Experimental and Molecular Pathology 115 (2020) 104440

Contents lists available at ScienceDirect

Experimental and Molecular Pathology

journal homepage: www.elsevier.com/locate/yexmp

RUNX1 variant as a genetic predisposition factor for acute myeloid leukemia

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ARTICLE INFO

Keywords:
AML
RUNX1
BAALC
Polymorphism
PCR-RFLP

ABSTRACT

Acute myeloid leukemia (AML) is the most common hematological malignancy among adults and is characterized by accumulation of immature myeloid cells. Different genetic factors have role in the occurrence of AML. Among different proteins, *RUNX1* and *BAALC* are involved in the development AML. It has been shown that BAALC overexpression is a factor that indicate shorter disease free survival in a subset of AML patients. RUNX1 has been implicated in the development of breast, prostate, lung, and skin cancers. The aim of this study is determination of the prevalence of common polymorphisms in *BAALC* (rs6999622 and rs62527607) and *RUNX1* (rs13051066 and rs61750222) in AML patients compared with healthy subjects. A total of 100 AML patients and 100 healthy control subjects were included in our study. Genomic DNA was isolated from peripheral blood and the polymorphisms were genotyped by applying ARMS and PCR-RFLP methods. Finally, data was analyzed using SPPSS software. Our results demonstrate a significant association between the *RUNX1* rs13051066 and AML in the co-dominant (odd ratio = 6.66, 95% Cl = 1.85–25, p = .006) and dominant (GT + TT versus GG: odd ratio = 6.15, 95% CI = 1.73–21.87, p = .002) models. The *RUNX1* rs13051066 polymorphism is associated with risk of AML in Iranian population. Future studies should consider larger sample size for assessment of *RUNX1* gene polymorphisms, and employ cytogenetic and molecular analyses in AML patients from different ethnic origins.

1. Introduction

Acute myeloid leukemia (AML) is a clinically and biologically heterogeneous disease with the clonal expansion of undifferentiated myeloid precursors (leukemic cells). The disorder usually leads to early death of older patients. Tiredness, anorexia, leukocytosis, signs of bone marrow failure such as anemia and thrombocytopenia are the clinical manifestations of AML that are seen in most patients (De Kouchkovsky and Abdul-Hay, 2016; Rubnitz et al., 2008). This disorder is categorized into different groups according to the type of lineage and degree of differentiation of blood cells (Lagunas-Rangel et al., 2017). The bone marrow in AML patients is disturbed and produces blood cells with inappropriate shape and function (Papaemmanuil et al., 2016; Heydt et al., 2018). Recent reports have shown that AML accounts for one-third of the all leukemia. Overall, 80% of AML occurs in adults above 60 years and the remaining occurs in children with the annual incidence of 5–7 per 100,000 (Rubnitz Rubnitz, 2012, Lagunas-Rangel et al., 2017). In most populations, men are more at risk than women (Tamamyan et al., 2017). Apart from genetic mutations, some factors such as chemicals, smoking, obesity and various diseases including Bloom and Down syndromes influence the risk of AML (Rubnitz et al., 2008; Lagunas-Rangel et al., 2017; Tamamyan et al., 2017; Mazraeh et al., 2020; Ketab et al., 2020). The European Leukemia Net have designed the prognostic model for AML and listed a group of risk-

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https://doi.org/10.1016/j.yexmp.2020.104440
Received 24 March 2020; Received in revised form 8 April 2020; Accepted 10 April 2020
Available online 12 April 2020
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associated genes including *CEBPA*, *HOXA9*, *TET2*, *WT1*, *NPM1*, *FLP3*, *CEBPA*, *FLT3–ITD*, *RUNX1*, *ASXL1* and *TP53* which have roles in early diagnosis and treatment of the disease (Antar et al., 2017; Sayad et al., 2017).

The human gene, *BAALC* has been introduced as a new marker for primary hematopoietic progenitor cells (Kühnl et al., 2010; Xu et al., 2012). It is located on chromosome 8 (8q22.3) between two *ATP6* and *FZD6* genes (Tanner et al., 2001). *BAALC* has 8 exons with various isoforms. Two isoforms (1–8) and (1–6-8) are highly conserved in mammals but other orthologues genes have been found in other organisms (Wang et al., 2005). This gene is expressed in CD34+ hematopoietic stem cells and neuronal tissues located in central nervous system (CNS) (Tanner et al., 2001). But high level of *BAALC* expression has been observed in AML patients with trisomy for chromosome 8 (Weber et al., 2014). The exact role of BAALC on cellular mechanism is not clear and it is assumed that it participates in cellular movement, adhesion and regulation of AML-related cells differentiation (Tanner et al., 2001; Xiao et al., 2015). It seems that over-expression of BAALC in AML and acute lymphoblastic leukemia (ALL) is related to undesirable prognosis of the illness (Lucena-Araujo et al., 2017; Azizi et al., 2015; Sayad et al., 2018; Ghafouri-Fard et al., 2020). Notably, *BAALC* gene contains two non-coding single nucleotide polymorphisms (SNPs) (rs6999622 and rs62527607) that are shown to influence expression of the encoded transcript. In a study in AML patients, it was demonstrated that patients having at least one copy of the T allele of either SNP express higher levels of *BAALC* gene. Moreover, the T allele of the rs62527607 produces a binding site for the transcription factor RUNX1(Eisfeld et al., 2012b).

RUNX1, as a transcription factor, is essential for normal hematopoietic development (Greif et al., 2012; Recouvreux et al., 2016). At first, it was discovered in AML linages with t(8:21) translocation (Tsai et al., 2015; Hayashi et al., 2017). This transcription factor is involved in apoptosis, cell cycle regulation, development of nociceptive neurons and immune response. The *RUNX1* gene is located on chromosome 21 and has two domains: RHD and TAD. Several studies have reported different types of mutations in TAD domain of this gene in patients with leukemia (Ganly et al., 2004; De Braekeleer et al., 2009; De Braekeleer et al., 2011). Current evidence suggests that loss of *RUNX1* is associated with many cancers. This gene has a role in suppressing aggressiveness particularly in the early stage of tumorigenesis. Nowadays, wide spread research is in progress for determining the role of *RUNX1* as tumor suppressor and novel marker in AML therapies. Furthermore, RUNX1 participates in the regulation of target genes that are needed for proper development in many tissues and cell lineages including bone, hair follicles, cartilage, blood, and mammary glands. RHD is one of the important domain of RUNX1 that forms heterodimer with CBFβ and binds to DNA suggesting *RUNX1* protein involvement in controlling the expression of genes such as *MPO*, *IL3*, *TCRB*, *BLK* and *FLT3* that are essential for hematopoiesis mechanism (Schnittger et al., 2011; Tsai et al., 2015). *RUNX1* has a number of SNPs which might affect its expression or function. Among these SNPs are rs13051066 and rs61750222 which are located at 3' UTR and coding region, respectively. Thus, they might affect expression or activity of the encoded protein. The role of *BAALC* rs13051066 and rs61750222 polymorphisms has not been verified in human disorders. Although the role of *BAALC* variants in AML has been assessed in other populations (Eisfeld et al., 2012a), there was no data regarding its role in Iranian patients. Based on the important roles of RUNX1 and BAALC in the pathophysiology of AML, we selected the different SNPs from the respective genes to assess their association with AML. In this study, we determined association between *RUNX1* (rs6999622 and rs62527607) and *BAALC* (rs13051066 and rs61750222) polymorphisms, and AML.

2. Materials and methods

2.1. Study population

Our study included 100 unrelated AML patients and 100 healthy people with the average age of 64 and 58, respectively. As there was no data about the allele frequencies of the mentioned polymorphisms in Iranian population, the present study was a pilot study in this regard. All participants were consecutively recruited from Shahid Ghazi Hospital and Tabriz Children's Hospital (between January 2017 and May 2018) after signing written informed consents. Controls were selected from volunteers referred for routine check up to the same hospital. They were matched in ethnicity, gender, geographical location and age with cases. The study was approved by the Medical Research Ethics Committee at Tabriz University of Medical Sciences. Demographics and clinicopathological data of participants were obtained from medical records.

2.2. DNA extraction and molecular genotyping

Five ml of peripheral blood samples were obtained from each participant and stored in EDTA tubes at 4 °C for DNA extraction. Genomic DNA was extracted from peripheral blood samples using DNA mini extract kit (Geneall, Korea) according to the manufacturer's protocol. DNA was stored in −20 °C freezer until the genotype analysis was performed. The four SNPs including the rs13051066, rs61750222 of *RUNX1* and the rs62527607 and rs6999622 of *BAALC* were genotyped using specific primers designed during the study using primer3 software (http//primer3.ut.ee).

Rs13051066 and rs61750222 polymorphisms of *RUNX1* were assessed by using RFLP-PCR (Restriction Fragment Length Polymorphism) method. Two primers pair which were used for evaluating these mutations are given in Table 1. The PCR-RFLP assay was performed by using 25 μL PCR reaction containing 12.5 μL Taq DNA Polymerase 2× Master Mix RED (2 mM MgCl), 10 pmol of each primer, 8.5 μL ddH_2O, 1 μL DMSO, 100 ng of DNA template. The optimum PCR condition were as follows: 95 °C for 7 min for cycle preparation, followed by 35 cycle of denaturation at 95 °C for 30 s, annealing at 56 °C for 30 s, polymerization at 72 °C for 30 s, and final extension at 72 °C for 5 min. For result visualization, 5 μL of PCR products were loaded along with 100 bp DNA marker in 2% agarose gel. PCR product of rs13051066 was digested with *Cfr42*I (*Sac*II) restriction enzyme (Thermo Scientific, United States) for 16 h in 37 °C. On the 2% agarose gel, two band were observed: 282 bp fragment for normal, 550 bp fragment for mutant and double bands 282 bp and 550 bp for heterozygotes variants. *BstX*I (Thermo Scientific, United States) restriction enzyme was used for detection of rs61750222 polymorphism. Reactions were incubated in 55 °C for 16 h. Three fragments were produced: 205 bp and 164 bp for normal, 369 for mutant and three bands for heterozygotes.

Table 1
Data of primer sequences, PCR product size and enzyme activation temperature.

SNP ID	Primer set and sequences	PCR product size	Enzyme	Enzyme activation temperature
rs13051066	F: 5′GAATCTTCCTGTTTGCTTTCCAG-3′ R: 5′-CCCTCCTACCACCTGTACTAC-3′	550	SacII	37
rs61750222	F: 5′-CTGACCTACAGCGAGATCCT-3′ R: 5′-CTCCTACCAGTTCTCCATGG-3′	369	BstXI	55

Table 2
PCR primers used in the tetra primer ARMS PCR for BAALC polymorphisms detection.

SNP ID	Primer set and sequences	Tm(°c)	Product size (bp)
rs62527607	Outer1F: 5′TGATCAGTGGACAGATGCAAGC 3′	54.8	364
	Outer1R: 5′ GCGACAAGAAAGACTCCACCC 3′	56.3	364
	Inner1F: 5′ CCTTGTCCAAAGCTTGCTCG 3′(G allele)	53.8	263
	Inner1R: 5′ ACCGGTCTCCCGACCAGA 3′ (T allele)	54.9	138
rs6999622	Outer2F: 5′ TCTGTTTCCTGTGCTCACCA 3′	51.8	456
	Outer2R: 5′ GTCTCCTCCTCCTCATGCTG 3′	55.9	456
	Inner2F: 5′ GCACTCCCTCCACCTTACAC 3′ (C allele)	55.9	143
	Inner2R: 5′ CAGGACTCTTCAACCTTGTTTTAGA 3′ (T allele)	54.4	357

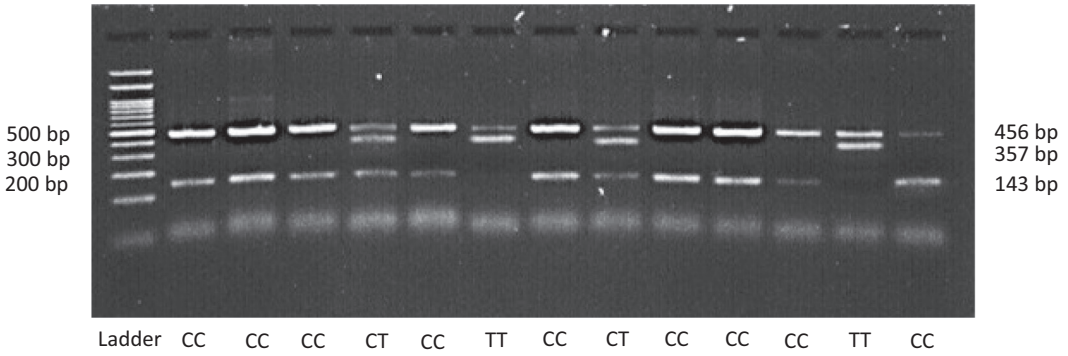

Fig. 1. Tetra-primer amplification refractory mutation system (ARMS)-PCR analysis of rs6999622.

Table 3
The clinical and demographic data of all study participants.

	Cases (n = 100)	Healthy controls (n = 100)
Sex		
Female, n (%)	42	44
Male, n (%)	58	56
WBC count, × 109/L (median, range)	9.2(0.11–665)	–
Platelet count, × 109/L /L (median, range)	61 (5–1117)	–
FAB subtype		
AML M0	15	
AML M1	22	
AML M2	36	
AML M4	14	
AML M5	4	
AML M6	8	
AML M7	1	
Cytogenetics available		
Normal karyotype, n (%)	23	
Trisomy 8, n (%)	18	
Trisomy 11, n (%)	12	
Trisomy 13, n (%)	13	
Trisomy 21, n (%)	7	
Other trisomies, n (%)	3	
−7/del(7q), n (%)	8	
del(5q), n (%)	2	
del(9q), n (%)	3	
del(20q), n (%)	2	
Other deletions, n (%)	2	
Others or combinations of 2 of above aberrations, n (%)	7	

The genotypes of the *BAALC* polymorphisms (rs62527607 and rs6999622) were determined by the tetra-primer amplification refractory mutation system (ARMS)-PCR method. Primers and products size for ARMS PCR were summarized in Table 2. PCR was performed in a total volume of 25 μL containing 7.5 μL ddH2O, 12.5 μL Taq DNA Polymerase 2× Master Mix RED (2 mM MgCl), 100 ng of DNA template, 1 μL of each outer primers and 10 pmol of each primer. PCR amplification was carried out at 95 °C for 7 min, followed by denaturation at 95 °C for 30 s, annealing for rs62527607 SNP at 65 °C for 30 s and for rs6999622 SNP at 62 °C for 30 s (30 cycle) and extension at 72 °C for 30 s, followed by a final extension at 72 °C for 5 min. The PCR products were electrophoresed on 2% agarose gel. Three fragments were seen for the rs62527607 SNPs: 364 bp and 263 bp for normal homozygotes; 364 bp and 138 bp for mutant and all three bands for heterozygotes. In rs6999622 polymorphism, normal individuals showed 456 bp and 143 bp bands and mutant individuals had 456 bp and 357 bp bands while heterozygotes showed all three fragments that are displayed in Fig. 1.

All PCR reactions had negative controls containing no DNA template. Enzyme digestions were performed with both positive and negative controls. Experiments were executed by a technician in a blinded manner i.e. samples had codes and disease status of samples were not obvious.

2.3. Statistical analyses

SPSS software version 18 (statistical package for social sciences Inc., Chicago, IL, USA) was used to analyze the data. The frequencies of the alleles and genotypes were compared using Chi-square (χ^2) test. Odds ratio (OR) was used to establish association between each genotype and AML in four inheritance models (co-dominant, dominant, recessive and over-dominant). *P* values were corrected for multiple comparisons using Bonferroni correction test. *P*-values less than 0.05 were considered as statistically significance.

3. Results

The general and clinical features of the participants in this project are presented in Table 3. The genotype frequencies revealed accordance with Hardy Weinberg equilibrium for all polymorphisms except for the rs13051066 and rs61750222 SNPs. The allelic and genotypic frequencies of the SNPs among patient and control groups are given in Table 4.

We investigated SNPs in different inheritance models. Our results

Table 4
Hardy Weinberg equilibrium statistics.

rs13051066		GG	GT	TT	P-value of HWE
	All subjects	19(0.1)	125(62%)	56(28%)	< 0.0001
	Patients	3(3%)	69(69%)	28(28%)	< 0.0001
	Controls	16(16%)	56(56%)	28(28%)	0.22
rs61750222		GG	GC	CC	P-value of HWE
	All subjects	49(24%)	118(59%)	33(16%)	0.01
	Patients	19(19%)	63(63%)	18(18%)	0.016
	Controls	30(30%)	55(55%)	15(15%)	0.31
rs62527607		GG	GT	TT	P-value of HWE
	All subjects	152(76%)	43(22%)	5(2%)	0.35
	Patients	74(74%)	23(23%)	3(3%)	0.42
	Controls	78(78%)	20(20%)	2(2%)	0.63
rs6999622		CC	CT	TT	P-value of HWE
	All subjects	173(86%)	25(12%)	2(1%)	0.27
	Patients	85(85%)	14(14%)	1(1%)	0.48
	Controls	88(88%)	11(11%)	1(1%)	0.34

demonstrate a significant association between the *RUNX1* rs13051066 and AML in the co-dominant (odd ratio = 6.66, 95% Cl = 1.85–25, p = .006) and dominant (GT + TT versus GG: odd ratio = 6.15, 95% CI = 1.73–21.87, p = .002) models (Table 5).

4. Discussion

Recent advances in cancer genome analysis revealed that AML is a complex hematological disease characterized by genetic and clinical heterogeneity (Lagunas-Rangel et al., 2017). Diverse genetic and non-genetic factors take part in the disease initiation and progression (Tamamyan et al., 2017; De Kouchkovsky and Abdul-Hay, 2016). Previous studies have uncovered alterations in dozens of new genes or pathways in AML patients. In the present study, *RUNX1* and *BAALC* genes were selected to be genotyped in AML patients. *RUNX1* acts as the key component in regulation of hematopoiesis in human cells and has a critical role in controlling cell cycle, ribosomal function and signaling pathways via interaction with *P53* and *TGFβ* (Sood et al., 2017). Considering the role of diverse *RUNX1*-associated transcription factors, this gene can have tumor activator or suppressor effects (De Braekeleer et al., 2011). The role of *RUNX1* in the growth and survival of leukemia cells has been mentioned in some studies (Hayashi et al., 2017).

BAALC is detected in hematopoietic precursor cells and CD34+ bone marrow cells, yet its role remained undefined. Some studies have pointed out the possible role of BAALC in cytoskeleton network as well as in cell synapses. The latest reports proposed *BAALC* collaboration with *HOXA9* in stopping the differentiation of myeloid cells and promoting leukemia (Soliman et al., 2016; Damiani et al., 2013). High level of BAALC expression was seen in malignancies such as melanoma and ALL in association with *SP1*, *WT1*, *CEBPA* and *RUNX1* proteins (Eisfeld et al., 2012a).

This study was set out with the aim of assessing the importance of two *RUNX1* polymorphisms (rs13051066 and rs61750222) and two *BAALC* polymorphisms (rs62527607 and rs6999622) in AML. The rs6999622 [CT] and rs62527607 [GT] are located in the promoter region of *BAALC*. The rs13051066 [GT] and rs61750222 [GC] are located in 3′UTR region and exon 6 of *RUNX1*, respectively. We compared genotype frequencies of these polymorphisms between AML patients and healthy individuals in four inheritance models. The outcome revealed that rs13051066 had significant difference in co-dominant (OR = 6.66, 95% Cl = 1.85–25, p = .006) and dominant (OR = 6.15, 95% CI = 1.73–21.87, p = .002) states. We found that this

Table 5
Association study of rs13051066, rs61750222, rs62527607, rs6999622 polymorphisms and AML.

SNPs	Models	Genotypes	Frequency in AML group, number (%)	Frequency in control group, number (%)	Odds ratio (95% confidence intervals)	P value
rs13051066	Co-dominant	GG	3(3%)	16(16%)	1.00	0.006
		GT	69(69%)	56(56%)	6.57(1.82–23.69)	
		TT	28(28%)	28(28%)	5.33(1.39–20.36)	
	Dominant	GG	3(3%)	16(16%)	6.15(1.73–21.87)	0.002
		GT + TT	97(97%)	84(84%)		
	Recessive	GG + GT	72(72%)	72(72%)	1(0.54–1.85)	1
		TT	28(28%)	28(28%)		
	Over dominant	GG + TT	31(31%)	44(44%)	1.75(0.98–3.12)	0.057
		GT	69(69%)	56(56%)		
rs61750222	Co-dominant	GG	19(19%)	30(30%)	1.00	0.19
		GC	63(63%)	55(55%)	1.81(0.91–3.57)	
		CC	18(18%)	15(15%)	1.88(0.77–4.54)	
	Dominant	GG	19(19%)	30(30%)	0.55(0.28–1.06)	0.07
		GC + CC	81(81%)	70(70%)		
	Recessive	GG + GC	82(82%)	85(85%)	0.80(0.38–1.70)	0.57
		CC	18(18%)	15(15%)		
	Over dominant	GG + CC	37(37%)	45(45%)	0.72(0.41–1.26)	0.25
		GC	63(63%)	55(55%)		
rs62527607	Co-dominant	GG	74(74%)	78(78%)	1.00	0.77
		GT	23(23%)	20(20%)	0.82(0.42–1.63)	
		TT	3(3%)	2(2%)	0.63(0.10–3.89)	
	Dominant	GG	74(74%)	78(78%)	0.80(0.42–1.54)	0.51
		GT + TT	26(26%)	22(22%)		
	Recessive	GG + GT	97(97%)	98(98%)	0.66(0.11–4.04)	0.65
		TT	3(3%)	2(2%)		
	Over dominant	GG + TT	77(77%)	80(80%)	0.84(0.43–1.65)	0.61
		GT	23(23%)	20(20%)		
rs6999622	Co-dominant	CC	85(85%)	88(88%)	1.00	0.81
		CT	14(14%)	11(11%)	0.76(0.33–1.77)	
		TT	1(1%)	1(1%)	0.97(0.06–15.69)	
	Dominant	CC	85(85%)	88(88%)	0.77(0.34–1.75)	0.53
		CT + TT	15(15%)	12(12%)		
	Recessive	CC + CT	99(99%)	99(99%)	1.00(0.06–16.21)	1
		TT	1(1%)	1(1%)		
	Over dominant	CC + TT	86(86%)	89(89%)	0.76(0.33–1.76)	0.52
		CT	14(14%)	11(11%)		

polymorphism probably plays a role in AML disease. Considering the location of this SNP in 3' UTR of the *RUNX1* gene, this SNP might affect expression level or activity of this gene or alter binding of regulatory factors. Future studies are needed to unravel the underlying mechanism of the observed association.

Interestingly, we did not find significant difference in other polymorphisms. A previous study has analyzed rs62527607 genotypes in 129 ALL and 16 AML patients (Nadimi et al., 2016). Authors have suggested a strong link between this polymorphism and the risk recurrence of ALL. Moreover, they found this polymorphism as a negative predictor in ALL. However, there was not any link between the risk of relapse of AML and this polymorphism. Furthermore, another study investigated the function of the rs6999622 and rs62527607 polymorphisms of the *BAALC* gene and found that T allele of rs62527607 induces a binding site for the *RUNX1* transcription factor which elevate BAALC expression level (Eisfeld et al., 2012a).

Our study is the first study to investigate the relationship between rs13051066/rs61750222 polymorphisms of *RUNX1* gene along with *BAALC* SNPs rs62527607/rs6999622 in AML patients in northwestern Iran. Considering the fact that three of the selected polymorphisms are in regulatory regions, nucleotide changes might affect expression or configuration of the encoded proteins.

We suggest conduction of similar studies with higher statistical power in different populations and geographic locations for approving our results. Considering our hypothesis, it is now possible to suggest that by using dynamic technologies such as CD34+ cell culture and in vitro assessment of function of mentioned SNPs might facilitate identification of molecular markers for AML risk assessment.

In conclusion, the *RUNX1* rs13051066 polymorphism is a possible risk factor for AML. Future studies should consider larger sample size for assessment of *RUNX1* gene polymorphisms, and employ cytogenetic and molecular analyses in AML patients from different ethnic origins.

Authors statement

MT, MR and SGF wrote the draft and revised it. JG and SD analyzed the data. MJ designed the study. SH, MH and SAM perfumed the experiment. SAB, FNG, KAR and AH collected the samples and data. All authors contributed equally and fully aware of submission.

Acknowledgements

This study was financially supported by Shahid Beheshti University of Medical Sciences.

References

Antar, A., Otrock, Z., El-Cheikh, J., Kharfan-Dabaja, M., Battipaglia, G., Mahfouz, R., Mohty, M., Bazarbachi, A., 2017. Inhibition of FLT3 in AML: a focus on sorafenib. Bone Marrow Transplant. 52, 344.

Azizi, Z., Rahgozar, S., Moafi, A., Dabaghi, M., Nadimi, M., 2015. mRNA overexpression of BAALC: a novel prognostic factor for pediatric acute lymphoblastic leukemia. Biomed. Rep. 3, 371–374.

Damiani, D., Tiribelli, M., Franzoni, A., Michelutti, A., Fabbro, D., Cavallin, M., Toffoletti, E., Simeone, E., Fanin, R., Damante, G., 2013. BAALC overexpression retains its negative prognostic role across all cytogenetic risk groups in acute myeloid leukemia patients. Am. J. Hematol. 88, 848–852.

De Braekeleer, E., Ferec, C., De Braekeleer, M., 2009. RUNX1 translocations in malignant hemopathies. Anticancer Res. 29, 1031–1037.

De Braekeleer, E., Douet-Guilbert, N., Morel, F., Le Bris, M.-J., Férec, C., de Braekeleer, M., 2011. RUNX1 translocations and fusion genes in malignant hemopathies. Future Oncol. 7, 77–91.

de Kouchkovsky, I., Abdul-Hay, M., 2016. Acute myeloid leukemia: a comprehensive review and 2016 update. Blood Cancer J. 6, e441.

Eisfeld, A.-K., Marcucci, G., Liyanarachchi, S., Döhner, K., Schwind, S., Maharry, K., Leffel, B., Döhner, H., Radmacher, M.D., Bloomfield, C.D., 2012a. Heritable polymorphism predisposes to high BAALC expression in acute myeloid leukemia. Proce. Nat. Acad. Sci. 201203756.

Eisfeld, A.-K., Marcucci, G., Maharry, K., Schwind, S., Radmacher, M.D., Nicolet, D., Becker, H., Mrózek, K., Whitman, S.P., Metzeler, K.H., 2012b. miR-3151 interplays with its host gene BAALC and independently affects outcome of patients with cytogenetically normal acute myeloid leukemia. Blood. J. Am. Soc. Hematol. 120, 249–258.

Ganly, P., Walker, L.C., Morris, C.M., 2004. Familial mutations of the transcription factor RUNX1 (AML1, CBFA2) predispose to acute myeloid leukemia. Leuk. Lymphoma 45, 1–10.

Ghafouri-Fard, S., Esmaeili, M., Taheri, M., 2020. Expression of non-coding RNAs in hematological malignancies. Eur. J. Pharmacol. 875.

Greif, P.A., Konstandin, N.P., Metzeler, K.H., Herold, T., Pasalic, Z., Ksienzyk, B., Dufour, A., Schneider, F., Schneider, S., Kakadia, P.M., 2012. RUNX1 mutations in cytogenetically normal acute myeloid leukemia are associated with poor prognosis and up-regulation of lymphoid genes. Haematologica Haematol. 2012, 64667.

Hayashi, Y., Harada, Y., Huang, G., Harada, H., 2017. Myeloid neoplasms with germ line RUNX1 mutation. Int. J. Hematol. 106, 183–188.

Heydt, Q., Larrue, C., Saland, E., Bertoli, S., Sarry, J., Besson, A., Manenti, S., Joffre, C., Mansat-de Mas, V., 2018. Oncogenic FLT3-ITD supports autophagy via ATF4 in acute myeloid leukemia. Oncogene 37, 787.

Ketab, F.N.G., Gharesouran, J., Ghafouri-Fard, S., Dastar, S., Mazraeh, S.A., Hosseinzadeh, H., Moradi, M., Javadlar, M., Hiradfar, A., Rezamand, A., 2020. Dual biomarkers long non-coding RNA GAS5 and its target, NR3C1, contribute to acute myeloid leukemia. Exp. Mol. Pathol. 104399.

Kühnl, A., Gökbuget, N., Stroux, A., Burmeister, T., Neumann, M., Heesch, S., Haferlach, T., Hoelzer, D., Hofmann, W.K., Thiel, E., Baldus, C.D., 2010. High BAALC expression predicts chemoresistance in adult B-precursor acute lymphoblastic leukemia. Blood J. Am. Soc. Hematol. 15 (18), 3737–3744.

Lagunas-Rangel, F.A., Chávez-Valencia, V., Gómez-Guijosa, M.Á., Cortes-Penagos, C., 2017. Acute myeloid leukemia—genetic alterations and their clinical prognosis. Int. J. Hematol. Oncol. Stem Cell Res. 11, 328.

Lucena-Araujo, A.R., Pereira-Martins, D.A., Koury, L.C., Franca-Neto, P.L., Coelho-Silva, J.L., de Deus Wagatsuma, V.M., Melo, R.A., Bittencourt, R., Pagnano, K., Pasquini, R., 2017. Clinical impact of BAALC expression in high-risk acute promyelocytic leukemia. Blood Adv. 1, 1807–1814.

Mazraeh, S.A., Gharesouran, J., Ghafouri-Fard, S., Ketab, F.N.G., Hosseinzadeh, H., Moradi, M., Javadlar, M., Hiradfar, A., Rezamand, A., Taheri, M., Rezazadeh, M., 2020. Association between WT1 and MEG3 polymorphisms and risk of acute myeloid leukemia. Meta Gene 23.

Nadimi, M., Rahgozar, S., Moafi, A., Tavassoli, M., Tanha, H.M., 2016. Evaluation of rs62527607 [GT] single nucleotide polymorphism located in BAALC gene in children with acute leukemia using mismatch PCR-RFLP. Cancer Gene Ther. 209, 348–353.

Papaemmanuil, E., Gerstung, M., Bullinger, L., Gaidzik, V.I., Paschka, P., Roberts, N.D., Potter, N.E., Heuser, M., Thol, F., Bolli, N., 2016. Genomic classification and prognosis in acute myeloid leukemia. N. Engl. J. Med. 374, 2209–2221.

Recouvreux, M.S., Grasso, E.N., Echeverria, P.C., Rocha-Viegas, L., Castilla, L.H., Schere-Levy, C., Tocci, J.M., Kordon, E.C., Rubinstein, N., 2016. RUNX1 and FOXP3 interplay regulates expression of breast cancer related genes. Oncotarget 7, 6552.

Rubnitz, J.E., 2012. How I treat pediatric acute myeloid leukemia. Blood J. Am. Soc. Hematol. 119 (25), 5980–5988.

Rubnitz, J.E., Gibson, B., Smith, F.O., 2008. Acute myeloid leukemia. Pediatr. Clin. N. Am. 55, 21–51.

Sayad, A., Hajifathali, A., Hamidieh, A.A., Roshandel, E., Taheri, M., 2017. HOTAIR long noncoding RNA is not a biomarker for acute myeloid leukemia (AML) in Iranian patients. Asian Pacific J. Cancer Prevent.: APJCP 18, 1581–1584.

Sayad, A., Hajifathali, A., Omrani, M.D., Arsang-Jang, S., Hamidieh, A.A., Taheri, M., 2018. Expression of TNF-and HNRNPL-related immunoregulatory long non-coding RNA (THRIL) in acute myeloid leukemia: is there any correlation? Iran. J. Allergy Asthma Immunol. 17, 274–280.

Schnittger, S., Dicker, F., Kern, W., Wendland, N., Sundermann, J., Alpermann, T., Haferlach, C., Haferlach, T., 2011. RUNX1 mutations are frequent in de novo AML with noncomplex karyotype and confer an unfavorable prognosis. Blood J. Am. Soc. Hematol. 117 (8), 2348–2357.

Soliman, A., Aal, A.A., Afify, R., Ibrahim, N., 2016. BAALC and ERG expression in Egyptian patients with acute myeloid leukemia, relation to survival and response to treatment. Open Access Macedo. J. Med. Sci. 4, 264.

Sood, R., Kamikubo, Y., Liu, P., 2017. Role of RUNX1 in hematological malignancies. Blood J. Am. Soc. Hematol. 129 (15), 2070–2082.

Tamamyan, G., Kadia, T., Ravandi, F., Borthakur, G., Cortes, J., Jabbour, E., Daver, N., Ohanian, M., Kantarjian, H., Konopleva, M., 2017. Frontline treatment of acute myeloid leukemia in adults. Crit. Rev. Oncol. Hematol. 110, 20–34.

Tanner, S.M., Austin, J.L., Leone, G., Rush, L.J., Plass, C., Heinonen, K., Mrózek, K., Sill, H., Knuutila, S., Kolitz, J.E., 2001. BAALC, the human member of a novel mammalian neuroectoderm gene lineage, is implicated in hematopoiesis and acute leukemia. Proce. Nat. Acad. Sci. 98, 13901–13906.

Tsai, S.C., Shih, L.Y., Liang, S.T., Huang, Y.J., Kuo, M.C., Huang, C.F., Shih, Y.S., Lin, T.H., Chiu, M.C., Liang, D.C., 2015. Biological activities of RUNX1 mutants predict secondary acute leukemia transformation from chronic myelomonocytic leukemia and myelodysplastic syndromes. Clin. Cancer Res. 21 (15), 3541–3551.

Wang, X., Tian, Q.B., Okano, A., Sakagami, H., Moon, I.S., Kondo, H., Endo, S., Suzuki, T., 2005. BAALC 1-6-8 protein is targeted to postsynaptic lipid rafts by its N-terminal myristoylation and palmitoylation, and interacts with α, but not β, subunit of Ca2+/calmodulin-dependent protein kinase II. J. Neurochem. 92, 647–659.

Weber, S., Alpermann, T., Dicker, F., Jeromin, S., Nadarajah, N., Eder, C., Fasan, A., Kohlmann, A., Meggendorfer, M., Haferlach, C., 2014. BAALC expression: a suitable marker for prognostic risk stratification and detection of residual disease in cytogenetically normal acute myeloid leukemia. Blood Cancer J. 4, e173.

Xiao, S.J., Shen, J.Z., Huang, J.L., Fu, H.-Y., 2015. Prognostic significance of the BAALC gene expression in adult patients with acute myeloid leukemia: a meta-analysis. Mol. Clin. Oncol. 3, 880–888.

Xu, B., Chen, G., Shi, P., Guo, X., Xiao, P., Wang, W., Zhou, S., 2012. ShRNA-mediated BAALC knockdown affects proliferation and apoptosis in human acute myeloid leukemia cells. Hematology 17, 35–40.