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mRNA expression profiling of histone modifying enzymes in pediatric acute monoblastic leukemia

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Histone modification is dysregulated in various types of cancers, including hematological malignancies. However, the expression profile of histone-modifying enzymes in pediatric acute monoblastic leukemia (AML FAB M5) has not been investigated. In this study, we evaluated the mRNA expression profile of 85 genes that encode enzymes involved in histone-modification in 27 pediatric AML FAB M5 samples by using a novel real-time PCR array. We obtained a gene cluster consisting of a total of 28 genes (15 up-regulated genes and 13 down-regulated genes). This gene signature revealed up-regulated expression of putative oncogenes *GCN5L2, SETD8, KDM5C, AURKA* and *AURKB*, and downregulated putative tumor suppressor genes (TSGs) *EP300, PRMT3, PRMT8* and *NOTCH2*. We investigated possible biological interactions between differentially expressed genes using ingenuity pathway analysis (IPA) and found 12 significant networks. Among these, gene expression, cancer, and embryonic development showed the highest number of networks with 39 focus molecules and had an associated significance score of 68. Further, *Rb, CDKN2C*, and *E2F1* were found to be upstream regulators of histone-modifying enzymes. This study provides additional insights into the molecular pathogenesis of pediatric AML FAB M5. These genes represent interesting targets with potential for diagnostic, prognostic and therapeutic application in pediatric AML patients.

1. Introduction

Acute myeloid leukamia (AML) is a heterogeneous clonal disorder of the myeloid lineage of white blood cells. It is caused by malignant transformation of self-renewing stem cells due to accumulation of genetic alterations which lead to their aberrant proliferation and differentiation. The etiology of AML has been linked to a variety of predisposing conditions that result from chromosomal instability, abnormal cytokine receptors, defects in DNA repair, aberrant activation of signal transduction pathways and abnormal histone modifications.

Epigenetic modifications are known to play a crucial role in carcinogenesis by at least two mechanisms: (i) by changing gene expression patterns through disordered regulation of tumor suppressor genes and/or oncogenes (Jin et al. 2013; Tao et al. 2013); (ii) by affecting genome integrity and/or chromosomal segregation (Boycheva et al. 2014). A posttranslational histone modification such as lysine acetylation, lysine and arginine methylation (Maze et al. 2014; Shechter et al. 2009; Nicklay et al. 2009), represents one of the epigenetic modifications. This modification affects chromosome function by any one of the two mechanisms, i.e., by altering the charge of histone which inturn causes structural alteration of histones that affects their binding ability to the DNA (Allahverdi et al. 2011); while the other leads to changes in the binding sites for protein recognition modules, such as, chromodomains or bromodomains that recognize methylated lysines or acetylated lysines, respectively. These epigenetic modifications are called histone code (Strahl et al. 2001; Strahl et al. 1999).

A wide body of evidence indicates an important role of posttranslational histone modifications in cancer biology and their association with cancer subtype, progression, and prognosis (Baptista et al. 2013; Albacker et al. 2013; Ramadoss et al. 2012; Toyokawa et al. 2011; Saeed et al. 2012; Müller-Tidow et al. 2010; Tickenbrock et al. 2011; Huang et al. 2011; Shi et al. 2011; Krivtsov et al. 2007). Up to date, there is very little information about abnormal changes in histone modification that may contribute to the development of pediatric AML. In a previous study, we have demonstrated that a real-time PCR array system could be a powerful tool for analyzing the expression of a focused panel of genes (Yan-Fang et al. 2012). In the present study, we analyzed the histone-modifying enzyme mRNA expression profiles in pediatric AML FAB M5 using this technique.

2. Investigations and results

2.1. Real-time PCR array design

We analyzed 88 pairs of real-time PCR primers for important gene expressions implicated in pediatric AML FAB M5. The primers for target genes are available on request. Using PCR array representing the human histone-modifying enzymes, we detected expression profiles of 85 crucial genes encoding enzymes that modify histones. This profile regulates chromatin accessibility and gene expression.

2.2. Gene expression and melting curve analysis by real-time PCR array

We used the real-time PCR array to analyze the expression of a panel of genes implicated in epigenetic chromatin modification. Gene expression and melting curve analysis was performed to confirm the primer specificity for the target genes (Fig. 1).

2.3. Expression profile analysis of pediatric AML FAB M5 and normal control samples

We analyzed the gene expression profile data obtained from real time PCR arrays using Multi Experiment View cluster software. We found that the gene expression profile of pediatric AML FAB M5 was significantly different from that of the normal controls (Fig. 2A). All the 28 genes were successfully clustered (Fig. 2B). Protein expression of GCN5L2 and HDAC7 in pediatric AML FAB M5 samples was validated by western-blot (Fig. 2C).

Among these, 15 genes were upregulated in pediatric AML FAB M5 (Table 1 and Fig. 3). In additon, we observed that 13 genes were downregulated in pediatric AML FAB M5 (Table 2 and Fig. 4).

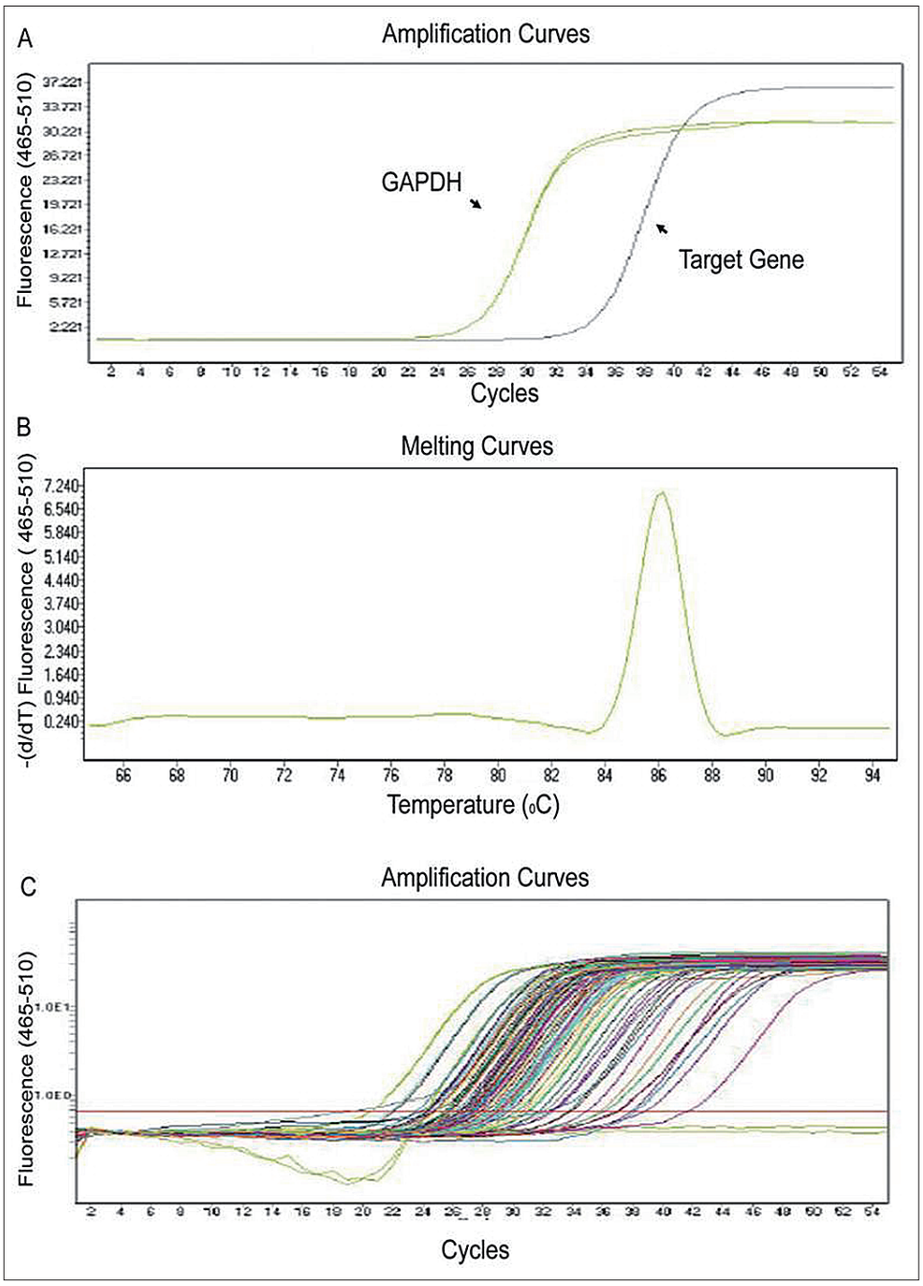

Fig. 1: Real-time polymerase chain reaction array design. a. Amplification of GAPDH and target genes in real-time polymerase chain reaction arrays. b. Melting curve analysis of the polymerase chain reaction product. c. Amplification of the whole genes in the polymerase chain reaction array.

2.4. Ingenuity pathway analysis of dysregulated genes in pediatric AML FAB M5

We investigated possible biological interactions between differentially expressed genes using IPA and found 12 significant networks. The top two networks are shown in Fig. 5A. Among these, gene expression, cancer, and embryonic development showed the highest number of networks with 39 focus molecules and had an associated significance score of 68 (Fig. 5D). The score refers to the possibility that a group of genes greater than or equal to the number of genes in a network could be obtained by chance alone. IPA groups the differentially expressed genes into different biological mechanisms that are related to hereditary breast cancer signaling (9.96×10^{8}), cell cycle G1/S checkpoint regulation (1.29×10^{7}), phospholipase C signaling (3.32×10^{7}), cyclins and cell cycle regulation (4.98×10^{7}), and chronic myeloid leukemia signaling (1.21×10^{6}).

3. Discussion

DNA methyltransferases and the enzymes demethylating CpG dinucleotides represented by the real-time array included NOTCH signaling genes (*NOTCH1, NOTCH2* and *EP300*) and DNA methyltransferases enzymes (*DNMT1, DNMT3A* and *DNMT3B*). The array also included 17 histone acetyltransferases (HATs), 14 histone methyltransferases (HMTs), 11 histone deacetylases (HDs), 8 histone ubiquitination enzymes, 7 histone phosphorylation enzymes, and 6 DNA/histone demethylases. The array also analyzed genes that encode the SET domain proteins containing a homologous domain with activity of histone methyltransferase in some of the family members, including *ASH1L, MLL3* and *MLL5*. Previous studies have reported that some of the dysregulated genes may be involved in acute lymphoblastic leukaemia (ALL) or other tumors. Elevated expression levels of *HDAC2* and *HDAC8* in 94 pediatric ALL samples, as compared to that in normal samples, has

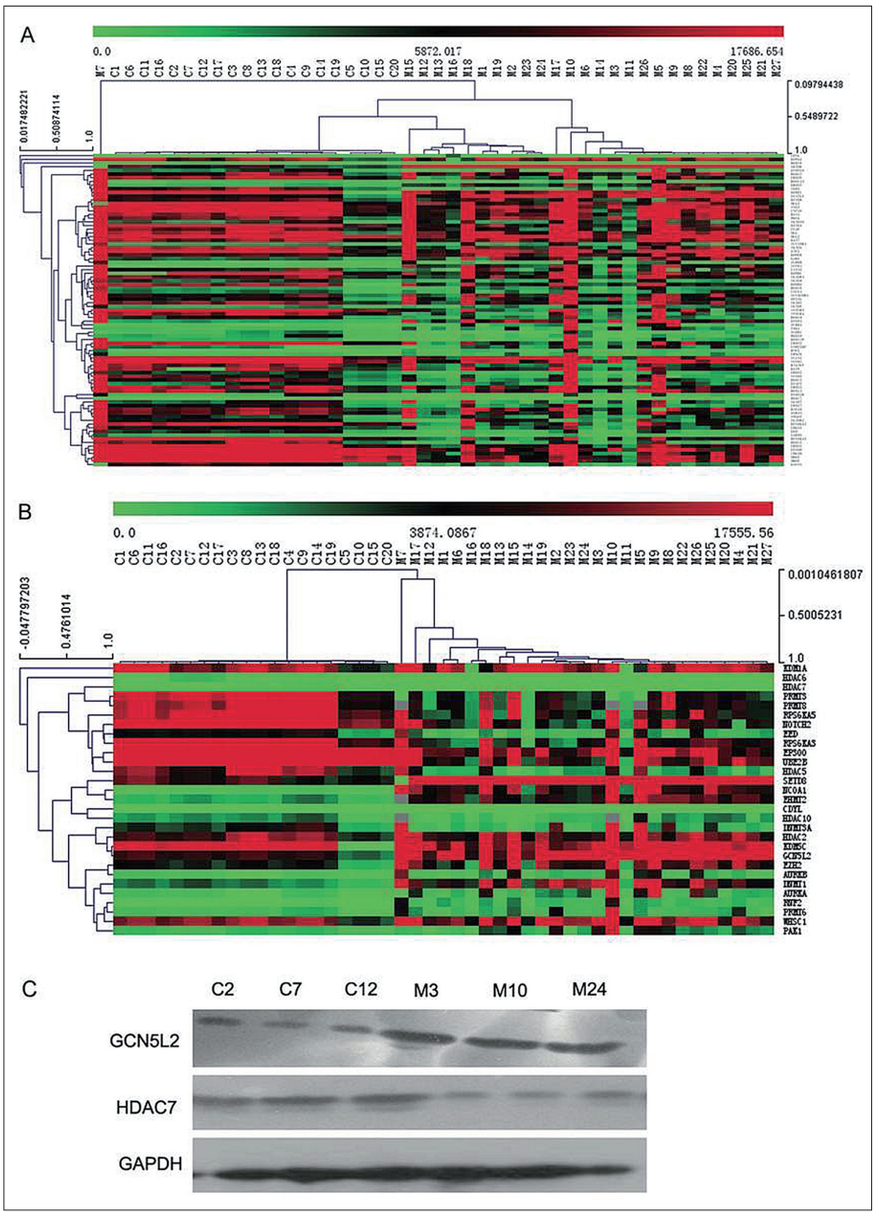

Fig. 2: Expression and cluster analysis of differentially expressed genes in pediatric acute monoblastic leukemia and normal controls. a. Cluster analyses of the data was performed for the gene cluster obtained .,rom the real-time polymerase chain reaction arrays. We used comparative Ct method to quantitate gene expression. First, we normalized gene expression levels for each sample to the expression level of the housekeeping gene encoding GAPDH within a given sample (-ΔCt). The relative expression of each gene was computed using the equation: $10^5 * \text{Log}_2$ (-ΔCt). Gene expression in acute myeloid leukemia and control samples was analyzed by Multi Experiment View cluster software. b. Twenty-eight genes were successfully clustered between acute myeloid leukemia and the normal control group. c. Western-blot analysis of proteins GCN5L2 and HDAC7 in pediatric acute monoblastic leukemia samples.

also been reported. *HDAC2* is also significantly over-expressed in solid tumors. Several studies have documented an over-expression of Aurora kinase A (*AURKA*) and B (*AURKB*), and its correlation with unfavorable prognosis in various malignancies including hematological malignancies. AURKA and AURKB inhibitors have been shown to be effective in early clinical trials. In addition, increased expression of p21-activated kinase 1 (PAK1 has been frequently reported in human malignancies, and has also been associated with reduced survival in patients with breast, ovarian and gastric cancers (Wang et al. 2010; Liu et al. 2009; Tharakan et al. 2008; Akinmade et al. 2008; Siu et al. 2010).

To the best of our knowledge, our study is the foremost to demonstrate upregulation of *GCN5L2* gene in pediatric AML. The *GCN5L2* gene encodes for histone acetyltransferase (HAT) enzyme. It acts primarily as a transcriptional activator as well as a repressor of NF-kB. The role of *GCN5L2* in carcinogenesis remains unknown. A research study found that GCN5L2 promotes growth of non-small cell lung cancer (Patel et al. 2004) . *GCN5L2* was shown to regulate the expression of E2F1, cyclin D1, and cyclin E, and promote lung cancer cell growth in a mouse tumor model (Xu et al. 2000). Therefore, GCN5L2 might be a potential target for the treatment of lung cancer. Our preliminary studies showed that GCN5L2 may inhibit apoptosis and promote proliferation of AML cells.

Our results showed that the *SETD8* gene was upregulated in pediatric AML FAB M5. SETD8 is a histone monomethyltransferase that serves to maintain genome integrity, is a transcription regulator, helps in DNA repair and causes tumor metastasis. It is required for progression S phase of cell cycle (Dhami et al. 2013). Inhibition of SETD8 expression decreases cell proliferation. SETD8 promotes carcinogenesis by deregulating proliferating cell nuclear antigen (PCNA) expression. SETD8 methylates PCNA at lysine 248, and depletion of SETD8 or substitution of lysine 248 destabilizes PCNA expression

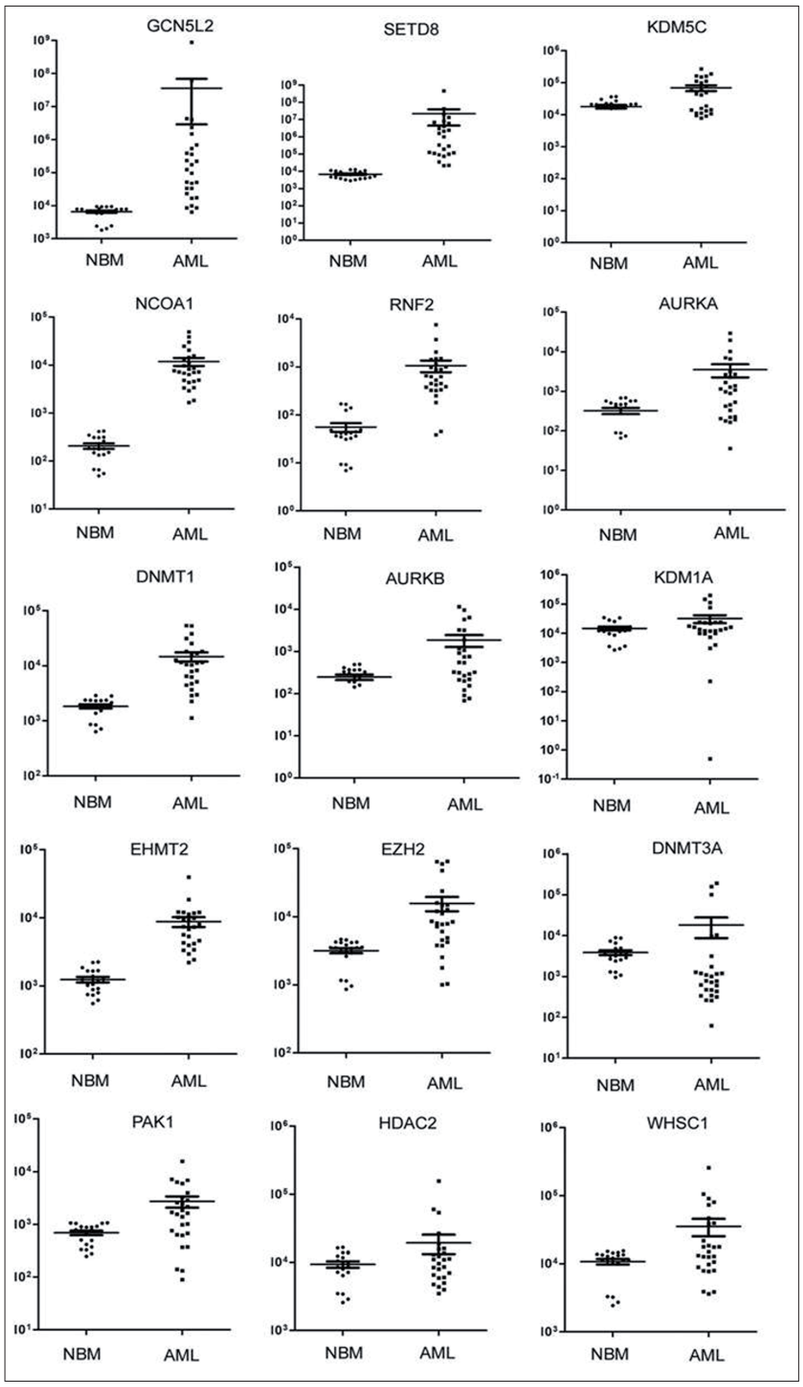

Fig. 3: Expression of up-regulated genes in pediatric acute monoblastic leukemia and normal controls. Data expressed as mean ±SE.

(Takawa et al. 2012). *SETD8* is physically associated with TWIST, a major regulator of epithelial-mesenchymal transition (EMT). Both SETD8 and TWIST promote EMT and enhance the invasive potential of breast cancer cells (Yang et al. 2012), which suggests that SETD8 might be a potential new therapeutic target for pediatric AML.

Tumor suppressor gene *EP300* is located on chromosomal region 22q13 and is associated with frequent loss of heterozygosity in breast, colon and ovarian cancers. However, no somatic mutations in EP300 have been reported in primary tumors (Tillinghast et al. 2003; Bryan et al. 2002). EP300 protein is a histone acetyltransferase, which regulates transcription via chromatin remodeling 3 and plays a key role in cell proliferation and differentiation (Gayther et al. 2000). The human *HDAC5* gene is located on chromosomal region 17q21 and its loss is frequently observed in a variety of cancers. Moreover, HDAC5 expression is often reduced in cancers (including in AML). Information pertaining to *EP300, PRMT3*, and *HDAC5* expression in pediatric AML is largely lacking. Therefore, our study is the first to demonstrate that these TSGs are down-regulated in pediatric AML FAB M5. In future their role further needs to be investigated in AML studies.

Our results suggest that the upstream regulators Rb (RB-1) and E2F1 signal for the histone-modifying enzymes in this cancer type. Loss of RB-1 commonly occurs in many human cancers. Rb functions as a tumor suppressor by blocking cell cycle progression. It is well known that the Rb/E2F pathway plays a key role in regulating DNA replication, and that this pathway is dysregulated in virtually all

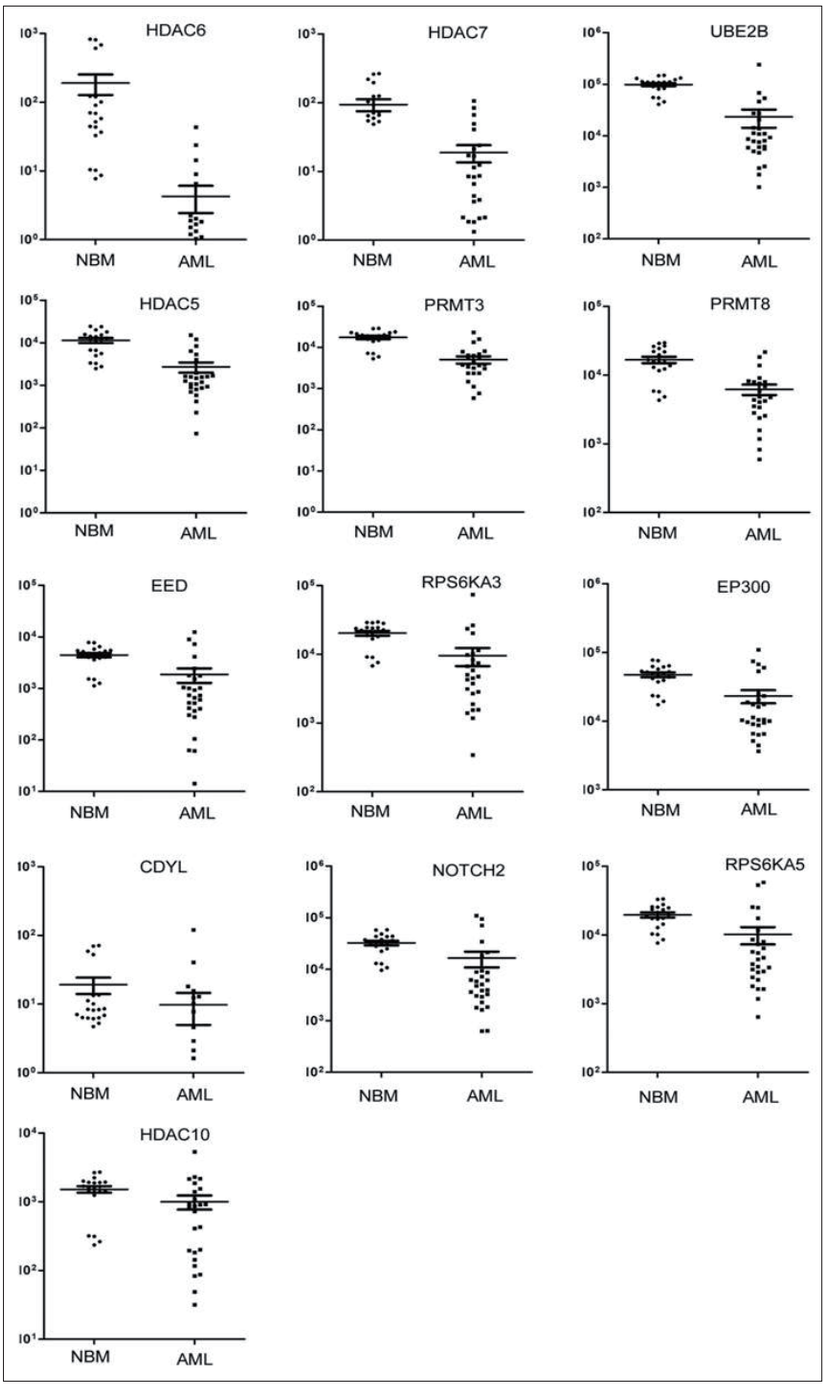

Fig. 4: Expression of down-regulated genes in pediatric acute monoblastic leukemia and normal controls. Data expressed as mean ±SE.

human malignancies (Nevins 2001). Rb binds the transcription activation domain of E2F at its gene promoters and inhibits its activity, thereby restricting cell proliferation. Recently, it was reported that the RB/E2F network may have a greater and a hitherto unrecognized role in gene expression rather than just transcription initiation (in regulation of RNA processing) (Ahlander et al. 2009).

Our analyses suggest CDKN2A as an additional upstream regulator. This gene encodes tumor suppressor p16 that induces cell cycle arrest at G1 checkpoint, and p14 that activates p53 by blocking MDM2-induced degradation of p53 and induces cell cycle arrest in both G1 and G2/M (Stott et al. 1998). CDKN2A also binds to E2F1 and MYC and blocks their transcriptional activation. Allelic loss of P53 and CDKN2A (present at 9p21 locus) and amplifications of the MDM2 gene were reported in cancer patients including those with hematological malignancies (Elnenaei et al. 2003; Biernat et al. 1998; Kraus et al. 2002; Moller et al. 1999). Till date there is no evidence for the regulation of histone modification enzymes by the up-stream signaling of RB-1/E2F1 and CDKN2A in pediatric AML. Therefore, our findings provide new leads for understanding the molecular-level pathogenetic mechanisms underlying pediatric AML FAB M5.

We found that 15 genes were upregulated and 13 genes were down-regulated in pediatric AML FAB M5. Oncogenes such as *GCN5L2, SETD8, KDM5C, AURKA, and AURKB* were upregulated and putative tumor suppressor genes *EP300, PRMT3, PRMT8, and NOTCH2*, were downregulated. Using IPA, we found that *Rb, CDKN2C, and E2F1* might be the upstream regulators of histone-modifying enzymes

Table 1: Genes upregulated in pediatric acute monoblastic leukemia

Gene	Description	NBM	AML	FC	*p*<-value
GCN5L2	K(lysine) acetyltransferase 2A	6554.99	695156.69	106.05	<0.001
SETD8	SET domain containing 8	6775.24	685112.26	101.12	<0.001
KDM5C	Lysine (K)-specific demethylase 5C	18000.96	1460628.44	81.14	<0.001
NCOA1	Nuclear receptor coactivator 1	206.64	11824.25	57.22	<0.001
RNF2	Ring finger protein 2	55.71	1068.57	19.18	0.002
AURKA	Aurora kinase A	324.19	3519.47	10.86	0.019
DNMT1	DNA (cytosine-5-)-methyltransferase 1	1829.27	14675.10	8.02	<0.001
AURKB	Aurora kinase B	246.54	1856.79	7.53	0.010
KDM1A	Lysine (K)-specific demethylase 1A	14620.75	104109.12	7.12	0.023
EHMT2	Euchromatic histone-lysine N-methyltransferase 2	1238.92	8775.83	7.08	<0.001
EZH2	Enhancer of zeste homolog 2 (Drosophila)	3175.47	15769.45	4.97	0.002
DNMT3A	DNA (cytosine-5-)-methyltransferase 3 alpha	3868.08	18243.65	4.72	0.015
PAK1	p21 protein (Cdc42/Rac)-activated kinase 1	690.79	2725.08	3.94	0.004
HDAC2	Histone deacetylase 2	9325.26	33231.17	3.56	0.0126
WHSC1	Wolf-Hirschhorn syndrome candidate 1	10767.62	35573.79	3.30	0.022

NBM: Normal bone marrow; AML: acute myeloid leukemia; FC: fold change

Table 2: Genes downregulated in pediatric acute monoblastic leukemia

Gene	Description	NBM	AML	FC	*p*-value
HDAC6	Histone deacetylase 6	190.74	4.26	0.02	0.008
HDAC7	Histone deacetylase 7	94.01	18.83	0.20	<0.001
UBE2B	Ubiquitin-conjugating enzyme E2B	99202.82	23332.22	0.24	<0.001
HDAC5	Histone deacetylase 5	11379.83	2719.44	0.24	<0.001
PRMT3	Protein arginine methyltransferase 3	17381.73	5039.38	0.29	<0.001
PRMT8	Protein arginine methyltransferase 8	16779.10	6221.27	0.37	<0.001
EED	Embryonic ectoderm development	4436.16	1864.57	0.42	<0.001
RPS6KA3	Ribosomal protein S6 kinase, 90kDa, polypeptide 3	20389.11	9555.98	0.47	<0.001
EP300	E1A binding protein p300	47487.19	23262.60	0.49	<0.001
CDYL	Chromodomain protein, Y-like	19.18	9.40	0.49	0.017
NOTCH2	Notch 2	32473.80	15912.17	0.49	0.016
RPS6KA5	Ribosomal protein S6 kinase, 90kDa, polypeptide 5	19647.31	9627.18	0.49	0.007
HDAC10	Histone deacetylase 10	1522.10	745.829	0.49	0.046

NBM: normal bone marrow; AML: acute myeloid leukemia; FC: fold change

in pediatric AML FAB M5. Future studies should investigate whether the alterations in these genes can serve as prognostic markers for pediatric AML FAB M5. Our findings reinforce the understanding that aberrant molecular pathways underlie the pathogenesis of pediatric AML FAB M5. Elucidation of these pathways may provide novel therapeutic targets and eventually facilitate the development of personalized treatment for pediatric AML FAB M5.

4. Experimental

4.1. Patient samples

We obtained bone marrow specimens from 27 patients with AML FAB M5, who were diagnosed at the Children's Hospital of Soochow University during 2010 to 2012. The study was approved by the Ethics Committee at the Children's Hospital. Written informed consent was obtained from all parents or from patient's guardians. AML FAB M5 diagnosis was made according the World Health Organization classification (Vardiman et al. 2009) .

4.2. RNA extraction and cDNA preparation

RNA was extracted from bone marrow samples of 20 normal healthy controls and 27 AML FAB M5 patients. An appropriate quantity of RNA was converted into complementary DNA (cDNA) using SuperScript II reverse transcriptase kit. (Invitrogen Corporation, Carlsbad, CA.)

4.3. Real-time PCR array

Primers were designed as described in our previous study (Yan-Fang et al. 2012). Primer selection parameters and the procedure for real-time PCR analysis were also as described in our previous study (Yan-Fang et al. 2012). Reactions were run on a Lightcycler 480 (Roche) using the following parameters: 95 °C for 5 min, 45 cycles of 10 s (sec) at 95 °C, 20 s at 60 °C, and 15 s at 72 °C.

Melting curves were completed using following parameters: 10 s at 95 °C, 60 s at 60 °C, and continuous melting. Primers were purchased from Invitrogen (genes and sequence of the primers were showed in supplementary material available on request). B2M, β-actin, and reference genes of low and medium copy number were also included.

4.4. Western blot analysis

Western blot analysis was performed using the methods described in our previous study . Antibodies against GAPDH (1:5000, Sigma, St. Louis, MO), GCN5L2 and HDAC7 (1:1000, Cell Signaling Technology, Inc. Danvers, MA), were used.

4.5. Ingenuity pathway analysis

Datasets that represent aberrant gene expression profile derived from real-time PCR array analyses were imported into the IPA tool (IPA Tool; Ingenuity H Systems, Redwood City, CA, USA; http://www.ingenuity.com), and processed as decribed elsewhere (Yan-Fang et al. 2012).

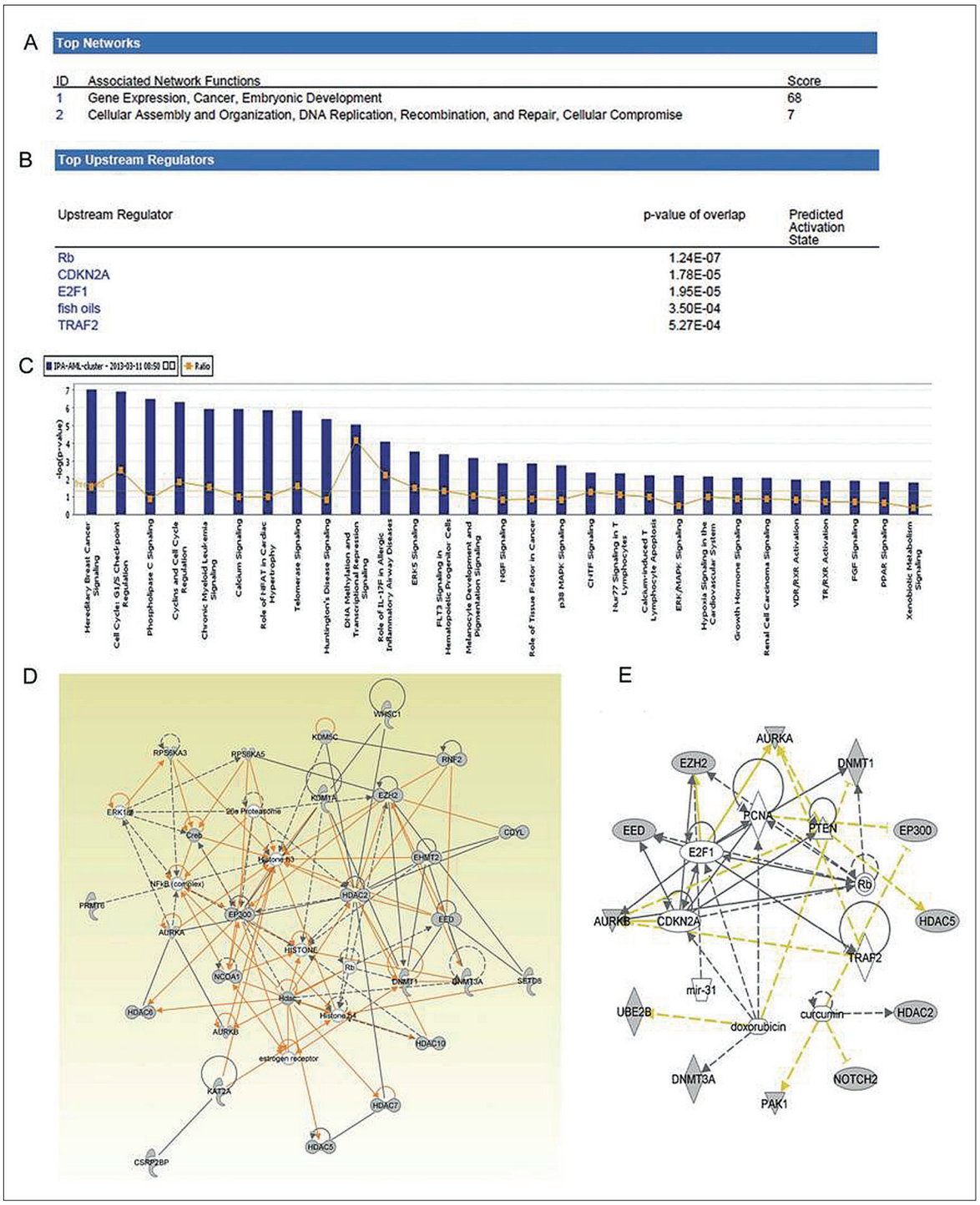

Fig. 5: IPA in pediatric acute monoblastic leukemia. a.The top two networks obtained on IPA. b. Upstream regulators included Rb, CDKN2C, and E2F1 genes. c. Toxicology pathways obtained from IPA. The X-axis denotes the top toxicology functions computed by IPA according to differentially expressed genes (highlighted). Y-axis denotes the ratio of number of genes from the dataset in the pathway and the number of all known genes in the pathway. The yellow line denotes the threshold of p-value < 0.05 as computed by Fischer's test. d. Most highly rated network in IPA analysis. The genes in shade were seen to be statistically significant. The dotted line denotes an indirect interaction and solid line represents a direct interaction of the two gene products. e. Genes associated with the upstream regulators.

4.6. Statistical analysis

Gene expression in AML FAB M5 and control samples were compared by Student's *t*-test using SPSS software ver.18 (SPSS Inc., Chicago, IL). The mRNA expression profiles of the pediatric AML FAB M5 samples and the control samples are presented as mean ± SE.

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Conflict of Interest: The authors declare that they have no conflict of interests.

Supplementary table

	Primer	Sequence
1	HTATIP-F	CTTGGCCAAAAGACACAGGT
2	HTATIP-R	CATCCTCCAGGCAATGAGAT
3	GCN5L2-F	CGGCGTAGGTGAGGAAGTAG
4	GCN5L2-R	GTGCTGTCACCTCGAATGAG
5	NCOA1-F	TGATGTTTTCCAGAGATGGCT
6	NCOA1-R	TCTGAGGGGCTTAGAAATTAACA
7	NCOA3-F	CAGTCAAAGGATGTTCAAGCA
8	NCOA3-R	CCGATTTAAAGCTGAGCTGC
9	NCOA6-F	AGTCGCAGTCCTGCTTGTTT
10	NCOA6-R	CGATCTTCTCGACCTGCTTC
11	CARM1-F	CCAGGGTGATGATGAAGGAC
12	CARM1-R	GGGCATCGCCCTCTACA
13	DNMT1-F	TTCTGTTAAGCTGTCTCTTTCCA
14	DNMT1-R	TGCTGAAGCCTCCGAGAT
15	DNMT3A-F	ATTCCTTCTCACAACCCGC
16	DNMT3A-R	TACTTCCAGAGCTTCAGGGC
17	DNMT3B-F	TCTCCATTGAGATGCCTGGT
18	DNMT3B-R	GAGATTCGCGAGCCCAG
19	ATF2-F	GCAGTCCTTTCTCAAGTTTCC
20	ATF2-R	GGTGCTTTGTAAACACGGCT
21	CDYL-F	CTCGCTGTCATAGCCTTTCC
22	CDYL-R	AGCAGCCTCCCGCTTTAC
23	CSRP2BP-F	TCTGGTGTCGAAATCCCTTC
24	CSRP2BP-R	CTGGACAAGCCACGTACAGA
25	ESCO1-F	CTGCCATCAGGGTATTCAGC
26	ESCO1-R	CAGCATCTGCTTTTCCACAA
27	HAT1-F	CATCCCCAAAGAGTTGATGG
28	HAT1-R	AACACCAACACAGCAATTGAA
29	PCAF-F	GGTGAGGGGTTAGGGTTTTT
30	PCAF-R	GGCCAAGAAACTGGAGAAACT
31	DOT1L-F	TCGATGATTTCATGAGCAGC
32	DOT1L-R	AGAAGCTGGAGCTGAGACTGA
33	MLL-F	TGAAGGAGACCTTGTGGGAC
34	MLL-R	CCGTGTTTGGGGAGAGC
35	MLL3-F	TCCCGCTCAGGACTAGATTC
36	MLL3-R	CAGAGCATTCAAGGGATGGT
37	SETDB2-F	TCAAGAGAGAGAATTTCCTTATTTCC
38	SETDB2-R	TCCAATGCATTTCCCTCTACA
39	SMYD3-F	TAGGCCAACACCAACTTCCT
40	SMTD3-R	CCTGCCTTTGACCTTTTTGA
41	SUV39H1-F	CTGACGGTCGTAGATCTGGC
42	SUV39H1-R	ATTCGCAAGAACAGCTTCGT
43	ASH1L-F	CCAAAACTCGAAACCAGCAT
44	ASH1l-R	ACCCCCTCTTCCCCACTT
45	MLL5-F	CAAACCCATGAGATGTCTGGTA
46	MLL5-R	GTTCGGGTGTCTCGTGTGT
47	NSD1-F	CATGGGCGTCTCTTGAATTT
48	NSD1-R	ATCTACTTCACAGGAATTGCCA
49	SUV420H1-F	TTGAGCTCCTCGAAGGAAAG
50	SUV420H1-R	ATGACCTAGCAACCAGTTTGG
51	WHSC1-F	ACTGGCGTGCACTCTTTTTC
52	WHSC1-R	GGTCCAAAGTGTCGGGTTAC
53	NEK6-F	TGTCCTGCCATCCTGCAT
54	NEK6-R	AAACTCGTGTGGGAGGCAC
55	PAK1-F	TGGGGGTTTGTCTTGAATGT
56	PAK1-R	AGGAGGAGCCGAGAGGAG
57	RPS6KA3-F	TGGGTTAATCTCCTCCTCTCC
58	RPS6KA3-R	GTGGCAGAAGATGGCTGTG
59	RPS6KA5-F	GGACCTTCAGGAGCTCAAAA
60	RPS6KA5-R	TCCTCACTGTCAAGCACGAG
61	DZIP3-F	TCTTATTCCACTGCTTGCCA
62	DZIP3-R	TGTGCGTCATCCTCTACCTG
63	RNF2-F	TGAGTTCCGTTTGTCTGCAC
64	RNF2-R	TGATACCAGAGTCTTGCTCCG
65	RNF20-F	AGCTTGCGATTTTTGGTTTG
66	RNF20-R	ATTCAGGGACCACAGTGGAA
67	UBE2A-F	TGGATGGAACATCTTAGAGACAAA
68	UBE2A-R	GAAGGGACCCCGTTTGAG
69	UBE2B-F	TGGACTCCATCGATTCTGAAG
70	UBE2B-R	TCCAAATAAACCACCAACTGTTA
71	USP16-F	GGTTCAGATCTTGGCGTCAG
72	USP16-R	AGCCTTCAGTTTGGCTGTGT
73	USP21-F	TAACAGGTGGCTCTCGGGTA
74	USP21-R	GCCTCACTGGGGGACAG
75	MBD2-F	CCTTTGGGTAGTTCCATGGTT
76	MBD2-R	CAACGAATGAATGAACAGCC
77	HDAC1-F	CATCTCCTCAGCATTGGCTT
78	HDAC1-R	CGAATCCGCATGACTCATAA
79	HDAC2-F	ATGAGGCTTCATGGGATGAC
80	HDAC2-R	ATGGCGTACAGTCAAGGAGG
81	HDAC3-F	CTGTGTAACGCGAGCAGAAC
82	HDAC3-R	GCAAGGCTTCACCAAGAGTC
83	HDAC4-F	CTGGTCTCGGCCAGAAAGT
84	HDAC4-R	CGTGGAAATTTTGAGCCATT
85	HDAC5-F	GAACTGGGCATGGCTCTTG
86	HDAC5-R	GGGAACCATCCTTGGAAATC
87	HDAC6-F	GCGGTGGATGGAGAAATAGA
88	HDAC6-R	CCGGAGGGTCCTTATCGTAG
89	HDAC8-F	GCGTGATTTCCAGCACATAA
90	HDAC8-R	ATACTTGACCGGGGTCATCC
91	HDAC9-F	GCCCACAGGAACTTCTGACT
92	HDAC9-R	GAACTCTAAGCCAGATGGGG
93	HDAC10-F	GAACAGCCACATCCAGGG
94	HDAC10-R	CCTCTTAGATGGGATGCTGG
95	HDAC11-F	AAGGAAGTTGGGGAGGAAGA
96	HDAC11-R	GCACACGAGGCGCTATCTTA
97	CIITA-F	CGCTGTTAAGAAGCTCCAGG
98	CIITA-R	GCTGGGTCCTACCTGTCAGA
99	KAT8-F	CCTCATGCTCCTTCTCCAAG
100	KAT8-R	GATCACTCGCAACCAAAAGC
101	KAT7-F	TGATACAGTGGGCATCCTGA
102	KAT7-R	TGAAGTGTCCTACACCAGGC
103	KAT6A-F	ACAGATGGGGATTGGTTCAG
104	KAT6A-R	CGTGGATGGGAAAGAGAGTT
105	KAT6B-F	CATATTGGAATGGGATCAGCA
106	KAT6B-R	AAAGGGGCACCTCAGTATCC
107	EHMT2-F	GAGGTCACCTTTCCCAGTGA
108	EHMT2-R	CCAAACCAGGAAATGGACAG
109	PRMT1-F	CTTCTCACTGCTTTCCGCCT
110	PRMT1-R	GCCGCGAACTGCATCAT
111	PRMT2-F	CGGGCATACAGGATGGACT
112	PRMT2-R	CAGAAGGTGGAGGATGTGGT
113	PRMT3-F	CCGTAGAGAACACGACCCTG
114	PRMT3-R	ACCCTGAAAATCACAAGGACA
115	PRMT5-F	CCTTCTTGGAATTGCTGCAT
116	PRMT5-R	TCTCATGGTTTCCCATCCTC
117	PRMT6-F	AGTTCCGAAGGATACCCAGG
118	PRMT6-R	ACGAGTGCTACTCGGACGTT
119	PRMT7-F	CCGATCAGCTCTGTGTCAAA
120	PRMT7-R	CAACAAGCATTCCACCGAG
121	PRMT8-F	CCGTAGAGAACACGACCCTG
122	PRMT8-R	ACCCTGAAAATCACAAGGACA
123	SETD1A-F	GGTGACTCCTCCGTGGG

	Primer	Sequence
124	SETD1A-R	ACCTGTGGAGGTGCCAGT
125	SETD2-F	TTCTGCTGTCTTGGGCTTTT
126	SETD2-R	GAGATGATGCCAGCCTTGAG
127	SETD3-F	GCCCACCTGTAGTCCTCGTA
128	SETD3-R	ACACAGCTCGACAGTACGCC
129	SETD4-F	AATCACTGTGTCCGTGGTGA
130	SETD4-R	CAGGTACAGGAAGAGGGCTG
131	SETD6-F	AGCCATTTGCCCATAAGTGT
132	SETD6-R	CCAATCACAACGCCAATCTA
133	SETD7-F	AGTGTAAACTCCCTGGCCCT
134	SETD7-R	GTTCACGGAGAAAAGAACGG
135	SETD8-F	TCATTCCTTCTTCCTTCCCA
136	SETD8-R	GAAGGAGCTCCAGGAAGAGC
137	SETDB1-F	TTCACGGAGCTTCTGGTCTT
138	SETDB1-R	TTCCCGGCCTACAGAAATAA
139	AURKA-F	GGGCATTTGCCAATTCTGT
140	AURKA-R	TGGAATATGCACCACTTGGA
141	AURKB-F	AGATGGGGTGACAGGCTCTT
142	AURKB-R	AGGAGAACTCCTACCCCTGG
143	AURKC-F	CTCCCCTCAATCATTTCTGG
144	AURKC-R	GATTGCAGATTTTGGCTGGT
145	KDM1A-F	CATCCGGTCATGAGGAAGTC
146	KDM1A-R	CCCCACCTGAGGAAGAAAAT
147	KDM5B-F	TGGATACGTGGCGTAAAATG
148	KDM5B-R	CGAGCAGACTGGCATCTGTA
149	KDM5C-F	CTCCTGCACACTGGTTTGTG
150	KDM5C-R	CTGACCTCCTGCACCAACTT
151	KDM4A-F	AGCTTGCTTAAAGGCTGACG
152	KDM4A-R	GAAGTTTCAGTGAGCGGGAG
153	KDM4C-F	CTTTCCTGCAAGTGCACAAT
154	KDM4C-R	CCATGCTGGTTTTAATCATGG
155	MBD2-F	CCTTTGGGTAGTTCCATGGTT
156	MBD2-R	CAACGAATGAATGAACAGCC
157	HDAC7-F	CCTGCTGTTGTCACCGC
158	HDAC7-R	TCCTCTCCAGCTCAGAGACC
159	NOTCH1-F	GTTGGGGTCCTGGCATC
160	NOTCH1-R	GGTGAGACCTGCCTGAATG
161	NOTCH2-F	CACAGGGTTCATAGCCATCTC
162	NOTCH2-R	GGAGGCGACCGAGAAGAT
163	EZH2-F	CTGATTTTACACGCTTCCGC
164	EZH2-R	GGAACAACGCGAGTCGG
165	SUZ12-F	AAGCTGCAAATGAGCTGACA
166	SUZ12-R	CCAGAACAAACATCAAAAGGA
167	EED-F	TCTTCCATCTTGCCAGGTTT
168	EED-R	ATGTTGATTGTGTGCGATGG
169	EP300-F	TCTGGTAAGTCGTGCTCCAA
170	EP300-R	GCGGCCTAAACTCTCATCTC
171	GAPDH-F	AGAAGGCTGGGGCTCATTTG
172	GAPDH-R	AGGGGCCATCCACAGTCTTC
173	ACTIN-F	AGCGAGCATCCCCCAAAGTT
174	ACTIN-R	GGGCACGAAGGCTCATCATT
175	B2M-F	TGCTGTCTCCATGTTTGATGTATCT
176	B2M-R	TCTCTGCTCCCCACCTCTAAGT

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