Int J Hematol (2014) 99:169–174
DOI 10.1007/s12185-013-1495-5

ORIGINAL ARTICLE

RUNX1 mutation associated with clonal evolution in relapsed pediatric acute myeloid leukemia with t(16;21)(p11;q22)

Olfat Ismael · Akira Shimada · Shaimaa Elmahdi · Momen Elshazley · Hideki Muramatsu · Asahito Hama · Yoshiyuki Takahashi · Miho Yamada · Yuka Yamashita · Keizo Horide · Seiji Kojima

Received: 23 October 2012 / Revised: 9 December 2013 / Accepted: 10 December 2013 / Published online: 28 December 2013

Abstract *TLS/FUS-ERG* chimeric fusion transcript resulting from translocation changes involving chromosomes 16 and 21 is a rare genetic event associated with acute myeloid leukemia (AML). The distinct t(16;21) AML subtype exhibits unique clinical and morphological features and is associated with poor prognosis and a high relapse rate; however, the underlying mechanism remains to be clarified. Recently, whole-genome sequencing revealed a large set of genetic alterations that may be relevant for the dynamic clonal evolution and relapse pathogenesis of AML. Here, we report three pediatric AML patients with t(16;21) (p11; q22). The *TLS/FUS-ERG* fusion transcript was detected in all diagnostic and relapsed samples, with the exception of one relapsed sample. We searched for several genetic lesions, such as *RUNX1*, *FLT3*, *c-KIT*, *NRAS*, *KRAS*, *TP53*, *CBL*, *ASXL1*, *IDH1/2*, and *DNMT3A*, in primary and relapsed AML samples. Interestingly, we found *RUNX1* mutation in relapsed sample of one patient in whom cytogenetic analysis showed the emergence of a new additional clone. Otherwise, there were no genetic alterations in *FLT3*, *c-KIT*, *NRAS*, *KRAS*, *TP53*, *CBL*, *ASXL1*, *IDH1/2*, or *DNMT3A*. Our results suggest that precedent genetic alterations may be essential to drive the progression and relapse of t(16;21)-AML patients.

Keywords *RUNX1* · AML · Translocation · *TLS/FUS-ERG*

Introduction

Translocation t(16;21) (p11;q22) is a rare reciprocal chromosomal change observed in Ewing's sarcoma [1], blast crisis of chronic myelocytic leukemia (CML) [2] and acute myeloid leukemia (AML) [3–5]. This translocation leads to the formation of *TLS/FUS-ERG* fusion gene, resulting in *TLS/FUS-ERG* chimeric protein [6]. The incidence of t(16;21) AML is estimated to be 1 % of all de novo and

O. Imael, S. Elmahdi contributed equally to this work.

O. Ismael (✉) · A. Shimada · S. Elmahdi · H. Muramatsu · A. Hama · Y. Takahashi · S. Kojima (✉)
Department of Pediatrics, Nagoya University Graduate School of Medicine, 65 Tsuruma-cho, Showa-ku, Nagoya, Aichi 466-8550, Japan
e-mail: olfat_80@yahoo.com

S. Kojima
e-mail: kojimas@med.nagoya-u.ac.jp

O. Ismael
Department of Pediatrics, EL-Hilal Hospital, General Authority for Health Insurance, Sohag, Egypt

A. Shimada · M. Yamada · Y. Yamashita · K. Horide
Clinical Research Center, National Hospital Organization, Nagoya Medical Center, Nagoya, Aichi, Japan

A. Shimada
Department of Pediatrics, Okayama University Hospital, Okayama, Japan

M. Elshazley
Department of Respiratory Medicine, Nagoya University Graduate School of Medicine, Nagoya, Aichi, Japan

M. Elshazley
Industrial Medicine and Occupational Diseases Unit, Faculty of Medicine, Sohag University, Sohag, Egypt

secondary AML cases [7]. The chromosomal translocation t(16;21)(p11;q22) has been diagnosed in adult and childhood AML with a wide range of age (1–60 years) and with no sex predilection [6]. AML with t(16;21) is a unique cytogenetic subtype that showed distinct clinical and hematological characteristics. Unfavorable prognosis and poor survival are considered the most prominent features of t(16;21) AML [7], while hemophagocytosis, micromegakaryocytes, increases of eosinophils in bone marrow (BM), and vacuolation of leukemic cells are frequently detected in t(16;21) AML [6, 7]. Treatment of AML with t(16;21)(p11;q22) represents significant therapeutic challenges with high relapse rate. Furthermore, post-relapse treatment outcomes remain unsatisfactory for this group of patients [6, 7]. Recently, it has been shown that CD56, a neural cell adhesion molecule, is frequently expressed in adult t(16;21)-AML patients and it could be a useful indicator for poor prognosis, failure of complete remission (CR) and extramedullary involvement [7]. Although some prognostic factors have been reported in AML patients with t(16;21) (p11;q22), the underlying mechanism of poor prognosis and high relapse rate in those patients is not yet fully understood. Thus, identification of new biological markers is urgently needed and would be useful to establish a new therapeutic strategy for t(16;21)-AML patients who are at extremely high risk of relapse. A subset of recurrent genetic alterations has been found in AML with potential clinical implications [8–12]; therefore, these genetic mutations are expected to hold prognostic importance for AML patients. More recently, genome-wide analysis using next generation sequencing have suggested two molecular models involved in the relapse pathogenesis of AML. First, the original clone in primary tumor is susceptible to gain new mutations and evolves into the relapse clone. Second, development of a subclone from the original clone, surviving initial therapy, acquires additional mutations and expands at relapse [13]. To investigate the genetic changes associated with primary and relapsed samples, we performed comprehensive genetic, clinical and morphological assessments for three pediatric cases diagnosed as having de novo AML with t(16;21)(p11;q22) to delineate new molecular features and better understand the mechanism of relapse for this unique cytogenetic subtype of AML.

Patients and samples

Three patients satisfying the diagnostic criteria for AML (according to the WHO classification) were included in this study. Morphological diagnosis was made according to the French–American–British (FAB) morphologic classification. Cytogenetic analysis was performed for all patients at diagnosis and after relapse. Peripheral blood (PB) and/or BM samples were collected from all patients at diagnosis, during CR or after relapse and mononuclear cell fractions were isolated using a Ficoll gradient. RNA and cDNA were prepared following the manufacturer's instructions (Roche Diagnostics, Mannheim, Germany) and Genomic DNA was isolated using the QIAmp DNA blood mini kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Informed consent was obtained from the guardians of all patients, in accordance with institutional guidelines, and study protocols were approved by the Institutional Review Boards of Nagoya University Graduate School of Medicine.

Cytogenetics analysis

Karyotyping of blood or BM cells from all patients was done by routine G-banding karyotype analysis.

Detection of TLS/FUS-ERG fusion transcript by RT-PCR

Reverse transcriptase polymerase chain reaction (RT-PCR) and real-time PCR were performed to detect *TLS/FUS-ERG* fusion transcript. In addition, sensitivity was evaluated as described previously [6].

Flow cytometric analysis

We performed detailed surface marker analysis including anti-CD13, CD10, CD33, CD34, CD15, CD41, CD38, CD56, cytoplasmic myeloperoxidase (MPO), and HLA-DR. (Positive and negative controls were examined along with samples. Positivity was defined as involving more than 20 % of the cells among the blast gate).

Mutation analysis of FLT3, c-KIT, NRAS, KRAS, RUNX1, TP53, CBL, TET2, ASXL1, IDH1/2 and DNMT3A

We investigated the following candidate genes at known mutational hot spots: *FLT3*, *c-KIT*, *IDH1/2*, *DNMT3A*, *NRAS* (exons 2 and 3), *KRAS* (exons 2 and 3), *TET2* (exons 3–11), *RUNX1* (exons 3–8), *ASXL1* (exon 12), *CBL* (exons 7, 8, 9), and the complete coding region for *TP53* were searched by sequencing analysis after PCR amplification of genomic DNA. PCR amplification and purification were performed in a 25-μl PCR mix containing at least 50 ng of template cDNA using quick Taq PCRTM HS Dye mix (Qiagen) under the following conditions: 94 °C for 2 min (first denaturing step); 94 °C for 30 s; 65 °C for 30 s; 35 cycles of 68 °C for 30 s to 1 min, depending on PCR product length; and 68 °C for 7 min (final extension step). PCR products were purified from the reaction mixture using the QIAquick PCR purification kit (Qiagen), and

were directly sequenced on a DNA sequencer (ABI PRISM 3100 Genetic Analyzer; Applied Biosystems, Japan) using a Big Dye terminator cycle sequencing kit (Applied Biosystems). All DNA sequence abnormalities were confirmed by three independent experiments.

Results

Clinical and hematological features

Three de novo AML patients (two females, one male) with a median age at onset of 2 years (range 2–10 years) were included. The main clinical and hematologic features of all patients are summarized in Table 1. The median white blood cell counts, percent of PB and BM blast, were 2.7 × 10^9/l (range 1.9–39.2 × 10^9/l), 53 % (range 23–62 %) and 57 % (range 9.3–73 %), respectively. Karyotypes of patient Nos. 1 and 2 at the onset were t(16;21)(p11;q22) with additional abnormalities observed in patient No. 2 in the form of 46, XY, t(16;21)(p11;q22), add(11)(q13), del(13)(q12q14). Patient No. 3 did not show the t(16;21)(p11;q22) and showed various additional chromosomal abnormalities (Table 2). Histopathologic examination of BM showed leukemic cells with hemophagocytosis (erythrophagocytosis and megakaryophagocytosis) and vacuolation (Fig. 1). Among three AML patients with t(16;21), two showed positivity for CD56, CD13+, 33+, 15+, 34+, MPO+, HLA-DR+, while the other patient expressed only CD13+, 33+, HLA-DR+. CD56-positive cell percentage was 73.3 and 33 % in the diagnostic samples, while at the relapsed one was 66 and 63 % in patient Nos. 2 and 3, respectively.

Table 1 Clinical features of 3 pediatrics patients with *TLS/FUS-ERG* chimera

Patient no.	Sex/ Age (Y)	WBC (x10^9/l)	FAB classification	CD56	DFS (months)	1st HSCT	2nd HSCT	Overall survival (months)	Survival outcome	Cause of death
1	F/2	39.2	M1	–	15	At 2nd CR, from HLA-mismatched mother	–	>121	Alive	–
2	M/2	2.7	M7	+	12	At 1st CR, from unrelated HLA-matched donor	At 1st relapse, from HLA-mismatched mother	27	Death	Relapse
3	F/10	1.9	M1 with MLD	+	10	At 2nd CR, from HLA-matched sibling	At 2nd relapse, from HLA-mismatched mother	45	Death	Hepatitis

MLD multilineage dysplasia, *DFS* disease-free survival, *HSCT* hematopoietic stem cell transplantation, *CR* complete remission

Table 2 Detailed cytogenetic analysis and mutations profile of 3 pediatrics patients with *TLS/FUS-ERG* chimera

ID	Disease stage	Karyotype	*TLS/FUS- ERG* chimera (copies/μgRNA)	Transcript type	Molecular alterations	
					RUNX1	Others
1	At diagnosis	46,XX,t(16;21)(p11;q22)[8]	497,814	B, C	–	–
2		46,XY,t(16;21)(p11;q22)[8]/46, idem, add(11)(q13), del(13)(q12q14) [6]	6,960	B	–	–
3		45,XX,der(1;17), +8[2]	1,237,000	B	–	–
1	At relapse	46,XX,t(16;21)(p11;q22)[14]	28,304	B, C	–	–
2		46,XY,t(16;21)(p11;q22),add(1) (q12), add(2)(p13),add(3)(q21), add(8)(p21), der (8),t(8;11) (q11.2;q11), −11, −15, add(15)(q24)[18]	0	–	+	–
3		47,X,t(X;3)(p22;p12), add(1)(p34),der(1)ad(1)add (1)(q21),add(7)(q32),−8, t(8;11)(q24;q13),add(12) (q24.1),add(17)(q21), +mar1[18]	132,168	B	–	–

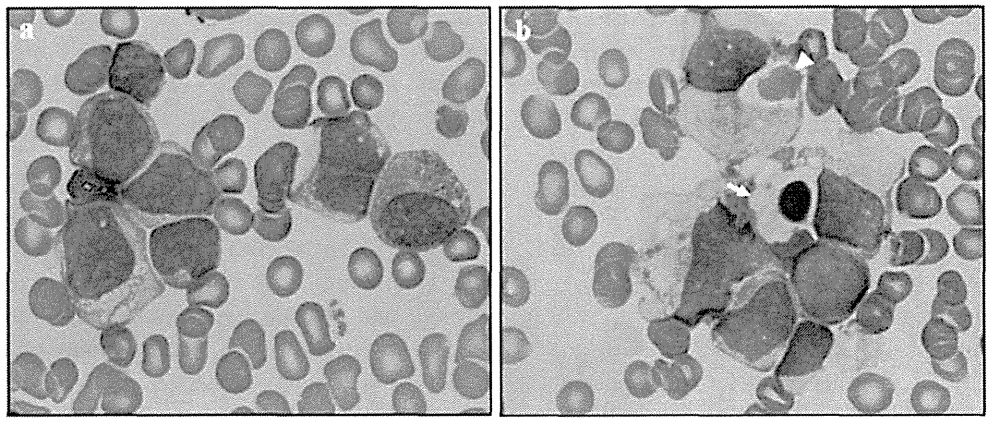

Fig. 1 Morphologic examination of leukemic cells obtained from bone marrow aspirates of t(16;21)-AML-CD56-positive pediatric patients. **a** Leukemic cells with cytoplasmic vacuolation. **b** Leukemic cells with erythrophagocytosis (*white arrow*) and megakaryocyte phagocytosis (*white arrow head*)

TLS/FUS-ERG fusion transcript

We detected the chimeric fusion *TLS/FUS-ERG* transcripts in all cases at the onset of diagnosis (patient 1 had type B and C fusion transcripts, patients 2 and 3 had type B fusion transcript). However, in patient 2, the band of fusion transcripts was absent at the relapse (Table 2). A serial tenfold dilution of cDNA of patients' samples was used to detect the chimeric transcripts; type B and C transcripts were found in very low-diluted solutions (10^{-6}).

Mutation profiling of pediatric patients with t(16;21) AML

We identified 203-bp (149_351) deletion that led to a frameshift mutation at codon 137 in the runt domain of *AML1/RUNX1* at relapse in RNA sample obtained from BM of patient No. 2 (Fig. 2a). Interestingly, missense point mutation in *TET2* (R835C) was detected in a diagnostic, complete remission and relapsed BM samples obtained from patient No. 3 (Fig. 2b), but 3 out of 50 healthy Japanese control were found to harbor this mutation, indicating that *TET2* (R835C) mutation could be encountered as single-nucleotide polymorphism (SNP). All t(16;21)-AML patients were negative for *FLT3*, *c-KIT*, *NRAS*, *KRAS*, *TP53*, *CBL*, *ASXL1*, *IDH1/2* and *DNMT3A* genetic alterations.

Therapy and clinical outcome

Patient Nos. 1, 2, 3 received AML-oriented chemotherapy (ECM regimen; VP16, AraC and Idarubicin), but they relapsed after 15, 12 and 10 months respectively from the onset of the disease. Patient No. 1 underwent HLA-haploidentical hematopoietic stem cell transplantation

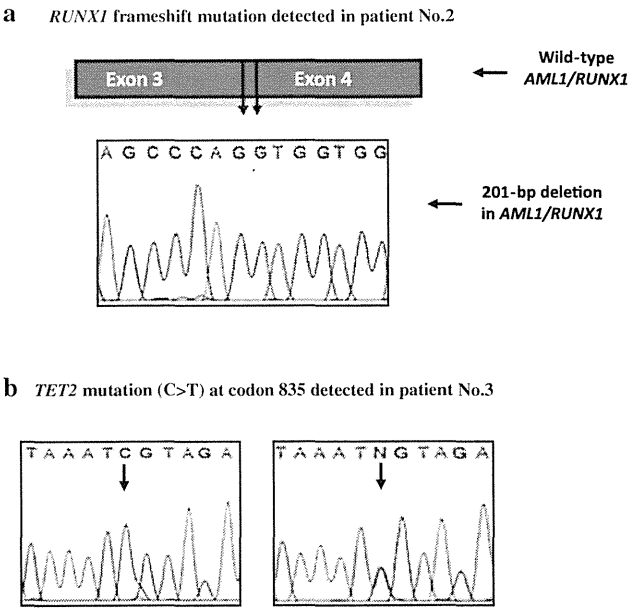

Fig. 2 *RUNX1* and *TET2* alterations detected in pediatric patients with t(16;21)(p11;q22). **a** Sequence data for *AML1/RUNX1* gene in patient No. 2. Nucleotide sequences showing wild-type exon 3 and 4 boundary of *AML1/RUNX1* in cDNA and 201-bp deletion from exon 3 resulting in a frameshift mutation. **b** Sequence traces showing *TET2* exon 3, C to T mutation at codon 835 in patient No. 3

(HSCT) from her mother during the 2nd complete remission (CR) (25 months after the 1st CR) and she is still alive. Patient No. 2 received HSCT from HLA-matched unrelated donor at 5 months after the 1st CR, but he relapsed and received the 2nd HSCT from HLA 3 loci-mismatched mother without induction chemotherapy. He achieved 2nd CR after HSCT but experienced 2nd relapse 12 months later and died soon due to the disease progression. Patient No. 3 received HSCT from HLA-matched related sibling at 6 months after the 2nd CR.

However, she relapsed 4 months after the 1st HSCT and underwent 2nd HSCT from HLA 1 locus-mismatched mother. She developed fulminant hepatitis (Caused by adenovirus) and died 4 months after the 2nd HSCT. Notably, two patients had poor prognosis with over all survival (OS) 27 and 45 months and the other patient is still alive (Table 1).

Discussion

Several studies demonstrated the clinical importance of genetics in prognostification of AML patients and this depends mainly upon the use of cytogenetic to categorize patients into favorable, intermediate, or adverse cytogenetic groups, in addition to identification of new molecular genetic abnormalities. We would like to emphasize the importance of cytogenetic analysis as well as genetic assessment in predicting the clinical outcome of AML patients. Herein, we report three pediatric AML patients with *TLS/FUS-ERG* fusion transcript. Although t(16;21)(p11;q22) karyotype was found in two out of three patients, chimeric fusion *TLS/FUS-ERG* transcript was detected in all cases at the onset of diagnosis and in two cases at the relapse phase, suggesting that detection of *TLS/FUS-ERG* chimeric transcript by RT-PCR has the upper hand in identification and monitoring of t(16;21)-AML patients. On the other hand, it is quite possible that absence of *TLS/FUS*-ERG fusion transcript at relapse phase in patients No. 2 may be attributed to RNA degradation eliminating the *TLS/FUS-ERG* transcripts. In line with our results, Kong et al. [6] demonstrated that RT-PCR analysis for detecting *TLS/FUS-ERG* chimeric fusion transcript is an important monitoring tool for t(16;21)-AML patients during various clinical stages. Remarkably, *RUNX1* mutation was detected in relapsed sample of one patient in whom cytogenetic analysis showed the emergence of a new additional clone, while *TET2* SNP was found at diagnosis, during complete remission (CR) and after relapse. Our study extends the findings of a recent study [13] showed that genetic alterations could contribute to clonal evolution and relapse pathogenesis. It has been shown that *RUNX1* mutations are frequently associated with AML disease [14–16] and particular t(16;21)-AML patients [7]. Auewarakul et al. [16] further demonstrated that a higher frequency of *RUNX1* mutation was found in southeast Asian t(16;21)-AML patients. Accumulated knowledge with strong evidence supports the integral and important role of *TET2* mutations in myeloid diseases [17, 18]. Approximately, 17 % of AML patients have been reported to carry *TET2* mutations [19], however, the prognostic impact of *TET2* mutation in AML remains enigmatic. Fathi et al. showed that there was no association between *TET2* mutations and patients' survival [19]. Conversely, other investigators have documented that *TET2* mutations were associated with low rate of complete remission, decrease overall survival and worse clinical outcome in patients with AML diagnosis [20]. In our study, immunophenotypic analysis of leukemic cells in t(16;21)-AML pediatric patients revealed positive expression for CD13, CD15, CD34, CD33, CD56, MPO, HLA-DR. Specifically, CD56 was strongly expressed in patients with decrease OS, but the small patients' number makes it difficult to draw a firm conclusion of this possible association. These findings are similar to previous reports that showed variable degree of surface markers expression in t(16;21)-AML patients [21]. Jekarl et al. [7] clearly described that high CD56 expression confer an unfavorable prognosis in AML patients with t(16; 21)(p11;q22). In conclusion, our findings lend significant credence for the use of CD56 as a prognostic marker for AML patients with t(16;21)(p11;q22). Further investigations are required to define precisely clonal evolutions and mutational changes that may be relevant for relapse pathogenesis of t(16;21) AML.

Conflict of interest The authors declare no conflict of interest.

References

1. Shing DC, McMullan DJ, Roberts P, Smith K, Chin SF, Nicholson J, Tillman RM, Ramani P, Cullinane C, Coleman N. FUS/ERG gene fusions in Ewing's tumors. Cancer Res. 2003;63:4568–76.
2. Ferro MR, Cabello P, Garcia-Sagredo JM, Resino M, San Roman C, Larana JG. t(16;21) in a Ph positive CML. Cancer Genet Cytogenet. 1992;60:210–1.
3. Mecucci C, Bosly A, Michaux JL, Broeckaert-Van Orshoven A, Van den Berghe H. Acute nonlymphoblastic leukemia with bone marrow eosinophilia and structural anomaly of chromosome 16. Cancer Genet Cytogenet. 1985;17:359–63.
4. Morgan R, Riske CB, Meloni A, Ries CA, Johnson CH, Lemons RS, Sandberg AA. t(16;21)(p11.2;q22): a recurrent primary rearrangement in ANLL. Cancer Genet Cytogenet. 1991;53:83–90.
5. Yao E, Sadamori N, Nakamura H, Sasagawa I, Itoyama T, Ichimaru M, Tagawa M, Nakamura I, Kamei T. Translocation t(16;21) in acute nonlymphocytic leukemia with abnormal eosinophils. Cancer Genet Cytogenet. 1988;36:221–3.
6. Kong XT, Ida K, Ichikawa H, Shimizu K, Ohki M, Maseki N, Kaneko Y, Sako M, Kobayashi Y, Tojou A, Miura I, Kakuda H, Funabiki T, Horibe K, Hamaguchi H, Akiyama Y, Bessho F, Yanagisawa M, Hayashi Y. Consistent detection of TLS/FUS-ERG chimeric transcripts in acute myeloid leukemia with t(16;21)(p11;q22) and identification of a novel transcript. Blood. 1997;90:1192–9.
7. Jekarl DW, Kim M, Lim J, Kim Y, Han K, Lee AW, Kim HJ, Min WS. CD56 antigen expression and hemophagocytosis of leukemic cells in acute myeloid leukemia with t(16;21)(p11;q22). Int J Hematol. 2010;92:306–13.
8. Rocquain J, Carbuccia N, Trouplin V, Raynaud S, Murati A, Nezri M, Tadrist Z, Olschwang S, Vey N, Birnbaum D, Gelsi-Boyer V, Mozziconacci MJ. Combined mutations of ASXL1, CBL, FLT3, IDH1, IDH2, JAK2, KRAS, NPM1, NRAS, RUNX1, TET2 and WT1 genes in myelodysplastic syndromes and acute myeloid leukemias. BMC Cancer. 2010;10:401.

9. Marcucci G, Metzeler KH, Schwind S, Becker H, Maharry K, Mrozek K, Radmacher MD, Kohlschmidt J, Nicolet D, Whitman SP, Wu YZ, Powell BL, Carter TH, Kolitz JE, Wetzler M, Carroll AJ, Baer MR, Moore JO, Caligiuri MA, Larson RA, Bloomfield CD. Age-related prognostic impact of different types of DNMT3A mutations in adults with primary cytogenetically normal acute myeloid leukemia. J Clin Oncol. 2012;30(7): 742–50.
10. Ruan M, Wang YQ, Zhang L, Liu TF, Liu F, Liu XM, Zhang JY, Zou Y, Chen YM. Zhu XF [FLT3 mutations in children with acute myeloid leukemia: a single center study]. Zhongguo Dang Dai Er Ke Za Zhi. 2011;13:863–6.
11. Metzeler KH, Becker H, Maharry K, Radmacher MD, Kohlschmidt J, Mrozek K, Nicolet D, Whitman SP, Wu YZ, Schwind S, Powell BL, Carter TH, Wetzler M, Moore JO, Kolitz JE, Baer MR, Carroll AJ, Larson RA, Caligiuri MA, Marcucci G, Bloomfield CD. ASXL1 mutations identify a high-risk subgroup of older patients with primary cytogenetically normal AML within the ELN Favorable genetic category. Blood. 2011;118:6920–9.
12. Ahmad EI, Gawish HH, Al Azizi NM, Elhefni AM. The prognostic impact of K-RAS mutations in adult acute myeloid leukemia patients treated with high-dose cytarabine. Onco Targets Ther. 2011;4:115–21.
13. Ding L, Ley TJ, Larson DE, Miller CA, Koboldt DC, Welch JS, Ritchey JK, Young MA, Lamprecht T, McLellan MD, McMichael JF, Wallis JW, Lu C, Shen D, Harris CC, Dooling DJ, Fulton RS, Fulton LL, Chen K, Schmidt H, Kalicki-Veizer J, Magrini VJ, Cook L, McGrath SD, Vickery TL, Wendl MC, Heath S, Watson MA, Link DC, Tomasson MH, Shannon WD, Payton JE, Kulkarni S, Westervelt P, Walter MJ, Graubert TA, Mardis ER, Wilson RK, DiPersio JF. Clonal evolution in relapsed acute myeloid leukaemia revealed by whole-genome sequencing. Nature. 2012;481:506–10.
14. Roumier C, Fenaux P, Lafage M, Imbert M, Eclache V, Preudhomme C. New mechanisms of AML1 gene alteration in hematological malignancies. Leukemia. 2003;17:9–16.
15. Tang JL, Hou HA, Chen CY, Liu CY, Chou WC, Tseng MH, Huang CF, Lee FY, Liu MC, Yao M, Huang SY, Ko BS, Hsu SC, Wu SJ, Tsay W, Chen YC, Lin LI, Tien HF. AML1/RUNX1 mutations in 470 adult patients with de novo acute myeloid leukemia: prognostic implication and interaction with other gene alterations. Blood. 2009;114:5352–61.
16. Auewarakul CU, Leecharendkeat A, Tocharoentanaphol C, Promsuwicha O, Sritana N, Thongnoppakhun W. AML1 mutation and its coexistence with different transcription factor gene families in de novo acute myeloid leukemia (AML): redundancy or synergism. Haematologica. 2007;92:861–2.
17. Li Z, Cai X, Cai CL, Wang J, Zhang W, Petersen BE, Yang FC, Xu M. Deletion of Tet2 in mice leads to dysregulated hematopoietic stem cells and subsequent development of myeloid malignancies. Blood. 2011;118:4509–18.
18. Klaus M, Psaraki A, Mastrodemou S, Pyrovolaki K, Mavroudi I, Kalpadakis C, Papadaki HA. Evaluation of TET2 deletions in myeloid disorders: a fluorescence in situ hybridization analysis of 109 cases. Leuk Res. 2011;35:413–5.
19. Fathi AT, Abdel-Wahab O. Mutations in epigenetic modifiers in myeloid malignancies and the prospect of novel epigenetic-targeted therapy. Adv Hematol. 2012;2012:469592.
20. Metzeler KH, Maharry K, Radmacher MD, Mrozek K, Margeson D, Becker H, Curfman J, Holland KB, Schwind S, Whitman SP, Wu YZ, Blum W, Powell BL, Carter TH, Wetzler M, Moore JO, Kolitz JE, Baer MR, Carroll AJ, Larson RA, Caligiuri MA, Marcucci G, Bloomfield CD. TET2 mutations improve the new European LeukemiaNet risk classification of acute myeloid leukemia: a Cancer and Leukemia Group B study. J Clin Oncol. 2011;29:1373–81.
21. Shikami M, Miwa H, Nishii K, Takahashi T, Shiku H, Tsutani H, Oka K, Hamaguchi H, Kyo T, Tanaka K, Kamada N, Kita K. Myeloid differentiation antigen and cytokine receptor expression on acute myelocytic leukaemia cells with t(16;21)(p11;q22): frequent expression of CD56 and interleukin-2 receptor alpha chain. Br J Haematol. 1999;105:711–9.