COMBINING *FLT3* STATUS MUTATION WITH POLYCOMB COMPLEX GENES EXPRESSION PROFILE IN BRAZILIAN CHILDREN WITH ACUTE MYELOID LEUKEMIA

Amanda Faria de Figueiredo[1,2], Marcelo Gerardin Poirot Land[3], Gerson Moura Ferreira[4], Andre Mencalha[5], Renata Binato[4], Roberto Rodrigues Capela de Matos[1,2], Thomas Liehr[6], Maria Luiza Macedo Silva[1,2], Eliana Abdelhay[4]

1. Genetics Laboratory, Pediatric and Puericulture Martagão Gesteira Institute, Federal University of Rio de Janeiro(UFRJ), Rio de Janeiro, Brazil
2. Cytogenetics Laboratory, Bone Marrow Unit, National Cancer Institute (INCA), Rio de Janeiro, Brazil; 3. Clinical Medicine Post-Graduation Program, College of Medicine, Federal University of Rio de Janeiro (UFRJ), Rio de Janeiro, Brazil; 4. Stem Cells Laboratory, Bone Marrow Unit (INCA), Rio de Janeiro, Brazil; 5. Department of Biophysics and Biometry, Roberto Alcântara Gomes Biology Institute, Rio de Janeiro State University (UERJ), Rio de Janeiro, Brazil; 6. Jena University Hospital, Institute of Human Genetics, Jena, Germany.

Objectives

Acute myeloid leukemia (AML) is a heterogeneous group of hematologic malignancies characterized by specific genetic, morphological and clinical aspects. The heterogeneous nature of AML is represented by the diversity of cytogenetic and molecular alterations described in recent decades. Despite great progress in understanding the pathogenesis of AML, the overall survival rate of pediatric patients only reaches about 55% and is still considered as an unacceptable cure rate. Recent studies have shown that pediatric AML has few genetic alterations, suggesting that a small number of them are necessary for the development of AML in this age group when compared to other cancers and adult AML. Epigenetic changes appear to be involved in the aberrant transcriptional program observed in AML. In this context, the *Polycomb group* (PcG) genes have emerged as important participants in this process. We compared the expression levels of 4 genes from the PcG repressive complexes, EZH2, YY1, BMI1 and SUZ12 in a cohort of 68 Brazilian children, according to selected clinical and biological features, including FLT3 gene mutations.

Material and Methods

Newly diagnosed cases of AML were included in the study. Diagnosis of AML was based on morphology on bone marrow (BM) aspirates, cytochemistry, and flow cytometric immunophenotyping. Chromosomal analysis was performed on BM by short-term unstimulated cultures using classical and molecular cytogenetic techniques. Screening for FLT3-ITD mutations was performed by PCR and PcG genes expression profile was performed by RT-qPCR.

Results an Discussion

The median age was 8 years (range 4months -18 years). Twenty-nine cases were classified as AML-M3, thirty-nine were classified as in AML M0/M1 (n=7), AML M2 (n=6), AML M4/M5 (n=24), AML M6 (n=1) and AML M7 (n=1) using the classical FAB morphology. FLT3-ITD mutations were detected in 20 cases. Our results show significant differences in the expression levels of *EZH2* and BMI1 between APL, AML patients, and healthy donors (Figures 1 and 2). When we compared APL and AML with clinical and laboratory data, we observed that APL was more associated with platelets count < 40,000 and patients' age < 8 years, *EZH2* and LN(BMI1) and *FLT3-ITD* in univariate analysis. In our predictive model, the *BMI1* gene and FLT3-ITD mutation were the main factors capable of predicting the APL phenotype. The clinical and laboratory data are in Table 1.

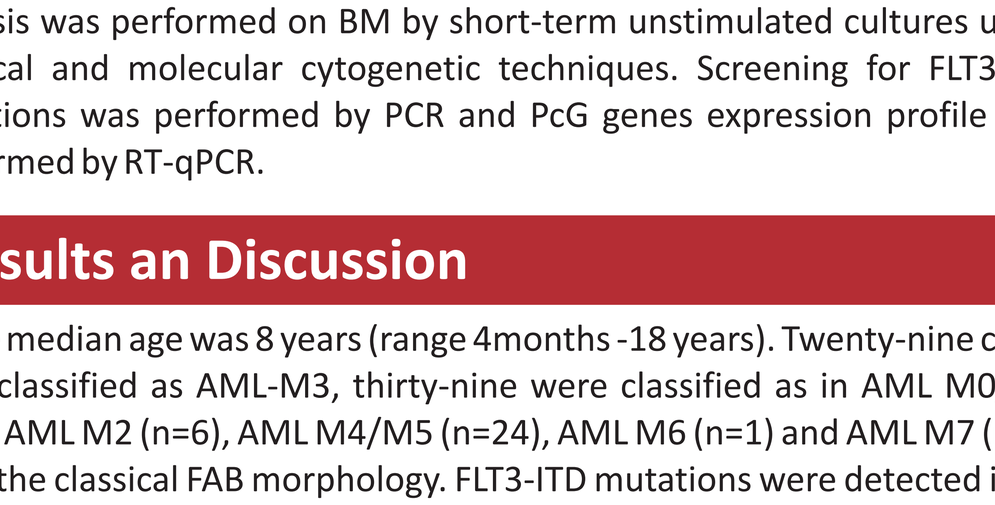

Figure 1: Expression profile of the Polycomb genes with a significant difference found between the control and patient samples. (A) Box plot showing the EZH2 expression profile of the control and the APL and AML patients. (B) Box plot showing the LN (BMI1) expression profile of the control and the APL and AML patients.

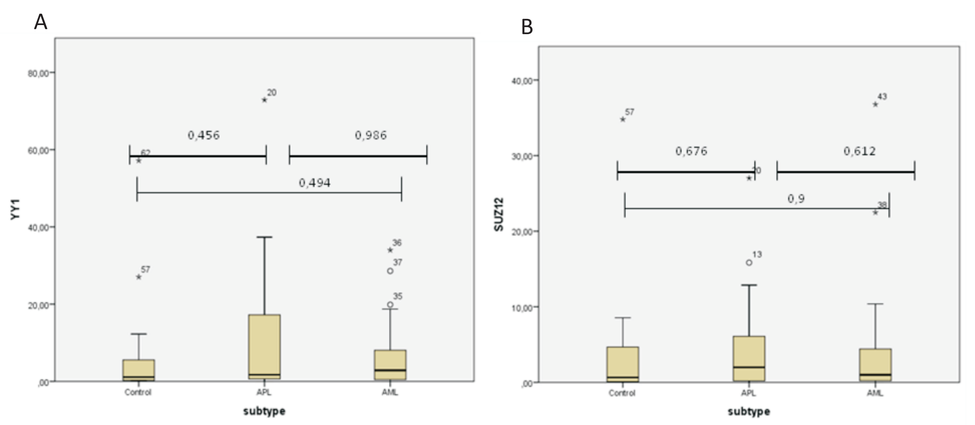

Figure 2: Expression profile of the Polycomb genes with no difference between the control and patient samples. (A) Box plot showing the YY1 expression profile of the control and the APL and AML patients. (B) Box plot showing the SUZ12 expression profile of the control and the APL and AML patients.

Table 1- Clinical and laboratory data from 68 children with AML.

Characteristics	All patients, 68 patients			APL			AML		
	No.	Median(range)		No.	Median(range)		No.	Median(range)	
Gender									
Female	29	42%		13	45%		19	49%	
Male	40	58%		16	55%		20	51%	
Age			8 (4 m-18y)			10(2-16y)			5 (4m-18y)
0-8 anos	36	53%		9	31%		27	69%	
9-18 anos	32	47%		20	69%		12	31%	
Leukocyte counts, x10^9/L			38.7 (1.14-500)			15.1(1.14-237)			65 (3-500)
≤10	13	20.3%		10	34.5%		3	8.6%	
out/50	24	37.5%		12	41.4%		12	34.3%	
≥50	27	42.2%		7	24.1%		20	57.1%	
Unkown	4	-		-	-		-	-	
Platelet count, x10^9/L			38 (3-442)			29 (3-367)			51.8 (13-442)
≤40	32	51.6%		20	69%		12	36.4%	
>40	30	48.4%		9	31%		21	63.6%	
Unkown	6	-		-	-		-	-	
Cytogenetics									
RUNX1/RUNX1T1	4	5.9%					4	10.3%	
KMT2A abnormalities	15	22.1%					15	38.5%	
Normal karyotype	4	5.9%					4	10.3%	
CBFb-MYH11	4	5.9%					4	10.3%	
Non recurrent abnormalities	8	11.8%					8	20.5%	
-7/7q and -5/5q	3	4.4%					3	7.7%	
No mitosis	1	1.5%					1	2.4%	
PML-RARA	21	30.8%		21	72.4%				
PML-RARA and additional cytogenetic abnormalities	8	11.7%		8	27.6%				
Morphologic subtype									
M0/M1	8	11.8%		8	20.5%				
M2	6	8.8%		6	15.4%				
M3	25	36.8%					25	86.2%	
M3v	4	5.9%					4	13.8%	
M4	5	7.3%		5	12.8%				
M5	18	26.5%		18	46.1%				
M6	1	1.5%		1	2.6%				
M7	1	1.5%		1	2.6%				
FLT3 Status									
FLT3-ITD	21	30.9%		13	44.8%		8	22.9%	
FLT3-WT	43	69.1%		16	55.2%		27	77.1%	
Unknown	4								
FLT3-ITD									
One allele	15	71.4%		8	61.5%		7	87.5%	
Two alleles	6	28.6%		5	38.5%		1	12.5%	

Dicussion and Conclusion

Our findings suggest that higher expression levels of *BMI1* and *FLT3-ITD* mutation were more significantly correlated with APL patients than with others AML subtypes in children and the expression levels of BMI1 presented a different landscape than the one previously reported in adult AML.

Projeto Gráfico: Área de Edição e Produção de Materiais Técnico-Científicos / INCA