACAGAGGGGGGGGGGG
DSPP	Forward	CCAGTTCCTCAAAGCAAACC
	Reverse	GGCATTTAACTCATCCTGTACTGA
DMP1	Forward	CTCCGAGTTGGACGATGAGG
	Reverse	
RUNX2	Forward	ACCATGGTGGAGATCATCGC
NONAZ	Reverse	
OSX	Forward	GCCAGAAGCTGTGAAACCTC
05/	Reverse	GCTGCAAGCTCTCCATAACC
OCN	Forward	
0en	Reverse	GCGCCTGGGTCTCTTCACT
DSPP (ChIP)	Forward	
	Reverse	GCATTTAACTCATCCTGTACTGA
DMP1 (ChIP)	Forward	
	Reverse	
OSX (ChIP)	Forward	ΔΓΓΔΤΕGTEGΔGΔΕΔΤΓΔΤΓΕ
	Roverse	
OCN (CHP)	Forward	
	Povorso	
	nevelse	GUIGCAAGUIUICCATAAUU

Table 1. (Continued).

Establishment of stable KDM5A knockdown by shRNA in hDPCs

KDM5A knockdown was performed by shRNA in hDPCs. The lentivirus vector hU6-MCS-CMV-Puromycin, containing either KDM5A shRNA (5'-GCCAAGAAC ATTCCAGCCTTT-3') or negative control shRNA, was obtained from GeneChem Biotechnologies (Shanghai, China). The lentiviral vectors were transfected into 293 T cells with the psPAX2, pMD.2 G packaging plasmid mix in serumfree Opti-MEM using the Lipofectamine 3000 reagent (Invitrogen Co, Carlsbad, CA, USA). The transfection mix was replaced with fresh DMEM plus 10% FBS 6 hours after transfection. The viruses were then harvested, filtered, and used to transfect hDPCs. Transfected hDPCs were selected with 1 μ g/mL puromycin 3 days after virus infection. The stable clones were then maintained in 0.25 μ g/mL puromycin to ensure a transfection rate greater than 90%. Knockdown was confirmed using real-time quantitative polymerase chain reaction (real-time qPCR) and western blot analysis.

Alkaline phosphatase (ALP) activity analysis

Transfected cells seeded into 24-well plates underwent odontogenic differentiation for 7 and 14 days as described above. The ALP activity of the hDPCs was then detected by utilizing an ALP assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's protocol. Briefly, cells were lysed with 0.1% Triton X-100 in 10 mM TrisHCl at 4°C overnight, and the absorbance of the solution was measured at 520 nm using an automated microplate reader (Sunrise). The total protein content in each individual sample was quantified with a BCA protein assay (Beyotime Biotechnology), and ALP activity was normalized to the total protein content.

Chromatin immunoprecipitation (ChIP) and qRT-PCR analysis

The promoters of DSPP, DMP1, OCN, OSX and H3K4me3 marks from the Encyclopedia of DNA Elements (ENCODE) were retrieved using UCSC Genome Browser (http://genome.ucsc.edu/cgi-bin). Primers specific to these regions with H3K4me3 enrichment were designed using Primer Express v3.0 software (Table 1) and were synthesized by BGI Technology (BGI, Shenzhen, China). For the ChIP the EZ-Magna ChIP[™] G Chromatin assay, Immunoprecipitation Kit (Millipore) was utilized according to the manufacturer's instructions. Approximately 1×10^7 transfected cells cultured in MM for 14 days were crosslinked with 1% formaldehyde (Sigma-Aldrich). Cells were then lysed and fragmented using a Dounce homogenizer and sheared by an ultrasonic processor (Sonics, Newtown, USA). The chromatin was then subjected to IP using 5 µg of H3K4me3-specific antibody (Abcam) overnight at 4° C with rotation. After washing, the protein-DNA cross-links were reversed, and the DNA was purified. qRT-PCR was performed to validate the sequences of interest with the primers. Normal rabbit IgG served as a negative control for IP.

Statistical analyzes

All experiments were repeated at least in triplicate. Experimental results are presented as the mean \pm standard deviation (SD). The data were analyzed

using the SPSS 19.0 software program (SPSS, Chicago, IL, USA). Student's t-test was used to compare two normally distributed groups. For multiple comparisons, the data passed normality and equal variance tests and were then subjected to one-way analysis of variance (one-way ANOVA) and the Student-Newman-Keuls multiple comparison test. Significantly different levels were set at the 95% and 99% confidence intervals and are marked with *(P < 0.05) and ** (P < 0.01), respectively.

Results

Odontogenic differentiation and HMT/HDM expression in hDPCs

To ensure the odontogenic differentiation of hDPCs, the expression levels of the odontoblast markers DSPP (Dentin Sialophosphoprotein) and DMP1 (Dentin Matrix Protein 1) and mineralized matrix deposition were assessed after mineralization induction. As shown in Figure 1(a,b), the expression levels of DSPP and DMP1 were upregulated, and the mineralized matrix deposition also increased after induction.

To investigate potential roles of histone methylation in the differentiation process of hDPCs and to identify the expression levels of the HMTs and HDMs during hDPC odontogenic differentiation, the mRNA expression levels of 25 HMTs and 21 HDMs were profiled in cells cultured in MM or GM for 14 days. Real-time qPCR revealed that eight enzymes were expressed differentially during the hDPC odontogenic differentiation process. The expression levels of 4 HMTs (PRDM12, SETD1A, SUV39H1, and EZH2) and 3 HDMs (KDM2B, KDM5 C, and JMJD6) decreased after mineralization induction; the HDM KDM5A was the only enzyme of those assessed that was induced after odontogenic differentiation (Figure 1(c,d)).

Expression features of the histone demethylase KDM5A in hDPCs

To evaluate the expression profiles of KDM5A in hDPCs during continuous passaging *in vitro*, the mRNA and protein expression levels of KDM5A in cells from the 1^{st} to 8^{th} passages were detected by



Figure 1. Differential HMT and HDM expression during the odontogenic differentiation of hDPCs. (a) The expression levels of DSPP and DMP1 were assessed using western blot analysis on days 0, 7, and 14 of culture in MM. GAPDH was used as an internal control. (b) The formation of mineralized nodules was assessed using alizarin red S staining (100X). (c) HMT mRNA expression was assessed by real-time qPCR. (d) HDM mRNA expression was assessed by real-time qPCR. The mRNA level of each product was normalized to the β -actin mRNA level. GM: growth medium; MM: odontogenic differentiation medium. All results are presented as the mean \pm SD of three independent experiments. Procedures were performed as described in the text (n = 3). **P* < 0.05.

using real-time qPCR and western blot, respectively. The expression level of KDM5A was elevated in the 1^{st} and 2^{nd} passages, maintained stably at a high level in the 3^{rd} to 6^{th} passages, and then decreased in the 7^{th} and 8^{th} passages (Figure 2(a,b)).

To examine the alterations to KDM5A expression during odontogenic differentiation, the mRNA and protein levels of KDM5A were analyzed in hDPCs induced to undergo odontogenic differentiation for 7 and 14 days. The results





(a) Real-time qPCR analysis of KDM5A mRNA expression at different passages. The amount of each product was normalized to β -actin. (b) The protein expression levels of KDM5A in the hDPCs at different passages were assessed by western blot analysis and densitometric evaluation (expressed as the ratio of KDM5A to β -actin). β -actin was used as an internal control. (c) KDM5A mRNA expression levels in hDPCs in MM for 0, 7 and 14 days were measured using real-time qPCR. The amount of each product was normalized to β -actin. (d) KDM5A protein expression levels in hDPCs in MM for 0, 7 and 14 days were assessed by western blot analysis and densitometric evaluation (expressed as the ratio of KDM5A to β -actin). β -actin was used as an internal control. All results are presented as the mean \pm SD of three independent experiments. Procedures were performed as described in the text (n = 3). **P* < 0.05.

showed that KDM5A increased in a timedependent manner during odontogenic induction (Figure 2(c,d)).

Effects of KDM5A knockdown on the mineralization potentiality of hDPCs

To explore the role of KDM5A in hDPC odontogenic differentiation, KDM5A was knocked down using shRNA. hDPCs without transfection were used as the normal control group to exclude the possibility that the properties of hDPCs might be affected by the lentivirus vector. Real-time qPCR and western blot analysis showed almost 70% depletion of KDM5A in the target shRNA group relative to that in control shRNA group, suggesting that KDM5A was effectively knocked down in hDPCs. There was no difference between the normal control group and control shRNA group (Figure 3(a,b)).

ALP activity and the formation of mineralized nodules were then examined in KDM5A-shRNA cells under normal culture conditions (GM group) and in MM (MM group). As shown in Figure 3(c), KDM5A silencing enhanced ALP activity relative to that of the control group under both normal culture and induction conditions. Furthermore, KDM5A knockdown increased the number of mineralized nodules after treatment with mineralization induction for 14 days, as assessed by alizarin red S staining. Under normal culture conditions, there was no significant difference in the number of mineralized nodules between the KDM5A-shRNA and the control group (Figure 3(d)).

Effects of KDM5A knockdown on odontogenic differentiation markers in hDPCs

To further investigate the differentiation potential of hDPCs after KDM5A knockdown, the mRNA levels of several matrix-related genes (DMP1, DSPP, RUNX2, OSX, and OCN) were detected after the cells were cultured in MM or GM for 14 days. As shown in Figure 4, KDM5A depletion significantly enhanced DMP1, DSPP, OSX (Zinc



Figure 3. Effects of KDM5A knockdown on the mineralization potentiality of hDPCs.

(a) Real-time qPCR analysis of KDM5A mRNA expression in KDM5A-shRNA- transfected, control-shRNA-transfected, and normal hDPCs. The amount of each product was normalized to β -actin. (b) The protein expression levels of KDM5A in KDM5A-shRNA-transfected, control-shRNA-transfected, and normal hDPCs were assessed by western blot analysis and densitometric evaluation (expressed as the ratio of KDM5A to β -actin). β -actin was used as an internal control. (c) Cells were cultured for 7 and 14 days in GM or MM, and ALP activity was measured. (d) Mineralization was analyzed using alizarin red S staining on day 14. The histogram shows the quantification of mineralization by densitometry. All results are presented as the mean \pm SD of three independent experiments. Procedures were performed as described in the text (n = 3). **P* < 0.05.

finger protein osterix, also known as transcription factor Sp7), and OCN (Osteocalcin, also known as OC or Bone Gamma-Carboxyglutamate Protein, BGLAP) expression (Figure 4(a)). No significant difference was found for RUNX2 in neither the MM nor the GM group. Western blotting was performed to confirm the significant results obtained by real-time qPCR (Figure 4(b)), which verified the upregulation of odontogenic differentiation markers in cells undergoing odontogenic induction (cultured in MM).

Effects of KDM5A knockdown on the methyl marks of H3K4 in hDPCs

Since KDM5A is a histone demethylase specific for tri- and dimethylated H3K4, the methyl marks of H3K4 were observed using western blot. As shown



Figure 4. Effects of KDM5A knockdown on the odontogenic differentiation markers of hDPCs. (a) Cells were cultured for 14 days in GM or MM. DMP1, DSPP, RUNX2, OSX, and OCN mRNA expression levels were detected using real-time qPCR. The amount of each product was normalized to β -actin. (b) Cells were cultured for 14 days in MM. DSPP, DMP1, OSX, OCN protein expression levels were detected using western blot and densitometric evaluation (expressed as the ratio to β -actin). The expression of β -actin was used as an internal control. All results are presented as the mean \pm SD of three independent experiments. Procedures were performed as described in the text (n = 3). **P* < 0.05.

in Figure 5(a), H3K4me3 increased in the KDM5A-shRNA group, whereas no significant difference was found for H3K4me2. Next, ChIP-PCR was implemented to determine whether KDM5A knockdown altered the modification of H3K4me3 on the promoters of odontogenic differentiation markers. The results revealed that KDM5A depletion prompted a significant increase in H3K4me3 levels on the promoters of DSPP, DMP1, OCN, and OSX (Figure 5(b)).

Discussion

Histone methylation is essential for many biological processes, such as transcriptional regulation, X-chromosome inactivation and heterochromatin formation [18,28]. It regulates gene expression and forms part of the epigenetic memory system, which modulates cell differentiation in embryonic

stem cells (ESCs), human bone marrow mesenchymal stem cells (MSCs), hDPSCs, and oncocytes [29-32]. Osteo/odontogenic differentiation is likely modulated by histone methylation. Gopinathan et al [24]. reported that early mineralization genes (i.e., RUNX2, MSX2, and DLX5) are predominantly marked with H3K4me3 active marks, while late mineralization markers (i.e., OSX, IBSP, and BGLAP) are enriched for H3K9me3 or H3K27me3 inactive marks in dental pulp progenitors and dental follicle progenitors. Increased enrichment for H3K4me3 correlates with increased expression levels of BMP2, BMP4, DLX5, and RUNX2 in both cell types during mineralization induction. Zheng et al. [22] demonstrated that the bivalent modifications characterized by H3K4me3 and H3K27me3 can be detected during murine tooth germ development. The methyltransferases SET7 and EZH2 and



Figure 5. Effects of KDM5A knockdown on methyl marks of H3K4 in hDPCs.

(a) Cells were cultured for 14 days in MM. H3K4me3/me2 levels were detected using western blot and densitometric evaluation (expressed as the ratio to H3). (b) Signals of H3K4me3 IP-DNA relative to the total amount of input DNA in hDPCs were extracted from the control group (no stripes) and KDM5A-knockdown group (stripes), as analyzed by qPCR using specific primer pairs for odontogenic differentiation markers. All results are presented as the mean \pm SD of three independent experiments. Procedures were performed as described in the text (n = 3). **P* < 0.05.

demethylases KDM5B and JMJD3 are expressed in a spatial-temporal manner. A recent study reported that four H3K9 methyltransferases (G9A, SETDB1, SUV39H1, and PRDM2) are highly expressed in the mesenchyme of the tooth germ in mice[23]. These studies suggested that histone methylation might play active roles in the differentiation and lineage commitment of dental mesenchymal stem cells. However, no data on the expression patterns and functions of the HMTs or HDMs in the odontogenic differentiation process of hDPCs are available. In the present study, we identified the expression profiles of HMTs and HDMs in hDPCs induced to undergo odontogenic differentiation. The expression levels of four HMTs (PRDM12, SETD1A, SUV39H1, and EZH2) and three HDMs (KDM2B, KDM5 C, and JMJD6) decreased after mineralization induction; KDM5A was the only enzyme that was induced after odontogenic differentiation. KDM5A has

been reported to play important roles in the osteogenic differentiation process [33,34]. Thus, KDM5A was identified for its regulatory role in the odontogenic differentiation of hDPCs.

KDM5A (JARID1A/RBP2), originally identified by virtue of its capacity to bind the retinoblastoma protein (pRB), is a member of the JmjC-containing JARID1 protein family, which is characterized by its ability to selectively remove methyl marks from H3K4 [26,35]. KDM5A has been detected in multiple cell lines, such as ESCs, human adiposederived stem cells (hASCs), spermatogonial stem cells, and oncocytes originating from numerous tissues [33,36-39]. In the present study, KDM5A mRNA and protein were detected in primary hDPCs, and their expression levels gradually increased in early passages and then decreased in later passages when the cells were serially passaged. It is possible that KDM5A expression is elevated in hDPCs undergoing proliferation and

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early spontaneous differentiation (from 1st to 6th passage), whereas its expression is attenuated following cell senescence (at 7th or 8th passage) by morphological abnormalities and self-renewal defects. Furthermore, its expression was also promoted during odontogenic differentiation. This result was consistent with the report that KDM5A is upregulated in leukemic HL60 cells with differentiation[40].

KDM5A has been reported to play important roles in controlling stemness and the differentiation fates of stem cells. In ESCs, it cooperates with KLF4 to promote the transcriptional activation of OCT4[36]. KDM5A is required to maintain the repressive state of genes involved in cell development, including the Wnt5a, Irx3, Igf2, and Hox genes, and KDM5A displaces from the promoters of related genes when ESCs are induced to differentiation undergo [41,42]. In hASCs, KDM5A physically and functionally interacts with RUNX2 and represses its transcriptional activity. The hypoactivity of RUNX2 then decreases the expression of the OSX and OCN genes. On the other side, KDM5A can directly repress OCN through its H3K4 demethylase activity. When hASCs are induced to osteogenic differentiation, KDM5A occupancy decreases dramatically, and its depletion enhances osteogenic differentiation [33,34]. According to these previous studies, KDM5A seemingly tends to maintain pluripotent status by inducing stem cellspecific genes and alleviate cell differentiation by reducing the expression of lineage genes. In the present study, after identifying KDM5A expression patterns in hDPCs, KDM5A was knocked down to explore its role in the odontogenic differentiation potential of hDPCs. ALP activity and mineralized nodule formation were analyzed under normal culture and induction conditions. ALP activity was enhanced under both conditions, and mineralized nodule formation was increased in KDM5A-knockdown cells in response to odontogenic induction for 14 days relative to the control group, but no significant differences were detected under normal culture conditions between the two groups. These findings were further confirmed by the increased expression levels of DMP1, DSPP, OSX, and OCN in cells undergoing mineralization induction. These results indicated that KDM5A

was negatively correlated with the odontogenic differentiation of hDPCs, consistent with the current understanding of its repressive effect on cell differentiation.

KDM5A is a histone demethylase specific for triand dimethylated H3K4; hence, ectopic expression of KDM5A reduces global levels of H3K4me3/me2 [41,43]. KDM5A has been suggested to function as a transcriptional repressor by binding to its targetgene promoter and to play an important role in cell differentiation. During NK cell activation, KDM5A binds to the suppressor of cytokine signaling 1 (SOCS1) promoter region, leading to a substantial decrease in its H3K4me3 modification and gene transcription[44]. Wang et al. reported that overexpression of KDM5A in MSCs inhibited BMP2-induced osteogenesis by decreasing H3K4me3 levels on promoters of RUNX2[45]. Knockdown of KDM5A resulted in significant increases in ALP, OCN and OSX expression in hASCs, since the levels of H3K4me3 on the promoters of OSX and OCN were maintained with lower KDM5A occupation[33]. In this study, KDM5A deficiency led to a significant increase in total H3K4me3 levels, whereas no significant difference was found for H3K4me2 in hDPCs induced to undergo odontogenic differentiation. To explore whether the enhanced levels of H3K4me3 in hDPCs were reflected in the upregulated odontogenic differentiation markers, ChIP-PCR was utilized to observe the H3K4me3 marks on the individual promoters of these genes. As expected, H3K4me3 levels on the promoters of DSPP, DMP1, OCN and OSX increased after KDM5A knockdown in hDPCs. These results revealed that KDM5A-dependent histone demethylation decreased the H3K4me3 marks on the promoters of matrix-related genes and impaired their expression, thereby restraining hDPC differentiation.

In conclusion, the present study estimated differentially expressed HMTs and HDMs during the odontogenic differentiation of hDPCs and demonstrated that KDM5A was highly expressed in odontogenically differentiated hDPCs. During an investigation into the role of KDM5A in regulating odontogenic differentiation, the results showed that KDM5A inhibited the odontogenic differentiation potentiality of hDPCs through its ability to remove H3K4me3 from specific gene promoters. These findings provide the first indication that histone methylation is involved in the process of odontogenic differentiation in hDPCs and might have potentially innovative implications for the field of regenerative endodontics and pulp regeneration.

Highlights

- (1) The expression levels of HMTs and HDMs were profiled in hDPCs.
- (2) HDM KDM5A expression increased during early passages and mineralization induction.
- (3) KDM5A depletion promoted odontogenic differentiation potentiality of hDPCs.
- (4) KDM5A knockdown upregulated H3K4me3 on promoters of mineralization markers.

Disclosure statement

The authors declare that there are no conflicts of interest.

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