NEW OPPORTUNITIES FOR ASSAY OF LEUKEMIA INTIATING CELLS (LIC) PARTICIPATING IN POST-TRANSLANT RELAPSE DEVELOPMENT IN THE PATIENTS WITH ACUTE MYELOID LEUKEMIA

Nikolay N. Mamaev, Alyena I. Shakirova, Ildar M. Barkhatov, Tatiana L. Gindina

RM Gorbacheva Research Institute of Pediatric Oncology, Hematology and Transplantation, Pavlov University, St. Petersburg, Russia

INTRODUCTION

Based on the recently obtained data concerning the transplantation of sorted leukemia initiating cells (LICs) from acute myeloid leukemia (AML) patients into combined immunodeficient (SCID) mice it was shown, that LIC population is heterogenic. The earliest LICs with the immunophenotype $CD34^+CD38^-$ are capable of specific increased expression of gene *BAALC* [1,2]. In turns, the most differentiated LICs with immunophenotype $CD34^-$, which are more characteristic for patients with acute promyelocytic leukemia (APL) or AML with *NPM1* mutations, similarly to blasts seemed to be predominantly associated with overexpression of gene *WT1*, although the latter may occur in $CD34^+$ cells too [3,4]. At the same time, it was recently shown, that intermediately differentiated LICs with immunophenotypes $CD34^+CD38^+$ and $CD34^+CD38^{++}$ in AML patient's bone marrow may be far more responsible for specific overexpression of gene *EVI1* [5]. The above facts prompted us to the conception, that indirect quantitative evaluation of variously differentiated LICs can be realized in clinical practice by means of *BAALC*, *EVI1* and *WT1* gene expression levels measurement using standard quantitative real-time PCR (QR-PCR).

RESULTS

We carried out a simultaneous measurement of *BAALC*, *EVI1* and *WT1* gene expression levels in specially selected groups of pediatric (n=9) and adult (n=13) patients with *EVI1*-positive and *EVI1*-negative AML after allogeneic hematopoietic stem cell transplantation (alloHSCT). The cutoff level was chosen as 250 copies /10^4 copies of *ABL1* reference gene for *WT1,* but 10 % and 31 % for *EVI1* and *BAALC*, respectively.

EVI1-negative AML patients

Among the EVI1-negative AML cases, 8 pts had overexpressed *BAALC* and *WT1* markers (№№ 1-4, 6, 9, 11 and 12), whereas 5 pts revealed only 1 overexpressed gene marker, i.e., *BAALC* (№ 5 and № 8) or *WT1* (№№ 7, 10 and 13).

№	Gender, age (y)	FAB	Karyotype at diagnosis	alloHSCT type	Conditioning	PTR				OS after alloHSCT (days)
						BAALC, %	*WT1*, copies	*EVI1*, %	Blasts, %	
1	F, 54	M1	NK	R	RIC	77	32981	0.5	35.4	61
2	M, 21	M1	45,X,-Y,t(8;21)(q22;q22)[20]	R	MAC	329	7858	0.03	70	306
3	M, 6	M4	46,XY,del(2)(q?33),del(5)(q?22),add(19)(q13)[2]/46,idem,add(X)(p22),del(5)(q31),add(6)(q25),add(9)(p24)[3]/46,XX[15]	H	RIC	7118	296	0.6	41.2	246
4	F, 15	M2	49,XX,+X,+4,t(8;21)(q22;q22),+15[20]	N	RIC	133	9929	0.4	75.6	235
5	F, 60	M1	NK	N	RIC	378	197	0.6	37	382
6	F, 48	M1	n/d	N	RIC	117	281	0.6	40	98
7	M, 28	M2	46XY,add(4)(q31),add(10)(q22)[1]/46,XY[3]/46,XX[16]	N	MAC	23	5518	3	24	103
8	M, 25	M0	46,XY,t(3;10)(p21;p11),del(11)(q21q23)[16]/46,XY[4]	N	MAC	82	5	0	28.2	162
9	M, 30	M2	46,XY,add(1)(p36),t(8;21)(q22;q22),add(17)(q25)[3]/46,XY[17]	N	RIC	153	2363	0.6	23	194
10	F, 55	M4	NK	N	RIC	0.06	1020	0.02	20	101
11	M, 5	M4	46,XY,del(7)(q32q36),t(8;21)(q22;q22)[19]/48,idem,+mar1,+mar2[3]/46,XY[1]	N	RIC	35	411	0.8	13	730
12	F, 45	M4	NK	N	RIC	43.8	3381	3	n/d	69
13	M, 17	M4	NK	H	RIC	6	362	2	7	197

EVI1-positive AML patients

Among $EVI1^+$ leukemias, overexpression of the 3 markers was shown in five pts (№№ 15-18 and 22), whereas 2 markers were overexpressed in 4 other cases (№№ 14, 19-21). The same overexpression of *EVI1* and *WT1* took place in the latter 4 cases, including M3 (n=1), M7 (n=2) and M5 (n=1) $EVI1^+$ FAB-variants.

№	Gender, age (y)	FAB	Karyotype at diagnosis	alloHSCT type	Conditioning	PTR				OS after alloHSCT (days)
						BAALC, %	*WT1*, copies	*EVI1*, %	Blasts, %	
14	F,3	M7	NK	H	MAC	0.04	6856	40	60.8	258
15	F,21	M4	45,XX,inv(3)(q21q26),t(2;3)(q?12;q21),-7[6]/46,XX[14]	N	RIC	400	26036	156	24	393
16	M,39	M2	NK	N	RIC	83	18872	102	26	350
17	F,26	M1	45,XO,der(11)add(p15)del(q23)[2]/46,idem,+21[1]/46,XX[1]/46,XY[16]	N	MAC	125	6500	22	51.2	692
18	F, 3	n/d	45,XX,t(6;12)(q26;q23),-7[10]	H	MAC	100	436	22	29.8	355
19	F, 1	M7	Complex karyotype	R	RIC	9	3315	99	60.4	150
20	F, 17	M3	t(15;17) PML-RARα	N	RIC	3	32684	321	24.4	160
21	M, 37	M5	46,XY,t(3;12)(q26;p13)[11]/45,XY,idem,-7[2]/46,XY,idem,+mar[7]	R	RIC	24	4542	88	21	423
22	M,8	AML from MDS	46,XY,del(11)(q13q23)[6]/46,XY[13]/46,XX[1]	N	RIC	121	10239	22	30.4	317

F – female, M – male, NK – normal karyotype, n/d – no data, R – relative, N – Nonrelative, H – haploidentical, MAC – myeloablative conditioning, RIC – reduced intensity conditioning, *BAALC*, *WT1* and *EVI1* gene expression levels over the cutoff are in red.

CONCLUSION

Our data show applicability of real-time PCR for studying participation of different LIC types in development of PTR, thus requiring further investigations.

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CONTACT

Mamaev Nikolay Nikolaevich: MD, DSci, Professor, leading researcher at Department of oncology, hematology and transplantation for young adults and adults RM Gorbacheva Research Institute of Pediatric Oncology, Hematology and Transplantation, Pavlov University, St. Petersburg, Russia

Adress: L. Tolstoy str., 6-8, 197022, St. Petersburg, Russia. E-mail: nikmamaev524@gmail.com.