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Original Article

MiR-675 downregulation correlates with favorable/intermediate karyotypes in *de novo* acute myeloid leukemia

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Abstract: *MicroRNA-675* (*miR-675*) plays an important role in tumorigenesis and development of various cancers. However, the expression status and the prognostic implication of *miR-675* in acute myeloid leukemia (AML) remain largely elusive. In this study, real-time quantitative PCR was carried out to examine *miR-675* level in 114 *de novo* AML patients and 26 controls. The level of *miR-675* expression was significantly down-regulated in AML patients (P=0.003). No significant differences were observed between *miR-675*^{low} and *miR-675*^{high} cases in clinical parameters including sex, age, white blood cell (WBC) counts, hemoglobin (HB), platelet (PLT) counts, and BM blasts. However, the patients with good/intermediate karyotypes had a higher incidence of *miR-675*^{low} expression as compared to those with poor karyotype (P=0.033). Moreover, *miR-675*^{low} group had an obvious tendency towards a lower frequency of complex karyotype than *miR-675*^{high} group (3% vs 16%, respectively, P=0.057). In regard to the molecular gene mutations, reduced *miR-675* expression was closely associated with *FLT3*-ITD wild type in CN-AML patients (P=0.015) and showed a trend in all AML patients (P=0.084). Unfortunately, our data failed to reveal the prognostic value of *miR-675* expression among all AML and non-M3 AML as well as CN-AML patients. Taken together, our study suggests that *miR-675* down-regulation is a frequent event and is associated with favorable/intermediate karyotypes in *de novo* acute myeloid leukemia.

Keywords: *miR-675*, expression, karyotype, acute myeloid leukemia

Introduction

Acute myeloid leukemia (AML), derived from clonal leukemic stem cells, is a heterozygous group of malignant proliferative diseases and results in a failure of hematopoiesis [1, 2]. Genetic abnormalities, such as somatic gene mutations as well as epigenetic alterations, have been widely concerned in decades [3]. Despite recent advances in the understanding of the mechanisms of leukemogenesis and the identification of markers for molecular-based stratification to risk-adapted therapies, it is still difficult to predict clinical outcome and response to the therapy of AML patients [2, 4]. Therefore, novel approaches are needed for better diagnosis and therapy planning of AML patients.

MicroRNAs (miRNAs) are short noncoding RNAs that regulate gene expression [5]. Numerous evidences demonstrate that miRNAs have critical functions across various biological processes, including cell cycle progression, differentiation, invasion, apoptosis, and immune responses [6-12]. MiRNAs are also functionally implicated into leukemogenesis [13-16], and some of these have prognostic value in the prediction for cancer patients received chemotherapy [17-19].

MiR-675, derived from the first exon of *H19*, is reported to participant in initiation and progression of various cancers, such as breast cancer, gastric cancer, non-small cell lung cancer and so on [20-22]. However, no study has so far been studied for the involvement of *miR-675* in

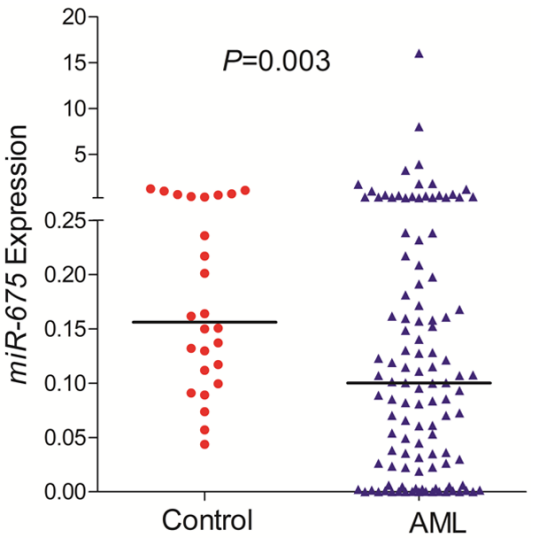

Figure 1. Relative expression levels of *miR-675* in AML patients and controls.

leukemia. Therefore, the present study is intended to investigate the pattern of *miR-675* expression and further analyze its clinical significance in *de novo* AML patients.

Materials and methods

Patients and samples

A total of 114 *de novo* AML patients treated at the Affiliated People's Hospital of Jiangsu University and 26 healthy persons were enrolled in the present study. Bone marrow (BM) was collected after written informed consent was signed. The diagnosis and classification of AML patients were on the basis of the revised French-American-British (FAB) and the 2008 World Health Organization (WHO) criteria [23, 24]. All trials approved by the Institutional Review Board of the Affiliated People's Hospital of Jiangsu University. Karyotypes were analyzed by conventional R-banding method, while karyotype risk was classified according to the reported study [25]. Therapeutic protocols for AML patients were described as reported previously [26].

RNA extraction and reverse transcription

Mononuclear cells from BM specimens (BMNCs) were isolated by Ficoll gradient centrifugation. Total RNA was extracted using Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions, and reverse transcription was carried out subsequently using miScript Reverse Transcription Kit (Qiagen, Duesseldorf, Germany) according to the manufacturer's protocol.

Real-time quantitative PCR

Real-time quantitative PCR (RQ-PCR) reactions were performed according to the manufacturer's instructions using miScript SYBR green PCR kit (Qiagen, Duesseldorf, Germany) on a 7300 Thermo cycler (Applied Biosystems, CA, USA). The specific forward primer of *miR-675* was 5'-GCGGAGAGGGCCCACAG-3'. The RQ-PCR reaction was composed of 1× QuantiTect SYBR Green PCR Master Mix, 1× miScript universal primer, and 1.0 μM of the specific forward primer in a final 20 μL volume. The RQ-PCR was carried out at 95°C for 15 min, 40 cycles at 94°C for 15 s, 55°C for 30 s and 72°C for 34 s, followed by a melting program (95°C for 15 s, 60°C for 60 s, 95°C for 15 s, and 60°C for 15 s) to validate the specificity of the expected PCR product. The relative expression level of *miR-675* was calculated using the following formula: $N_{miR\text{-}675} = (E_{miR\text{-}675})^{\Delta CT\ miR\text{-}675\ (control\text{-}sample)} \div (E_{U6})^{\Delta CT\ U6\ (control\text{-}sample)}$.

Gene mutation detection

NPM1, C-KIT, N/K-RAS, *IDH1/2*, *DNMT3A* and *U2AF1* mutations were detected by high-resolution melting analysis (HRMA) on the LightScanner™ platform (Idaho Technology Inc, Salt Lake City, Utah) as reported previously [27-30]. All positive samples were further sequenced for confirmation. *CEBPA* and *FLT3*-ITD mutations were detected by direct DNA sequencing [27, 31].

Statistical analysis

Statistical analyses of experimental data were implemented on SPSS 20.0 software package (SPSS, Chicago, IL). In order to compare the difference of categorical variables, Pearson Chi-square analysis or Fisher exact test were used. Comparisons between two different groups were determined by the Mann-Whitney's *U* test. Receiver operating characteristic curve (ROC) and area under the ROC curve (AUC) were applied to evaluate the value of *miR-675* expression in discriminating AML patients from normal controls. Kaplan-Meier survival curves were used to determine any significant relation-

Table 1. Comparison of clinical manifestations and laboratory features between *miR-675*^{low} and *miR-675*^{high} patients

Patient's parameters	Status of *miR-675* expression in AML		
	Low (n=38)	High (n=76)	*P*
Sex, male/female	22/16	46/30	0.841
Median age, years (range)	60 (25-87)	55.5 (20-93)	0.112
Median WBC, $\times10^9$/L (range)	7.35 (1.3-136.1)	12.40 (0.3-528.0)	0.723
Median hemoglobin, g/L (range)	74 (40-120)	75 (32-138)	0.748
Median platelets, $\times10^9$/L (range)	38 (4-399)	32 (3-447)	0.557
BM blasts, % (range)	45.5 (5.5-94.5)	46.5 (1.0-97.5)	0.904
FAB			0.047
M0	1 (3%)	0 (0%)	
M1	4 (10%)	4 (5%)	
M2	17 (45%)	39 (51%)	
M3	8 (21%)	9 (12%)	
M4	7 (18%)	15 (20%)	
M5	0 (0%)	9 (12%)	
M6	1 (3%)	0 (0%)	
WHO			0.166
AML with t (8;21)	4 (10%)	12 (16%)	
APL with t (15;17)	8 (21%)	9 (12%)	
AML with 11q23	0 (0%)	1 (1%)	
AML with minimal differentiation	1 (3%)	0 (0%)	
AML without maturation	4 (10%)	4 (5%)	
AML with maturation	13 (34%)	27 (36%)	
Acute myelomonocytic leukemia	7 (19%)	16 (21%)	
Acute monoblastic and monocytic leukemia	0 (0%)	7 (9%)	
Acute erythroid leukemia	1 (3%)	0 (0%)	
Karyotype classification			0.110
Favorable	12 (32%)	21 (28%)	
Intermediate	23 (60%)	40 (52%)	
Poor	1 (3%)	13 (17%)	
No data	2 (5%)	2 (3%)	
Karyotype			0.168
normal	20 (53%)	30 (39%)	
T (8;21)	4 (10%)	12 (16%)	
T (15;17)	8 (21%)	9 (12%)	
11q23	0 (0%)	1 (1%)	
complex	1 (3%)	12 (16%)	
others	3 (8%)	10 (13%)	
No data	2 (5%)	2 (3%)	
Gene mutation*			
CEBPA (+/-)	2/35 (5%)	13/61 (18%)	0.138
NPM1 (+/-)	4/33 (11%)	8/66 (11%)	1.000
FLT3-ITD (+/-)	2/35 (5%)	14/60 (19%)	0.084
c-KIT (+/-)	1/36 (3%)	2/72 (3%)	1.000
N/K RAS (+/-)	1/32 (3%)	7/64 (10%)	0.431
IDH1/2 (+/-)	2/31 (6%)	4/67 (6%)	1.000
DNMT3A (+/-)	4/29 (12%)	5/66 (7%)	0.460

U2AF1 (+/-)	2/31 (6%)	3/68 (4%)	0.651
CR (+/-)	17/18 (49%)	31/43 (42%)	0.541

WBC, white blood cells; FAB, French-American-British classification; AML, acute myeloid leukemia; CR, complete remission; *, percentage was equal to the number of mutated patients divided by total cases in each group.

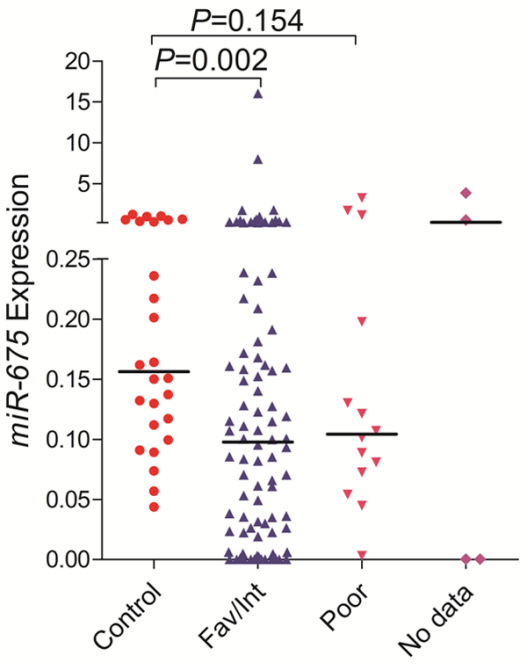

Figure 2. Relative levels of *miR-675* expression in AML patients according to karyotypic risking.

ship between *miR-675* expression and clinical outcome. A two-tailed value of $P<0.05$ was determined as statistically significant.

Results

MiR-675 expression in normal controls and AML patients

The level of *miR-675* ranged from 0.044 to 1.252, with a median level of 0.156 in normal controls, while *miR-675* expression (0.000-16.044, median 0.100) showed a significant down-regulation in whole AML ($P=0.003$, **Figure 1**). And decreased *miR-675* expression was also presented in both non-M3 AML and cytogenetically normal AML (CN-AML) patients ($P=0.005$ and $P=0.001$).

Discriminative capacity of miR-675 expression

ROC curve, conducted to assess the discriminative capacity of *miR-675* expression, suggested that *miR-675* level might serve as a predictor for distinguishing AML from healthy controls (AUC=0.686, 95% CI: 0.592-0.779, $P=0.003$). The cutoff value (0.042) was that point closest to both maximum sensitivity and specificity. Moreover, significant differentiating value was also revealed in non-M3 patients (AUC=0.679, 95% CI: 0.581-0.777, $P=0.005$) and CN-AML patients (AUC=0.722, 95% CI: 0.610-0.834, $P=0.002$).

Association between miR-675 expression and clinical characteristics of AML patients

A cohort of 114 patients with *de no* AML was divided into two groups, namely, the *miR-675* low-expressed (*miR-675*^{low}) group (<0.042) and the *miR-675* high-expressed (*miR-675*^{high}) group (>0.042). Compared with patients with high *miR-675* expression, those with low *miR-675* expression less frequently had FAB-M5 subtypes ($P=0.028$). The patients with good/intermediate karyotypes had a significantly higher incidence of *miR-675*^{low} expression as compared to those with poor karyotype ($P=0.033$, **Table 1**). Moreover, the level of *miR-675* in patients with favorable/intermediate karyotypes (median 0.098) was significantly reduced than those in controls ($P=0.002$), whereas those with poor karyotype (median 0.104) had similar level of *miR-675* expression as controls ($P=0.154$) (**Figure 2**). Apart from these, *miR-675*^{low} group had an obvious tendency towards a lower frequency of complex karyotype than *miR-675*^{high} group (3% vs 16%, respectively, $P=0.057$). Regarding the molecular gene mutations (**Tables 1** and **2**), higher *miR-675* expression was closely associated with *FLT3*-ITD mutation in CN-AML patients ($P=0.015$) and showed a trend in overall AML patients ($P=0.084$). However, no significant differences were observed between the two groups in other clinical parameters including sex, age, white blood cell (WBC) counts, hemoglobin (HB), platelet (PLT) counts, and BM blasts ($P>0.05$, **Table 1**). There was also no significant difference in the distribution of WHO subtypes between the two groups. Additionally, no significant correlations were observed

Table 2. Comparison of gene mutations between *miR-675*^{low} and *miR-675*^{high} in CN-AML patients

Gene mutation*	Status of *miR-675* expression in AML		*P*
	Low (n=20)	High (n=30)	
CEBPA (+/-)	2/18 (10%)	8/22 (27%)	0.279
NPM1 (+/-)	2/18 (10%)	7/23 (23%)	0.285
FLT3-ITD (+/-)	0/20 (0%)	8/22 (27%)	0.015
c-KIT (+/-)	1/19 (5%)	0/30 (0%)	0.400
N/K RAS (+/-)	1/19 (5%)	4/26 (13%)	0.636
IDH1/2 (+/-)	2/18 (10%)	4/26 (13%)	1.000
DNMT3A (+/-)	3/17 (15%)	3/27 (10%)	0.672
U2AF1 (+/-)	1/19 (5%)	2/28 (7%)	1.000

*: percentage was equal to the number of mutated patients divided by total cases in each group.

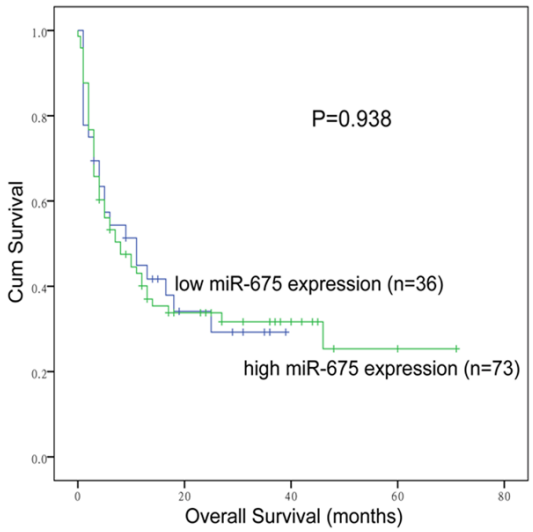

Figure 3. The impact of *miR-675* expression on overall survival of all AML patients.

between *miR-675* expression and other gene mutations (*P*>0.05, **Table 1**).

Correlation between miR-675 expression and clinical outcome

After induction chemotherapy, *miR-675*^{low} patients and *miR-675*^{high} patients had similar complete remission (CR) rate in the whole AML patients (49% vs 42%, *P*=0.541, **Table 1**). The same results were also presented among non-M3 patients (45% vs 35%, *P*=0.491) and CN-AML patients (42% vs 36%, *P*=0.763). Survival analyses were implemented in 109 patients with follow-up data ranged from 0 to 71 months (median 7 months). In parallel, Kaplan-Meier analysis revealed a similar overall survival (OS) time between *miR-675*^{low} and *miR-675*^{high} patients (median 7.5 months vs 7 months, *P*=0.938, **Figure 3**). There were no significant differences among non-M3 patients and CN-AML patients (median 5 vs 5.5 months and 6 vs 5 months, *P*=0.988 and 0.995, respectively). Furthermore, multivariate analysis also failed to demonstrate the prognostic value of *miR-675* expression in AML patients (data not shown).

Discussion

The underlying role of *miR-675* in transcriptional regulation and oncogenesis was increasingly investigated so far. Extensive researches about the aberrant expression of *miR-675* and its function have been reported in a variety of tumors. In gastric cancer, it was demonstrated that *miR-675* upregulation promoted cell proliferation, migration and invasion in vitro, which could also be verified in mice models [21]. Vennin et al reported that overexpression of *H19/miR-675* enhanced the aggressive phenotype of breast cancer cells including increasing cell proliferation and migration in vitro, and accelerating tumor growth and metastasis in vivo [20]. In addition, *miR-675* increased the stability and the activation of *EGFR* and *c-Met*, leading to sustained activation of *Akt* and *Erk* by directly binding *c-Cbl* and *Cbl-b* mRNA [20]. Tsang et al regarded *miR-675* to be a regulator in colorectal cancer development through downregulation of its target gene *RB* [32]. Furthermore, abnormal enhanced *miR-675* expression could increase bladder cancer growth by regulating *p53* activation [33]. Interestingly, Zhu and colleagues unveiled that lncRNA *H19/miR-675* axis repressed prostate cancer metastasis by targeting *TGFBI*, which implicated a potential tumor-suppressive function of *miR-675* [34]. Similarly, Lv et al disclosed that inhibition of lncRNA *H19* and *miR-675* expression promoted migration and invasion of hepatocellular carcinoma cells via *AKT/GSK-3β/Cdc25A* signaling pathway [35]. These results suggested that *miR-675* might play different role during the process of carcinogenesis. However, the pattern of *miR-675* expression in leukemia is rarely studied.

The original aim of the current study was to explore the *miR-675* expression pattern and

further analyze the clinical significance of *miR-675* expression in *de novo* AML patients. This study showed for the first time that *miR-675* transcript was frequently down-regulated in AML, and indicated *miR-675* to be a potential biomarker for discriminating all AML, non-M3 AML and CN-AML patients from healthy controls. Moreover, our data demonstrated that *miR-675* expression was significantly decreased in patients with favorable/intermediate karyotypes especially in complex karyotype. Unexpectedly, our data failed to elaborate the prognostic role of *miR-675* in AML through Kaplan-Meier analysis. These results indicated that the function of *miR-675* during the process of leukemogenesis was dependent on the context of different cytogenetics. Accordingly, experimental studies are urgently needed to determine the specific mechanism of *miR-675* in leukemogenesis.

To the best of our knowledge, *FLT3*-ITD mutation is a frequent event and is found to be an independent prognostic factor in AML [36-38]. Mutations in *FLT3* induced constitutive activation of *FLT3* and activated multiple signaling pathways and induced leukemic transformation [39, 40]. To date, increasing researches have linked *FLT3* mutation to several microRNAs. Whitman et al expounded their idea that an *FLT3*-ITD-associated microRNA-expression signature consisted of overexpressed *miR-155* and *miR-125b-2* and underexpressed *miR-144* and *miR-451* [41]. Salemi and the team concluded that activating mutation of *FLT3* in AML could lead to an increased expression of *miR-155*, which then caused down-regulation of *SPI1* and *CEBPB* and consequently resulted in blocking of myeloid differentiation [42]. Apart from these, Ramiro et al reported an upregulation in AML patients with *FLT3*-ITD mutation than *FLT3* wild type patients or normal controls, suggesting that *miR-155* was strongly but independently associated with *FLT3*-ITD mutation [43]. Our study displayed that low *miR-675* expression was also associated with *FLT3*-ITD wild type in all AML and CN-AML patients. However, the mutual regulation between *miR-675* and *FLT3*-ITD remains unclear. Consequently, prospective trials with independent cohort will be needed to explain the underlying association.

In summary, our study suggests that *miR-675* down-regulation is a frequent event and reduced *miR-675* expression is associated with favorable/intermediate karyotypes in *de novo* AML patients.

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Disclosure of conflict of interest

None.

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References

[1] Smith M, Barnett M, Bassan R, Gatta G, Tondini C, Kern W. Adult acute myeloid leukaemia. Crit Rev Oncol Hematol 2004; 50: 197-222.

[2] Estey E, Döhner H. Acute myeloid leukaemia. Lancet 2006; 368: 1894-1907.

[3] Chen J, Odenike O, Rowley JD. Leukaemogenesis: more than mutant genes. Nat Rev Cancer 2010; 10: 23-36.

[4] Estey EH. Acute myeloid leukemia: 2014 update on risk-stratification and management. Am J Hematol 2014; 89: 1063-1081.

[5] Yan W, Xu L, Sun Z, Lin Y, Zhang W, Chen J, Hu S, Shen B. MicroRNA biomarker identification for pediatric acute myeloid leukemia based on a novel bioinformatics model. Oncotarget 2015; 6: 26424-26436.

[6] Lu J, Getz G, Miska EA, Alvarez-Saavedra E, Lamb J, Peck D, Sweet-Cordero A, Ebert BL, Mak RH, Ferrando AA, Downing JR, Jacks T, Horvitz HR, Golub TR. MicroRNA expression profiles classify human cancers. Nature 2005; 435: 834-838.

[7] Chen T. The role of MicroRNA in chemical carcinogenesis. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev 2010; 28: 89-124.
[8] Ufkin ML, Peterson S, Yang X, Driscoll H, Duarte C, Sathyanarayana P. MiR-125a regulates cell cycle, proliferation, and apoptosis by targeting the ErbB pathway in acute myeloid leukemia. Leuk Res 2014; 38: 402-410.
[9] Wang XS, Gong JN, Yu J, Wang F, Zhang XH, Yin XL, Tan ZQ, Luo ZM, Yang GH, Shen C, Zhang JW. MicroRNA-29a and microRNA-142-3p are regulators of myeloid differentiation and acute myeloid leukemia. Blood 2012; 119: 4992-5004.
[10] Zhu J, Feng Y, Ke Z, Yang Z, Zhou J, Huang X, Wang L. Down-regulation of miR-183 promotes migration and invasion of osteosarcoma by targeting Ezrin. Am J Pathol 2012; 180: 2440-2451.
[11] Carleton M, Cleary MA, Linsley PS. MicroRNAs and cell cycle regulation. Cell Cycle 2007; 6: 2127-2132.
[12] Lindsay MA. MicroRNAs and the immune response. Trends Immunol 2008; 29: 343-351.
[13] Pulikkan JA, Dengler V, Peramangalam PS, Peer Zada AA, Muller-Tidow C, Bohlander SK, Tenen DG, Behre G. Cell-cycle regulator E2F1 and microRNA-223 comprise an autoregulatory negative feedback loop in acute myeloid leukemia. Blood 2010; 115: 1768-1778.
[14] Marcucci G, Radmacher MD, Maharry K, Mrózek K, Ruppert AS, Paschka P, Vukosavljevic T, Whitman SP, Baldus CD, Langer C, Liu CG, Carroll AJ, Powell BL, Garzon R, Croce CM, Kolitz JE, Caligiuri MA, Larson RA, Bloomfield CD. MicroRNA expression in cytogenetically normal acute myeloid leukemia. N Engl J Med 2008; 358: 1919-1928.
[15] Pulikkan JA, Dengler V, Peramangalam PS, Peer Zada AA, Müller-Tidow C, Bohlander SK, Tenen DG, Behre G. Cell-cycle regulator E2F1 and microRNA-223 comprise an autoregulatory negative feedback loop in acute myeloid leukemia. Blood 2010; 115: 1768-1778.
[16] Nowek K, Sun SM, Dijkstra MK, Bullinger L, Döhner H, Erkeland SJ, Löwenberg B, Jongen-Lavrencic M. Expression of a passenger miR-9* predicts favorable outcome in adults with acute myeloid leukemia less than 60 years of age. Leukemia 2016; 30: 303-9.
[17] Marcucci G, Maharry KS, Metzeler KH, Volinia S, Wu YZ, Mrózek K, Nicolet D, Kohlschmidt J, Whitman SP, Mendler JH, Schwind S, Becker H, Eisfeld AK, Carroll AJ, Powell BL, Kolitz JE, Garzon R, Caligiuri MA, Stone RM, Bloomfield CD. Clinical role of microRNAs in cytogenetically normal acute myeloid leukemia: miR-155 upregulation independently identifies high-risk patients. J Clin Oncol 2013; 31: 2086-2093.
[18] Butrym A, Rybka J, Baczyńska D, Tukiendorf A, Kuliczkowski K, Mazur G. Low expression of microRNA-204 (miR-204) is associated with poor clinical outcome of acute myeloid leukemia (AML) patients. J Exp Clin Cancer Res 2015; 34: 68.
[19] Yan W, Xu L, Sun Z, Lin Y, Zhang W, Chen J, Hu S, Shen B. MicroRNA biomarker identification for pediatric acute myeloid leukemia based on a novel bioinformatics model. Oncotarget 2015; 6: 26424-26436.
[20] Vennin C, Spruyt N, Dahmani F, Julien S, Bertucci F, Finetti P, Chassat T, Bourette RP, Le Bourhis X, Adriaenssens E. H19 non coding RNA-derived miR-675 enhances tumorigenesis and metastasis of breast cancer cells by downregulating c-Cbl and Cbl-b. Oncotarget 2015; 6: 29209-29223.
[21] Li H, Yu B, Li J, Su L, Yan M, Zhu Z, Liu B. Overexpression of lncRNA H19 enhances carcinogenesis and metastasis of gastric cancer. Oncotarget 2014; 5: 2318-2329.
[22] He D, Wang J, Zhang C, Shan B, Deng X, Li B, Zhou Y, Chen W, Hong J, Gao Y, Chen Z, Duan C. Down-regulation of miR-675-5p contributes to tumor progression and development by targeting pro-tumorigenic GPR55 in non-small cell lung cancer. Mol Cancer 2015; 14: 73.
[23] Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DA, Gralnick HR, Sultan C. Proposed revised criteria for the classification of acute myeloid leukaemia. A report of the French-American-British Cooperative Group. Ann Intern Med 1985; 103: 620-625.
[24] In: Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, editers. WHO classification of tumours of haematopoietic and lymphoid tissues. Lyon, France: IARC Press; 2008.
[25] Li Y, Lin J, Yang J, Qian J, Qian W, Yao DM, Deng ZQ, Liu Q, Chen XX, Xie D, An C, Tang CY. Overexpressed let-7a-3 is associated with poor outcome in acute myeloid leukemia. Leuk Res 2013; 37: 1642-1647.
[26] Lin J, Yao DM, Qian J, Chen Q, Qian W, Li Y, Yang J, Wang CZ, Chai HY, Qian Z, Xiao GF, Xu WR. Recurrent DNMT3A R882 mutations in Chinese patients with acute myeloid leukemia and myelodysplastic syndrome. PLoS One 2011; 6: e26906.
[27] Wen XM, Lin J, Yang J, Yao DM, Deng ZQ, Tang CY, Xiao GF, Yang L, Ma JC, Hu JB, Qian W, Qian J. Double CEBPA mutations are prognostically favorable in non-M3 acute myeloid leukemia patients with wild-type NPM1 and FLT3-ITD. Int J Clin Exp Pathol 2014; 7: 6832-6840.
[28] Yang X, Qian J, Sun A, Lin J, Xiao G, Yin J, Chen S, Wu D. RAS mutation analysis in a large cohort of Chinese patients with acute myeloid leukemia. Clin Biochem 2013; 46: 579-583.

[29] Lin J, Yao DM, Qian J, Chen Q, Qian W, Li Y, Yang J, Wang CZ, Chai HY, Qian Z, Xiao GF, Xu WR. IDH1 and IDH2 mutation analysis in Chinese patients with acute myeloid leukemia and myelodysplastic syndrome. Ann Hematol 2012; 91: 519-525.

[30] Qian J, Yao DM, Lin J, Qian W, Wang CZ, Chai HY, Yang J, Li Y, Deng ZQ, Ma JC, Chen XX. U2AF1 Mutations in Chinese patients with acute myeloid leukemia and myelodysplastic syndrome. PLoS One 2012; 7: e45760.

[31] Wen XM, Hu JB, Yang J, Qian W, Yao DM, Deng ZQ, Zhang YY, Zhu XW, Guo H, Lin J, Qian J. CEBPA methylation and mutation in myelodysplastic syndrome. Med Oncol 2015; 32: 605.

[32] Tsang WP, Ng EK, Ng SS, Jin H, Yu J, Sung JJ, Kwok TT. Oncofetal H19-derived miR-675 regulates tumor suppressor RB in human colorectal cancer. Carcinogenesis 2010; 31: 350-358.

[33] Liu C, Chen Z, Fang J, Xu A, Zhang W, Wang Z. H19-derived miR-675 contributes to bladder cancer cell proliferation by regulating p53 activation. Tumour Biol 2015; [Epub ahead of print].

[34] Zhu M, Chen Q, Liu X, Sun Q, Zhao X, Deng R, Wang Y, Huang J, Xu M, Yan J, Yu J. LncRNA H19/miR-675 axis represses prostate cancer metastasis by targeting TGFBI. FEBS J 2014; 281: 3766-3775.

[35] Lv J, Ma L, Chen XL, Huang XH, Wang Q. Downregulation of LncRNAH19 and MiR-675 promotes migration and invasion of human hepatocellular carcinoma cells through AKT/GSK-3β/Cdc25A signaling pathway. J Huazhong Univ Sci Technolog Med Sci 2014; 34: 363-369.

[36] Badar T, Patel KP, Thompson PA, DiNardo C, Takahashi K, Cabrero M, Borthakur G, Cortes J, Konopleva M, Kadia T, Bohannan Z, Pierce S, Jabbour EJ, Ravandi F, Daver N, Luthra R, Kantarjian H, Garcia-Manero G. Detectable FLT3-ITD or RAS mutation at the time of transformation from MDS to AML predicts for very poor outcomes. Leuk Res 2015; 39: 1367-74.

[37] Garg M, Nagata Y, Kanojia D, Mayakonda A, Yoshida K, Haridas Keloth S, Zang ZJ, Okuno Y, Shiraishi Y, Chiba K, Tanaka H, Miyano S, Ding LW, Alpermann T, Sun QY, Lin DC, Chien W, Madan V, Liu LZ, Tan KT, Sampath A, Venkatesan S, Inokuchi K, Wakita S, Yamaguchi H, Chng WJ, Kham SK, Yeoh AE, Sanada M, Schiller J, Kreuzer KA, Kornblau SM, Kantarjian HM, Haferlach T, Lill M, Kuo MC, Shih LY, Blau IW, Blau O, Yang H, Ogawa S, Koeffler HP. Profiling of somatic mutations of acute myeloid leukemia, FLT3-ITD subgroup at diagnosis and relapse. Blood 2015; 126: 2491-501.

[38] Zuffa E, Franchini E, Papayannidis C, Baldazzi C, Simonetti G, Testoni N, Abbenante MC, Paolini S, Sartor C, Parisi S, Marconi G, Cattina F, Bochicchio MT, Venturi C, Ottaviani E, Cavo M, Martinelli G. Revealing very small FLT3-ITD mutated clones by ultra-deep sequencing analysis has important clinical implications in AML patients. Oncotarget 2015; 6: 31284-31294.

[39] Kim KT, Baird K, Ahn JY, Meltzer P, Lilly M, Levis M, Small D. Pim-1 is up-regulated by constitutively activated FLT3 and plays a role in FLT3-mediated cell survival. Blood 2005; 105: 1759-1767.

[40] Kim KT, Baird K, Davis S, Piloto O, Levis M, Li L, Chen P, Meltzer P, Small D. Constitutive Fms-like tyrosine kinase 3 activation results in specific changes in gene expression in myeloid leukaemic cells. Br J Haematol 2007; 138: 603-615.

[41] Whitman SP, Maharry K, Radmacher MD, Becker H, Mrózek K, Margeson D, Holland KB, Wu YZ, Schwind S, Metzeler KH, Wen J, Baer MR, Powell BL, Carter TH, Kolitz JE, Wetzler M, Moore JO, Stone RM, Carroll AJ, Larson RA, Caligiuri MA, Marcucci G, Bloomfield CD. FLT3 internal tandem duplication associates with adverse outcome and gene- and microRNA-expression signatures in patients 60 years of age or older with primary cytogenetically normal acute myeloid leukemia: a Cancer and Leukemia Group B study. Blood 2010; 116: 3622-3626.

[42] Salemi D, Cammarata G, Agueli C, Augugliaro L, Corrado C, Bica MG, Raimondo S, Marfia A, Randazzo V, Dragotto P, Di Raimondo F, Alessandro R, Fabbiano F, Santoro A. MiR-155 regulative network in FLT3 mutated acute myeloid leukemia. Leuk Res 2015; 39: 883-896.

[43] Garzon R, Garofalo M, Martelli MP, Briesewitz R, Wang L, Fernandez-Cymering C, Volinia S, Liu CG, Schnittger S, Haferlach T, Liso A, Diverio D, Mancini M, Meloni G, Foa R, Martelli MF, Mecucci C, Croce CM, Falini B. Distinctive microRNA signature of acute myeloid leukemia bearing cytoplasmic mutated nucleophosmin. Proc Natl Acad Sci U S A 2008; 105: 3945-3950.