Clinical and molecular features of acute promyelocytic leukemia with variant retinoid acid receptor fusions

Acute promyelocytic leukemia (APL) is a unique disease entity in acute myeloid leukemia (AML), characterized by the expansion of leukemic cell block at the promyelocytic stage. The vast majority of APL patients bear t(15;17)(q24;q21) involving the promyelocytic leukemia (PML) gene at chromosome band 15g24 and the retinoic acid receptor alpha (RARA) gene at 17g21, generating an aberrant PML-RARA fusion gene.^{1,2} However, in a subset of APL patients, a t(15;17)(g24;g21)and *PML-RARA* fusion cannot be detected.³ Many *RARA*, RARB, or RARG fusions have been reported so far, with APL patients presenting at least 17 alternative partner genes, including PLZF, NPM1, NUMA, STAT5B, PRKAR1A, BCOR, FIP1L1, OBFC2A, GTF2I, TBLR1, IRF2BP2, NUP98, FNDC3B, PML, STAT3, CPSF6, among others.^{1,4-12} Whereas RARA fusions with PML, NPM, NUMA, FNDC3B, and IRF2BP2 are sensitive to ATRA, PLZF-RARA, STAT3-RARA, STAT5B-RARA, CPSF6-RARG fusions are significantly resistant. Nevertheless, the molecular landscape of APL patients lacking classic t(15;17)(q24;q21)/PML-RARA remains to be delineated. Here, we report our investigations into the clinical and molecular features of APL patients lacking classic t(15;17)(q24;q21)/PML-RARA.

From January 2003 to December 2016, a total of 1401 patients with suspected APL were enrolled in this study.

Patients were considered eligible for inclusion only if the following criteria were satisfied: morphological and immunophenotypic features were consistent with the diagnosis of APL. This study was approved by the ethics committee of the First Affiliated Hospital of Soochow University in Suzhou, P.R. China, in accordance with the Declaration of Helsinki.

We performed cytogenetic and molecular characterizaon 1401 suspected APL patients for tion t(15;17)(q24;q21) and/or PML-RARA via karvotyping. fluoresecence in situ hybridization (FISH), RT-PCR, or RNAseq (Figure 1A). Twenty patients negative for rearrangements of RARA genes were excluded. We identified t(15;17)(q24;q21) and/or PML-RARA via karyotyping, FISH, RT-PCR, or RNA-seq in 98.6% (1362 out of 1381) of cases (Figure 1A). In total, 19 patients with alternative RARA or RARG fusions were identified: PLZF-RARA fusions in 10 patients, STAT5B-RARA in 4, STAT3-RARA in 2, CPSF6-RARG in 2, and TBLR1-RARA in 1 patient, respectively (Figure 1A and B). We observed a significantly higher number of males with alternative RARA or RARG fusions when compared to APL with classic PML-RARA (84.2% vs. 52.6%; P=0.04). Primary patients' characteristics are summarized in Tables 1 and 2. Median white blood cell (WBC) count at presentation for the alternative RARA or RARG fusions cohort was significantly higher than the PML-RARA cohort (19.7x10⁹/L vs. 2.5×10^{9} /L; P=0.01) (Table 1). In addition, the median platelet count for the alternative RARA or RARG fusions cohort was significantly higher than that in the PML-RARA cohort (78x10⁹/L vs. 25x10⁹/L; P<0.001).

Table 1. Clinical and laboratory features of 1381 patients with acute promyelocytic leukemia (APL).

	APL with t(15;17) <i>/ PML-RARA</i>	APL with X-RARA/RARG	Р	
Cases	1362	19		
Age, years, median (range)	39(4-91)	42(24-70)	0.952	
Gender, M/F	716/646 (1.1/1)	16/3 (5.3/1)	0.04	
WBC, 10 ⁹ /L, median (range)	2.5(0.2-200)	19.7(1.34-72.7)	0.01	
Hb, gd/L, median (range)	85(24-162)	83(45-125)	0.89	
PLT, 10 ⁹ /L, median (range)	25(1-212)	78(24-282)	< 0.001	
Blast cell (%), median (range)	79(46~98)	77(48~96)	0.91	
Karyotype				
Normal	78 (5.7%)	-		
t(15:17) alone	1012 (74.3)	-		
t(15:17) with ACAs	260 (19.1%)	-		
Failure	12 (0.9%)			
PML-RARA transcripts				
L type	838 (65.7%)	-		
S type	426 (33.4%)	-		
V type	12 (0.9%)	-		
Sanz risk stratification			< 0.001	
High	361 (26.5%)	13 (68.4%)		
Middle	685 (50.3%)	1 (5.26%)		
Low	316 (23.2%)	5 (26.31%)		
Complete remission (%)	791/835 (94.8%)	8/15 (53.3%)	< 0.001	
Relapse rate (%)	58/471 (12.3%)	5/8 (62.5%)	< 0.001	

M: male; F: female; WBC: white blood cell count; Hb: hemoglobin level; PLT: platelet count; ACAs: additional chromosomal abnormalities

Case	Gender/		HB,	PLT,	es of acute promyelocytic l Karyotype	FISH probe		Transcript	In vivo	Outcome
	Age	x10 ⁹ /L		x10°/L	ini jospo		RNA-seq	type	ATRA	Catoonio
	1400	4.0.4		400					response	
	M/38	1.34	47	129	46,XY,t(11;17)	PML-RARA	ND	PLZF-RARA	NA	Lost to
					(q23;q21)[20]	negative		(exon3-exon3)		follow up.
	M/39	3.1	62	106	46,XY,t(11;17)[8]/45,X,-Y,	PML-RARA	ND	PLZF-RARA	NA	Lost to
					t(11;17) [9]/45,X,-Y,t(3;6) (q26;p25),t(11;17) [3]	negative		(exon4-exon3)		follow up.
	F/62	72.7	125	201	46,XX,t(11;17)(q23;q21)	PML-RARA	ND	PLZF-RARA	NA	Lost to
					[10]	negative		(exon3-exon3)		follow up.
	M/42	31.7	96	163	46,XY,del(9),t(11;17)	PML-RARA	ND	PLZF-RARA	insensitive	Died in
					(q23;q21)[10]	negative		(exon3-exon3)		RP2 at 58 mo.
M/32	M/32	20	84	80	46,XY,t(11;17)(q23;q21)	PML-RARA	ND	PLZF-RARA	insensitive	Alive in
					[9]/46,XY[1]	negative		(exon4-exon3)		CR2 at 72 mo (allo-HSCT in CR2 at 60 mo).
	M/58	23.7	84	25	45,X,-Y,t(11;17)(q23;q21)	PML-RARA	ND	PLZF-RARA	NA	Died at 1 mo.
	11/30	20.1	04	20		negative	ND	(exon3-exon3)	1 V/A	(Patient decision
					[8]/46,XY[3]	negative		(620113-620113)		
	M/46	10.54	111	31	45 V V+(11.17) (~99.~91) [1		ND	PLZF-RARA	registent	to stop therapy).
	IVI/40	10.54	111	91	45,X,-Y,t(11;17)(q23;q21)[1	-	ND		resistant	Died at day 4
						negative		(exon3-exon3)		(cerebral
	1405	0.01	0 7	100			ND			hemorrhage).
	M/37	3.31	65	109	46,XY,t(11;17)	PML-RARA	ND	PLZF-RARA	resistant	Died at 10 mo.
					(q23;q21)[5]/46,XY[5]	negative		(exon3-exon3)		
M/25	M/25	35	77	55	46,XY,t(11;17)	PML-RARA	ND	PLZF-RARA	insensitive	Alive in CR1
					(q23;q24)[10]	negative		(exon3-exon3)		at 24 mo (allo-HSCT in
0	1.670	00 F	0.0	01	40 387 (11 17)		ND		• • •	CR1 at 3 mo).
0 M/70	M/70	22.5	96	61	46,XY,t(11;17)	PML-RARA	ND	PLZF-RARA	resistant	Lost to follow up
	14/00	0.0	0.0	0.0	(q23;q24)[10]	negative		(exon3-exon3)	• • •	
1 M/32	M/32	3.8	83	28	46,XY[20]	RARA	STAT5B-RARA	STAT5B-RARA	resistant	Alive in CR1
						positive		(exon15-exon3)		at 68 mo (allo-HSCT in
2	M/40	10.7	74	94	10 VV[90]	DADA	מתאתבם האהא	CTATED DADA	ungintent	CR1 at 19 mo).
2	M/49	19.7	74	24	46,XY[20]	RARA	STAT5B-RARA	STAT5B-RARA	resistant	Died in RP1
	1405	10	45	10	40 18 (100)	positive		(exon15-exon3)	• • •	at 13 mo
}	M/35	13	45	43	46,XY[20]	RARA	STAT5B-RARA	STAT5B-RARA	resistant	Died at 1 mo.
						positive		(exon15-exon3)		
1	M/46	17.18	77	78	45,X,-Y,1q-,	RARA	STAT5B-RARA	STAT5B-RARA	resistant	Alive in RP2
					11q+[8]/46,XY[2]	positive		(exon15-exon3)		at 14 mo.
5	M/26	6.6	73	94	46,XY[20]	RARA	STAT5B-RARA		resistant	Died at 6 mo.
						positive		(exon23-exon3)		
5	M/24	32.3	123	89	45,X,-Y[6]/46,XY[8]	RARA	STAT5B-RARA	STAT5B-RARA	resistant	Died in RP1
						positive		(exon21-exon3)		at 36 mo (cerebral
7	M/E9	NA	NI A	NIA	AC VV[90]	DIDI		א מא מ	nonistant	hemorrhage).
7	M/52	NA	NA	NA	46,XY[20]	RARA	TBLR1-RARA	TBLR1-RARA	resistant	Lost to
	D //0	0.0	0.0	P 0	00 377777 [0]	negative		(exon5-exon3)	• • •	follow up.
8	F/48	22	69	73	92,XXXX[2]	RARA	CPSF6-RARG	CPSF6-RARG	resistant	Refractory to
						negative		(exon4-exon4)		ATRA, ATO, and chemotherap died of cerebral
										hemorrhage in 1
9	F/51	20.15	65	45	46,XX,del(12)(p12)	RARA	CPSF6-RARG	CPSF6-RARG	resistant	hemorrhage in 10 mo. Alive in CR1

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WBC: white blood cell count; Hb: hemoglobin level; PLT: platelet count; M: male; F: female; ND: not determined; NA: not available; allo-HSCT: allogeneic stem cell transplantation; ATO: arsenic trioxide; ATRA: all-trans retinoic acid; CR: complete remission; mo: months; RP: relapse. We observed poor responses to ATRA in most patients with *PLZF-RARA, STAT3-RARA, STAT5B-RARA,* or *CPSF6-RARG* fusion transcripts (Tables 1 and 2). Only 8 out of 15 (53.3%) of cases with alternative *RARA* or *RARG* fusions acquired complete remission (CR) by chemotherapy combined with ATRA and/or arsenic trioxide. Furthermore, 62.5% of alternative *RARA* or *RARG* fusion cases acquiring CR eventually underwent relapse (Table 1). To further analyze the prognostic impact of APL with alternative *RARA* or *RARG* fusions, we compared the overall survival (OS) and leukemia-free survival (LFS) in APL with t(15;17)(q24;q21)/*PML-RARA*. APL patients with alternative *RARA* or *RARG* fusion showed poor outcomes: the 3-year OS rate was 26.7% compared

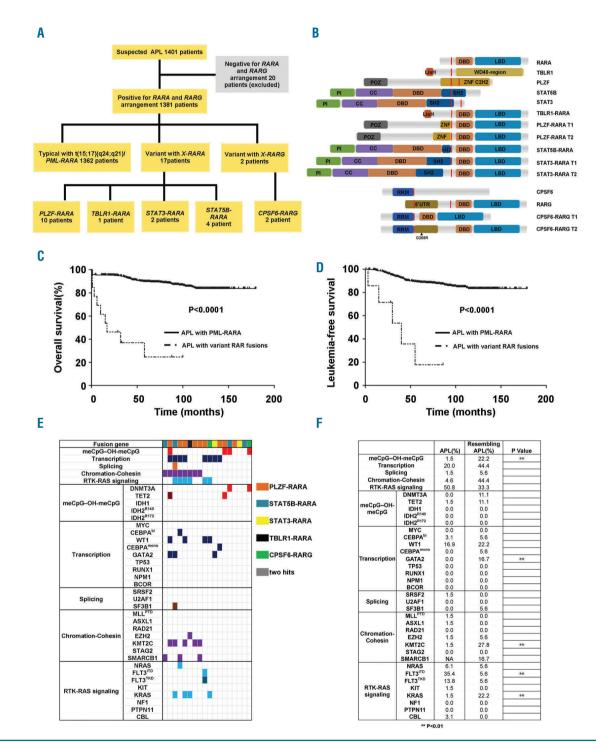


Figure 1. The clinical and molecular characteristics of resembling acute promyelocytic leukemia (APL) with RARA/RARG fusions. (A) Flowchart of the patient cohort. (B) Schematic representation of alternative RARA and RARG fusions identified in this study. Breakpoints are indicated with red lines. Different patterns and colors are used to represent various functional regions of the RARA, RARG, TBLR1, PLZF, STAT5B, STAT3, and CPSF6 proteins. (C) Overall survival (OS) of APL compared with APL with alternative RARA/RARG fusions. (D) Leukemia-free survival (LFS) of APL compared with APL with alternative RARA/RARG fusions. (E) Mutational spectrum in APL with alternative RARA or RARG fusions. Each column represents one of the 18 resembling APL samples sequenced here. The rows in the graph represent individual genomic lesions. (F) Distribution of mutations in APL patients with PML-RARA or alternative RARA/RARG fusions.

to 92.1% in APL with PML-RARA (P<0.0001) (Figure 1C). The 3-year LFS was also clearly poorer than that of the APL cohort (20.0% vs. 86.5%; P<0.0001) (Figure 1D). The risk stratification of APL patients with alternative RARA or RARG was also significantly poorer than the PML-RARA cohort (P<0.001) (Table 1). Among the 13 patients with alternative RARA or RARG fusion with follow up, 8 (61.5%) patients received combinational induction therapy by all-trans retinoic acid (ATRA) and arsenic trioxide, 5 (38.5%) patients received combinational induction therapy by ATRA and chemotherapy. In addition, three patients who received allo-HSCT were still alive at 24, 68 and 72 months, respectively (Table 2). This suggests that allo-HSCT may be an effective way to improve the survival of the APL with alternative RARA or RARG fusions.

Next-generation sequencing (NGS) has identified novel genetic variants in many hematologic malignancies, including APL.¹³ High frequency of FLT3 and WT1 mutations were identified in APL patients with PML-RARA. Nevertheless, the molecular landscape of APL patients with alternative RARA or RARG fusions remains to be delineated. To decipher the mutational spectrum of patients with alternative RARA or RARG fusions, we performed NGS on the target DNA with a panel of 382 genes in a cohort of 18 patients (*PLZF-RARA* n=9, *STAT5B-RARA* n=4, *STAT3-RARA* n=2, *CPSF6-RARG* n=2, and TBLR1-RARA n=1). Mutations were detected in 15 out of 18 patients (83.3%): 7 out of 18 (38.9%) patients carried 1 mutation. 3 out of 18 (16.7%) carried 2. 3 out of 18 carried 3, and 2 out of 18 carried 4, i.e. an average 1.7 mutations per sample (Figure 1E). We identified high frequencies of mutations, KMT2C (27.8%), WT1 (22.2%), K-RAS (22.2%), GATA2 (16.7%), SMAR-CB1 (16.7%), followed by DNMT3A (11.1%), TET2 (11.1%), CEBPA (11.1%), SF3B1 (5.6%), FLT3-TKD (5.6%), FLT3-ITD (5.6%), EZH2 (5.6%), and N-RAS (5.6%), etc. We further compared the mutational spectra of patients with alternative RARA or RARG to those with PML-RARA fusion.¹³ APL with alternative RARA or RARG presented with more mutations of KMT2C (27.8% vs. 1.5%; P<0.01), K-RAS (22.2% vs. 0.5%; P<0.01), GATA2 (16.7% vs. 0%; P<0.01), and fewer mutations of FLT3-ITD (5.6% vs. 35.4%; P<0.01) (Figure 1F).

Only 53.3% of APL with alternative RARA or RARG fusions achieved CR by chemotherapy combined with ATRA and/or ATO; the relapse rate was as high as 62.5%. The 3-year OS and LFS of APL with alternative RARA or RARG were worse than those of the PML-RARA cohort. Among the 19 patients in our study with suggested APL, 15.79% (3 out of 19) were insensitive and 63.16% (12 out of 19) were resistant to ATRA treatment. It has been reported that the three mutations in the PML part of PML-RARA could attenuate the negative regulation of arsenic on PML-RARA. The resistant effect can be overcome by either increasing the concentration of arsenic trioxide or by combination with ATRA.^{14,15} The APL patients with alternative RARA or RARG fusions in our study were resistant to ATO and/or ATRA treatment. Fusion gene moiety was not involved in the NGS panel; however, we noticed a high proportion of mutation in signaling pathways, especially the K-RAS mutation, and epigenetics compared with APL patients. We speculated that the gene mutation might partly be the reason for these APL patients to be resistant or insensitive to ATRA and/or ATO.

In summary, we retrospectively analyzed 1381 patients with APL diagnosis and identified 1.4% patients with alternative *RARA* or *RARG*. We observed poor response to all-trans retinoic acid in most APL patients with PLZF-RARA, STAT3-RARA, STAT5B-RARA, or CPSF6-RARG fusion transcripts. NGS performed on APL patients with alternative RARA or RARG fusions revealed more mutations of KMT2C, K-RAS, and GATA2, but fewer mutations of FLT3-ITD when compared to APL patients with the PML-RARA fusion. We suggest that routine karyotypic analysis, FISH, and real-time polymerase chain reaction should be performed in patients with morphological and immunophenotypic features consistent with the diagnosis of APL. In suspected APL patients lacking a PML-RARA fusion, RNA sequencing should be performed to exclude variant fusion involving RARA/RARB/RARG genes. This study highlights the importance of combining multiple molecular techniques for the characterization optimal management of APL and lacking t(15;17)(q24;q21)/PML-RARA fusions.

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