Transcription Analysis of a Histones Modifiers Panel Coupled with Tumor Suppressor Genes Displayed Frequent Changes in Acute Myeloid Leukemia-Related Genes

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Research

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Abstract

Introduction Epigenetic alterations could cause leukemia through the activation of normally silent loci or silencing of normally active loci. We herein aimed to compare the expression patterns of a histone modifiers panel consisted of SUV39H1, PRDM16, UHRF2, KDM2B, and KDM3C between acute myeloid leukemia(AML) cells and normal cells and to assess the correlation of these genes with the expression of vital tumor suppressor genes, including p16^{INK4A} and p53.

Materials and methods Bone marrow or peripheral blood samples of 50 AML patients at diagnosis and also 18 subjects with a normal hematopoietic system as a control group were obtained after informed consent. Then, qRT-PCR was performed to determine the expression levels of the aforementioned genes.

Results We found a broad alteration in the expression signature of five out of seven studied genes in AML patients as compared with the control group. UHRF2 and p53 were remarkably downregulated in AML patients ($P < 0.001$), while SUV39H1, PRDM16, and KDM3C significantly overexpressed ($P < 0.01$). Based on the Spearman rank correlation, SUV39H1, KDM2B, and age of the patients negatively regulated both p16^{INK4A} and p53 expression.

Conclusion Taken together, our findings provided preliminary evidence regarding the pervasive mRNA perturbation of histone modifiers and their plausible influences on critical tumor suppressor genes. Future studies in this area would be required to assist in establishing these results in the clinical practice of AML patients.

1. Introduction

Despite the numerous efforts in the last 15 years to identify the defected signaling pathways that could be targeted to treat acute myeloid leukemia (AML), still, the success rate of these approaches is dismal in the leukemic patients[1]. The results of the genome-wide association showed that the nucleotide sequences of many genes involved in signaling pathway are intact in a significant proportion of AML patients[2], suggesting that the solution toward cancer treatment is not in targeting of signaling pathways. This study also showed that epigenetic regulators are frequently defected in the majority of AML patients either by gene mutations or by non-mutational mechanisms[2]. Albeit, it is difficult to alleviate the devastating effects of mutations in malignant cells, epigenetic alterations are most amenable and fortunately would be excellent targets for development of new anticancer agents[3].

Among the cell cycle regulators, it has been proved that p16^{INK4A} and p53 are by far the most frequently altered gene in many types of human cancers[4]. The mutation in the genes encoding p16^{INK4A} and p53 are roughly detected in 40% and 50% of cancer-derived cell lines and human malignancies, respectively[4]. However, when it comes to AML, the rate of mutations in these genes are significantly lower as compared to solid tumors[5, 6]. Given the critical role of p16^{INK4A} and p53 in negative regulation of cell cycle, it is not surprising that many non-mutational mechanisms dampen the functions of these genes and lead to uncontrolled cell growth in AML patients. Among diverse non-mutational mechanisms, which could distort the normal functions of p16^{INK4A} and p53 in AML cells, hypermethylation of CpG islands, changes in chromatin

configuration mediated by histones' modifiers, and suppression by negative regulatory RNAs and proteins are the most important ones[6-11]. Concerning the prominent role of DNA and histone methyltransferase in the regulation of p16^{INK4A} and p53 expressions[8, 10, 12], we assumed that epigenetic alterations might have an impact on the suppression of these genes. Epigenetic regulators are divided into three groups, known as "epigenetic writers," "epigenetic readers," and "epigenetic eraser," which produce epigenetic marks, recognize and remove them, respectively[13, 14]. Previous studies showed that methylated DNA and H3K9me3 are of the main epigenetic-related suppressive marks for p16^{INK4A} [15, 16] and p53 genes in cancers[8]. It is well-established that H3K9me3 could be recognized by epigenetics readers, such as UHRF1/2, which in turn could precipitate DNA methyltransferase (DNMTs) in CpG islands or prepare them for breakdown by ubiquitin-proteasome system[17, 18]. On the other hand, H3K36me3 is a euchromatic mark which induced p16^{INK4A} and p53 expressions[8, 19]. Given these, we selected five important epigenetic regulators, including two H3K9 methylation writers, named PRDM16 and SUV39H1, an H3K9me reader, known as UHRF2, and two epigenetic erasers consisted of KDM3C(H3K9me3 demethylase) and KDM2B(H3K36me3 demethylase), to evaluate their correlation with p16^{INK4A} and p53 in AML patients.

Mounting body of evidence had declared a critical role for histone modifiers in normal and malignant hematopoiesis. PRDM3 and PRDM16 are two specific methyltransferases that convert H3K9 to H3K9me1[20]. Through further methylation by SUV39H1 enzyme, H3K9me1 turned into a repressive H3K9me3 histone mark at heterochromatic foci[20]. PRDM16 encodes two isoforms; a full-length (fPRDM16) and a short (sPRDM16) isoforms, which are associated with tumor suppressor and tumor promoter functions, respectively. The opposite functions of PRDM16 isoforms are attributed to presence or absence of an N-terminal PR domain. The N-terminal PR domain is deleted in some rare translocations presented in AML and MDS patients. It has also been demonstrated that enforced overexpression of sPRDM16 could induce AML in mice, only when accompanied by the absence of p53 [21]. In this line, previous studies showed that SUV39H1 could repress p53 expression, either by induction of heterochromatic at its promoter [22] or by impinging the function of p53 protein [23]. These findings arose a question of whether there is a possibility that PRDM16 and SUV39H1 over-expression could take part in AML development. KDM2B and KDM3C are of two recently appreciated histones modifiers that play a role in H3K36me2 and H3K9me1/2 demethylation, respectively[19, 24]. The function of KDM2B and KDM3C results in reduction and elevation of accessibility of chromatin to transcription apparatus, respectively[19, 24]. The results of the study in MDS patients revealed that KDM2B could indirectly reduce the expression of p16 via let7b/EZH2 pathway[25]. Several lines of evidence have also demonstrated that KDM3C is necessary for self-renewal of AML cells and could be applied as a potential target for anticancer therapy[26]. Regarding the paradoxical functions of SUV39H1 and KDM3C in catalyzing and erasing of H3K9me, it is interesting to evaluate the correlation of these genes with the expression of TSGs in AML patients.

Given this background, several reports are suggesting that these epigenetic modifiers are involved in the pathogenesis of AML; however, their precise mechanisms of action are not yet fully clarified. In this regard, to dissect the probable underlying molecular mechanisms, we aimed to investigate the expression patterns of these epigenetic regulators, including UHRF1, PRDM16, SUV39H1, KDM2B and KDM3C and also two TSGs named p16^{INK4A} and p53 in AML patients using RTq-PCR analysis.

2. Materials And Methods

2.1 Subjects characteristics

From June 2016 to August 2017, both peripheral blood (PB) and bone marrow (BM) samples of 50 patients with AML at the time of diagnosis and 18 subjects with normal PB/BM features were obtained after taking informed consent. This study was confirmed by ethics committee of Shahid Beheshti University of Medical Sciences. The diagnosis of AML was established according to morphology characteristics, immunophenotyping and molecular studies at Taleghani Hospital (Tehran, Iran). The control group consisted of patients with lymphoma who were referred to roll-out the bone marrow involvement with malignant cells and patients with suspected immune thrombocytopenic purpura (ITP). This group had a normal bone marrow features in terms of cellular composition and absence of abnormal cells, as revealed by immunophenotyping with flow cytometry.

2.2 Translocation Analysis

RQ-PCR was applied to investigate the frequent translocations in AML patients. Briefly, five translocations, such as t(8;21) (RUNX1-RUNX1T1), t(15;17) (PML-RAR- α), t(9;11) (MLL rearrangement), t(16;16) (CBF- β MYH11), and t(9;22) (BCR-ABL1) were analyzed using ABI (ThermoFisher, USA) instrument.

2.3 Immunophenotypic Analysis

Samples were labeled with 5 μ L antibodies against CD45, CD34, CD-11b, CD13, CD33, CD117, CD16, CD64, HLA-DR, CD3, CD5, CD19 and isotype control (Dako, Denmark). Antibodies were conjugated with FITC, PE or APC. After incubation of the samples in refrigerator for 20 min and lysing RBC by using a hypotonic formic acid solution, the formic acid was first naturalized with a basic solution, and then the cells were fixed with a formalin based solution. Finally, cells were analyzed by a flow cytometry instrument (Attune, USA). Based on the composition of positive CD-markers and cytomorphology, patients were divided in AML with myeloblastic phenotype (AML-M0, -M1 and -M2), AML with promyelocytic phenotype (AML-M3) and AML with myelomonocytic features (AML-M4 and -M5).

2.4 RNA extraction and cDNA synthesis

Mononuclear cells were isolated using Ficoll-Hypaque (INTRON, South Korea) density gradient centrifugation as outlined by manufacture. Leukemic blasts and or promyelocytes accounted for upward 80% of the total cells in patients with AML, especially after Ficoll separation. Total RNA was extracted using Trizol (Invitrogen™) according to the single-step method, then the quantity and quality of the RNA were examined by Nanodrop (Thermo Scientific, USA) and agarose gel electrophoresis. Total RNA was exposed to amplification grade DNase I to eliminate any residual genomic DNA. Approximately 1000 ng RNA from each sample was applied for cDNA conversion using an ABI kit.

2.5 Real-time quantitative PCR (RQ-PCR)

RQ-PCR primers for target genes and control gene were designed using gene runner x64 v 6.0.28 beta (Table 1). Primer specificity was checked by NCBI primer-blast tool. An SYBR Green I real-time PCR assay was

performed in an ultimate volume 25µl containing: 5µl cDNA (equivalent 100ng RNA), 0.75µl primers (equivalent 300nM) (Takapou zist, Iran), 12.5 universal Master Mix (Amplicon, Denmark), and nuclease-free water (Sincolon, Iran) to reach total volume. The expression levels were measured using Rotor-Gene® 6000 machine. The specificity of the amplicons was confirmed by melting curve analysis and gel electrophoresis. The standard curves were constructed using serial dilutions of an appropriate cDNA. Transcript levels of genes of interest were normalized to ABL transcripts as a control gene; then, the relative fold changes were calculated using $2^{-\Delta\Delta CT}$ formula due to concordant of RQ-PCR efficiencies. We denoted the 5th and 95th percentile of expression in the control group as the lower and upper limit of normal expression.

2.6 Statistical analysis

Data are expressed by mean± SD. all samples were tested in triplicate. For analyzing of difference among multi-state variables One-Way ANOVA or Kruskal-Wallis were applied; for two-state variables, t-test or Mann-Whitney U test were performed. The selection between these tests was according to the distribution of data. For analysis of correlation, Spearman rank test was performed. Statistical analyses were performed with SPSS software (version 23), and a two-tailed P value less than 0.05 was taken as significant. Graphs were designed using GraphPad prism (version 7.0)

3. Results

3.1 Patients' characteristics

Of the 65 subjects, 50 from AML patients and 15 from individuals with normal bone marrow or peripheral blood were used for gene expression analysis. The age of AML patients ranged from 2 years to 89 years (median 47 years); 42% were male and 58% were female. Chromosomal translocations, including t(8;21), t(15;17), t(9;11) and t(16;16) were detected in 2, 18, 1 and 2 patients, respectively. None of the patients carried the t(9;22). Owing to the limited number of patients in each group, we did not perform statistical analyses to evaluate the effect of various translocations on gene expression signature; however, patients with t(15;17) were categorized as AML M3 and distinctly were compared with other FAB sub-groups. Other characteristics of patients are summarized in Table 2. The control group included five males and 13 females, aged 13-87 years (median 35 years). 12 BM and 6 PB samples were obtained from these subjects.

3.2 The expression signatures were not relevant to gender, type of samples (BM vs PB) and blast percentage

To define whether the expression patterns of studied genes were being affected by the gender and samples' types, Man-Whitney U test was performed. The resulting data showed that the expression of all the genes was independent of these variables in both AML and control subjects, suggesting that these variables were not a confounder in the present study. So, for the next analyses, we neglected the effects of these variables and combined the data to compare other variables or measuring the correlations. When the Spearman rank test was performed, we found that the expression levels also had no statistically significant correlation with the blast percentage at the diagnosis.

3.3 There was a difference between the expression of the epigenetic regulatory genes in AML and control groups

The association between the expression of epigenetic-related genes and development of human malignancies has been examined in several studies; however, in many cases, there are conflicting results. Given evidences showing the expression of p16^{INK4A} and p53 may be dampened by epigenetic mechanisms in cancer cells, we elected these genes to evaluate their correlation with epigenetic regulators. As presented in Fig 1, while the expression levels of UHRF2 and p53 were much lower in AML patients than that of control subjects (P-value 0.005 and 0.005 respectively), the expression of SUV39H1, PRDM16 and KDM3C were significantly elevated in AML patients (P value 0.005, 0.005 and respectively). Moreover, KDM2B and p16^{INK4A} had a comparable expression level between these groups (P-value >0.05). Strictly, the 5th and 95th percentiles of expressions of the control group were chosen as cutoff values of normal expression and were further used to define significantly increased and decreased expression in AML patients, respectively. The percentages of underexpression, normal expression, and upregulation of each gene are summarized in Table 3.

3.4 Analyses among FAB sub-types showed a significant difference in the expression of SUV39H1, KDM3C, and p53

To investigate whether there is a correlation between FAB subtypes and gene expression, we classified the patients into three groups; patients with acute myeloblastic leukemia (M0, M1, and M2), patients with acute promyelocytic leukemia (APL, M3) and patients with acute myelomonocytic/monocytic leukemia (M4 and M5).. Non-parametric analysis using showed the APL patients displayed significant overexpressed KDM3C, while the expression of p53 was decreased as compared to M0-M2 patients. SUV39H1 had a significantly higher expression in patients with M0-M2 than patients with M4 and M5 phenotypes. Other genes represented a relative similar patterns of expression among different FAB subtypes.

3.5 Correlation of TSGs with epigenetic regulator panel

Table 4 represented the results obtained from the Spearman rank correlation. Concisely, the results showed the lower expression of p16^{INK4A} and p53 genes were significantly correlated with the higher expression of SUV39H1 and KDM2B. Moreover, we found that the expression of p16^{INK4A} had a borderline negative correlation with PRDM16 expression in AML patients. Interestingly, we found a positive strong correlation between p16^{INK4A} and p53 gene expression in the healthy subjects, but not in AML patients ($r = 0.813$, P-value <0.001 and $r = 0.097$, P value= 0.548, respectively).

3.6 The gene expression patterns of p16^{INK4A} and p53 changed by the age

We previously showed that p16^{INK4A} expression varies among different age groups of AML patients and healthy subjects, as we found that the p16^{INK4A} expression was inversely correlated with aging among AML patients, contrariwise the healthy subjects[27]. In the present study, we found that the lower levels of p53 expression were also significantly skewed to older patients ($r = -0.348$, P-value = 0.019). It was of particular interest to dissect whether there is an association between the expression pattern of epigenetic regulatory genes and age both in AML and control groups. Of note, the resulting data did not show any significant correlation between these parameters in the present study. In previous study, we showed that UHRF1 expression increased by age and may be involved in the down-regulation of p16^{INK4A} in elder patients with AML. In this respect, we sought to examine the correlation of p53 with UHRF1. Interestingly, results showed a

significant negative correlation between the expression of these genes in the AML patients ($r = -0.421$, P -value = 0.006); however, this finding was not significant in the control group.

4. Discussion

Since the dawn of whole-genome sequencing in cancers, it well-defined that AML cells carry the lowest mutation number as compared with other malignant cells[2]. This characteristic may be in part due to the genome-wide hypermethylation, which occurs in many AML patients, a feature that could guard leukemic cells against genome instability but simultaneously increased the chance of point mutation in CpG islands and dampened the expression of TSGs[28]. On the contrary, genome-wide hypomethylation is a marker of a dozen types of solid tumors, which is accompanied by genome instability and consequently a higher mutation rate per TSGs[29]. However, disfavoring cancer cells, general hypomethylation could attenuate the net suppressive effect of epigenetic on TSGs[29]. In this study, to shed light on the molecular mechanisms of p16^{INK4A} and p53 suppression in AML patients, we investigated the expression of a panel of important epigenetic regulators and compared their expression with the control group.

Epigenetic could inactivate or silent the gene expression. It has been corroborated that the role of epigenetic in the silencing of TSGs in cancer cells is at least as effective as gene mutations[30]. DNA methylation is even thought to be the primary mechanism of silencing TSGs in patients with myelodysplastic syndrome (MDS) and AML[30]. We previously showed that p16^{INK4A} expression significantly reduced in AML patients and its expression pattern had a tight association with the age of the patients. Our recent findings also showed that there was a remarkable and widespread decrease in the expression level of p53 by the mean of 12.5-fold in AML patients as compared with healthy control. DNA methylation is a possible mechanism in the suppression of p16^{INK4A} and p53 genes. In this study, we demonstrated for the first time that akin to that seen in p16^{INK4A}, p53 expression was also significantly reduced with advancing age in AML patients. Declining p53 function in the aging process has been suggested as a possible mechanism for the development of cancer in older populations[31]. Conversely, an augmented activation of many of TSGs, including p53, p21, p16, p27, and p15 have previously been documented in the cells isolated from normal tissues of elder individuals[32]. This feature confers cellular senescence, a process acting as a strict physiological antitumor response to counteract the oncogenic insults in cells with an accumulated oncogenic mutation [32]. Indeed, a recent study demonstrates that non-mutational p53 dysfunction may exist in roughly all AML patients[6]. Overall, the reduction of p53 protein and overexpression of their negative regulators named MDM2/4 have been previously attributed to p53 aberrancy in AML[6].

Our results showed a strong positive correlation with a prominent statistical significance between p16^{INK4A} and p53 expressions in the control group, but not in the AML patients. A study on normal fibroblasts and osteoblasts of mouse organs indicates p53 enforced expression could repress p16^{INK4A} gene expression in normal condition, but not in response to noxious stimuli. The authors have proposed an indirect mechanism for p16^{INK4A} suppression mediated by Ets1 transcription factor activity [33]. Another study showed that p16^{INK4A} up-regulation after exposure of the cells to ultraviolet light is required for efficient p53 over-

expression [34]. So, it could be concluded that same to similarities that exist between p16^{INK4A} and p53 functions, their expressions also could changes in parallel in response to stimulant environmental factors such as genotoxic stresses. The absence of p16^{INK4A} and p53 correlation among AML patients coupled with the downregulation of these genes provokes further investigation to determine if AML cells properly respond to genotoxic stimuli through induction of p16^{INK4A} and p53.

With this background, we were avid for looking for the plausible mechanisms behind the suppression of these critical TSGs in the AML patients with a special focus on histones modifiers. In the present study, of the five epigenetic regulators, four genes represented a grossly altered expression in AML patients. For the epigenetic reader, analyzing the expression level of UHRF2 showed that there was a 20-fold decrease in the expression of this gene as compared to the control group, suggestive of the tumor suppressor property of this gene in the patients. We did not find any significant correlation between UHRF2 expression and TSGs. UHRF2 repression in AML patients is in keeping with this fact that the UHRF2 coding region located at 9p24 is subjected to deletion in a variety of malignancies[35]. Moreover, Lu and colleagues have been indicated that the protein level of UHRF2 significantly reduced or mislocalized in diverse human cancers, in particular AML[36]. In sharp contrast, there are many studies in explanation of the oncogenic role of UHRF2 and its clinical relevance. UHRF2 was uncovered as a breast cancer promoter by suppression of p16, p21, and p27, through inducing DNA methylation and histone modification[37]. Furthermore, UHRF2 overexpression was attributed to poor prognosis in colon cancer, likely through transcriptional induction of ERK1/2 axis [38]. Regarding the various function of UHRF2 in the regulation of cell cycle, epigenetic and apoptosis, it seems that UHRF2 serves either as a tumor promoter or a tumor suppressor in a context-dependent manner[38]. According to the role of UHRF2 in the degradation of DNMTs and genome-wide hypomethylation[39], the down-regulation of this gene in AML patients could be defensible by the evidence of DNA hypermethylation and elevation of DNMTs in patients[40]. The absence of correlation between UHRF2 and TSGs could be explained in part by the impressive reduction of mRNA, which may attenuate their function in AML patients. In keeping with the previous studies, we found a significant negative correlation between the expression of UHRF1 and p53 in AML patients. UHRF1 is frequently activated in various malignancies and plays various oncogenic roles particularly by repression of TSGs[41].

Noteworthy, both p16^{INK4A} and p53 gene expressions have been demonstrated to be influenced by histones modifiers [42, 43]. In this study, we found that two epigenetic writers named PRDM16 and SUV39H1 had significant high transcriptional expressions in 50% and 36% of AML patients respectively. PRDM16 was previously detected to drive a prognostically unfavorable inflammatory signature and also to regulate the expression of cytokines and chemokines, including VEGF, HGF, and TNF in AML patients[44]. Overexpression of PRDM16 has been demonstrated in approximately 30% of AML patients, which was associated with poor prognosis[44]. A great deal of evidence is already in hand that explores the suppressive role of SUV39H1 on TSGs in the AML patients[45]. The synoptic conclusion from these studies could be that SUV39H1 is required for the formation of AML largely through transcriptional repression of p15, p21 and E-cadherin[45]. Moreover, the results of their study also declared that SUV39H1 inhibition could induce differentiation and apoptosis in AML cells by restoring the expression of epigenetically silenced genes[45].

In corroboration with the previous studies[46], our results demonstrated that SUV39H1, which acts as an important H3K9 methyltransferase, adversely correlated with the expression of p16^{INK4A} and p53 gens. PRDM16 overexpression coupled with p53 inactivation could be enough for the development of AML[21]. Interestingly, we found that SUV39H1 may have repressive effects on p53 expression, so, further studies are required to uncover whether parallel overexpression of PRDM16 and SUV39H1 could create similar results.

The mounting body of evidence has shown that both KDM3C and KDM2B have a crucial role in the maintenance and the self-renewal of AML cells through epigenetic regulation of a wide array of genes[19, 24]. Notably, the results of the qRT-PCR analysis revealed a significant upregulation of KDM3C in AML patients, while the expression level of KDM2B in AML patients was similar to the control group. Moreover, we found that the expression level of KDM3C was higher in M3 patients in comparison with other subtypes of AML. Previous reports have suggested that via regulation of IL-3/RAS/JAK pathway and also through cooperation with the HOXA9/MEIS1 axis, KDM3C could exert pro-oncogenic function in AML[47]. On the other hand, in contrast to Oncomine databases, which assigned an elevated expression of KDM2B in acute leukemias, we found a normal expression for this gene in the AML patients[19]. An elevated level of this gene was also attributed to the advanced stages of pancreatic ductal adenocarcinoma [48]. KDM2B has been reported to have a remarkable suppressive effect for p15^{ink4a} and modestly for p16^{ink4a} and p19^{arf} in a murine model of AML[19]. In keeping with this study, we found that KDM2B expression was negatively correlated with both p16^{INK4A} and p53 expressions. The lack of correlation between KDM3C and TSGs in our study could be explained by this fact that KDM3C is mainly involved in erasing the suppressive marks, such as H3K9me3, particularly from promoters of genes involved in metabolic pathways.

We demonstrated that the epigenetic regulators were remarkably perturbed at the level of expression in the majority of AML patients. Distorted expression of some genes was correlated with repression of "guardians of the genome" namely p16^{INK4A} and p53. We also delineated that the expression pattern of some genes is dependent on the age, and maybe involved in a greater tendency of AML occurrence toward advanced ages. Prospectively, our introduced panel consisting of epigenetic reader, writer and eraser enzymes would be promising to recruit by further investigation in the terms of prognosis determination, risk stratification, and monitoring in AML patients.

Declarations

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Declaration:

Ethics approval and consent to participate: All samples were obtained after taking informed consent according to the Declaration of Helsinki. This study was confirmed by ethics committee of Shahid Beheshti University of Medical Sciences (IR.SBMU.RETECH.REC.1396.800).

Consent for publication: not applicable

Availability of data and material: The set of data used and / or analyzed during the present study by the relevant author Available upon reasonable request.

Competing interests: The authors declare that they have no competing interests

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Authors' contributions

All authors contributed to the design of the research. VA, MAF, MM, MR and BG collected the data. VA,MAF, MHM, BG, SS and MM conducted analysis and interpretation of data. All authors drafted the first version. VA,MAF, MHM, BG, SS and MM edited the first draft. All authors reviewed, commented and approved the final draft.

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Tables

Table 1. Real-Time PCR oligonucleotide primers				
Genes	Primers	Sequence	TM	Amplicon size(bp)
CDKN2A	P16 ^{INK4A} .F	GCACCAGAGGCAGTAACCA	59.63	101
	P16 ^{INK4A} .R	AGTTTCCCGAGGTTTCTCAGAG	59.70	
UHRF2	UHRF2.F	GATATGATGGCATTATAAGGTG	53.2	151
	UHRF2.R	AGACATAATCTCCTTGACCG	54.3	
ABL	ABLF	TGGAGATAACACTCTAAGCATAACTAAAG	59.1	124
	ABLR	GATGTAGTTGCTTGGGACCCA	60.0	
CDKN1C	p53.F	AGGCTGTGTTCAAGTGGTTCTGA	62.29	83
	p53.R	ACAAAAATTAGCGGGGCGTGG	62.07	
PRDM16	PRDM16.F	ACGTGAAGGAAGGCGTCTA	58.4	96
	PRDM16.R	ACTGGAAGAGTTCGTCACAC	57.5	
SUV39H1	SUV39H1.F	CGAGTACCTGTGCGATTAC	55.9	134
	SUV39H1.R	GGAAGTCTTGAGGATACG	55.4	
KDM2B	KDM2B	TGTCCCAGTTTGTGCGTTAC	58.7	130
	KDM2B	ACCAGGTCTACCACAGTCG	58.4	
KDM3C	KDM3C	ACAACTCTCTTCAACATCCACTG	58.6	114
	KDM3C	ATCACCCATCTCTTCTGTCTCAC	59.5	

Table 2. Summary of patient's demographic data(n=50)	
Age median, y (Range, y)	35(3-89)
Sex(Male/Female)	21/29
Sample type (Peripheral blood/Bone marrow)	12/38
Blast percent (Range %)	80(20-98)
Immunological Classification (%)	
AML M0-M2	26(52)
AML M3	18(36)
AML M4,M5	6(12)

Table 3. the expression patterns of histones modifiers and TSGs in AML patients compared with control group					
	Underexpression (%)	Normal expression (%)	Upregulation (%)	Fold change	P value
UHRF2	24	76	0	0.05	<0.001*
PRDM16	8	42	50	3.00	0.007*
SUV39H1	0	64	36	10.40	0.004*
KDM2B	0	100	0	0.82	0.617
KDM3C	10	76	14	3.50	0.004*
p53	34	66	0	0.08	<0.001*
p16 ^{INK4A}	16	74	10	3.11	0.352

Table 4. Spearman Correlation of p16 ^{INK4A} and p53 gene expression with epigenetic regulators and also age of the subjects (AML, n=50 and control, n=18)									
			UHRF2	PRDM16	SUV39H1	KDM2B	KDM3C	p53	Age
p16 ^{INK4A}	AML	r	0.212	-0.301	-0.429	-0.683	0.117	0.097	-0.346
		P value	0.183	0.05	0.007*	<0.001*	0.198	0.548	0.018*
	Control	r	0.291	0.295	-0.170	-0.713	0.221	0.813	0.502
		P value	0.241	0.286	0.499	0.003*	0.211	<0.001	0.034*
p53	AML	r	0.228	0.086	-0.443	-0.598	0.228		-0.348
		P value	0.147	0.628	0.006*	<0.001*	0.147		0.019*
	control	r	0.254	0.261	-0.323	-0.574	0.035		-0.071
		P value	0.343	0.348	0.191	<0.005*	0.896		0.794

Figures

Figure 1

The differences between the expression of studied genes among AML subtypes(M0-M2, M3 and M4,M5) and control group. As illustrated in fig 2, KDM3C and p53 significantly overexpressend in M3 patients compared with M0-M2 patients while SUV39H1 higher in M0-M2 patients

Figure 2

The mRNA expression level of studied genes in patients and control groups. Expression level of each genes are demonstrated by dot plots and column graphs. Dot plots represents the mean $\pm$ SD of patients and control groups. The 5 and 95 percentails of control group are depicted by dotted lines. Fold changes of each genes are showed at the top of related coulmnns. The expression levels of SUV39H1, KDM3C and PRDM16 significantly increased, while UHRF2 and p53 profoundly deacreated in AML patients. p16INK4A and KDM2B mRNA expressions were comparable to control groups.

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