Cytogenetic and Epigenetic Alterations in Pediatric Myelodysplastic Syndrome: Investigating Diagnostic and Prognostic

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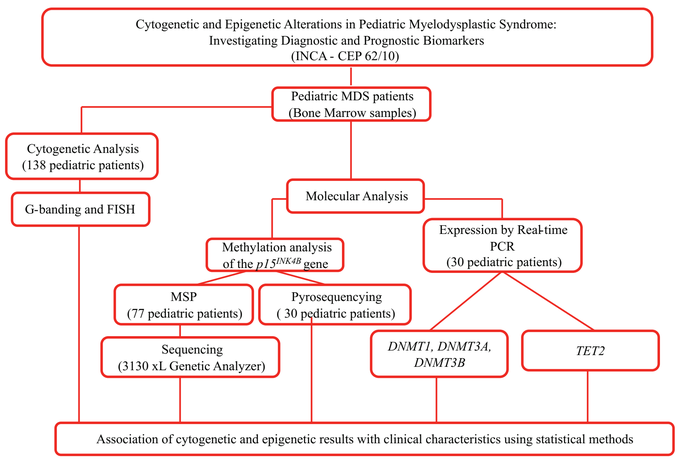
INTRODUCTION

Pediatric MDS is an uncommon disorder, accounting for less than 5% of hematopoietic malignancies. MDS comprises a heterogeneous group of clonal bone marrow disorders characterized by varying degrees of pancytopenia, morphological and functional abnormalities of hematopoietic cells and increased risk of transformation into acute myeloid leukemia (AML). Some studies in children have been shown that MDS appears with distinct clinical and laboratory characteristics when compared with adults, which may reflect special biological issues of MDS during childhood. Due, the MDS heterogeneity, little is known about the molecular basis of development and evolution of MDS in pediatric patients.

The field of cancer epigenetics is evolving rapidly on several aspects. In myelodysplastic syndrome (MDS), some research groups have been shown the importance to study epigenetic alterations as new diagnostic, prognostic and risk stratification biomarkers. The aim of this study was to analyze the cytogenetic alterations, the methylation status of *p15*^{INK4B} gene, the expression of *DNMTs* and *TET2* genes in pediatric primary MDS, the correlation with the different MDS subtypes and the role of *p15*^{INK4B}, *DNMTs* and *TET2* genes in the development and in the evolution of MDS toward AML.

METHODOLOGY



RESULTS AND DISCUSSION

A total of 138 patients with a mean age of 8 years (3 months - 18 years) were studied, 77 male and 61 female. The presence of abnormal karyotypes was observed in 54% (75/138). The monosomy of chromosome 7 was the most frequent cytogenetic alteration (19%), followed by deletion (11q23) and complex karyotypes (Figure 1). The distribution of pediatric patients according to the MDS classification was: refractory cytopenia (CR) in 67% of the cases (93/138), refractory anemia with blasts (RAEB) in 17% (24/138) and refractory anemia with excess of blasts in transformation (RAEB-t) in 15%(21/138).

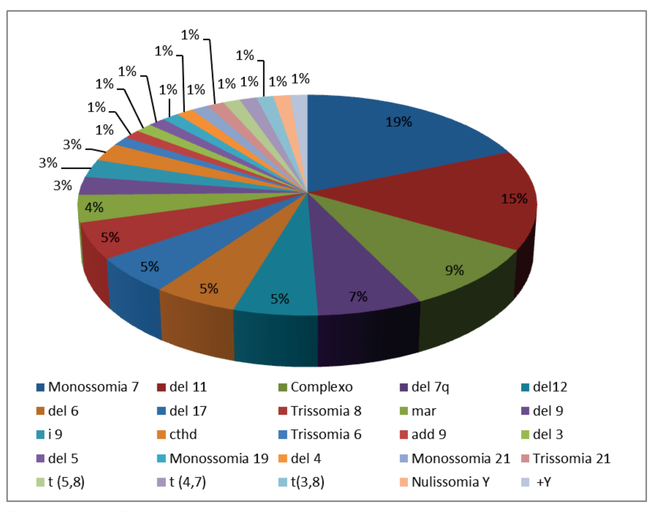


Figure 1: Frequency of clonal chromosomal alterationss in pediatric primary MDS.

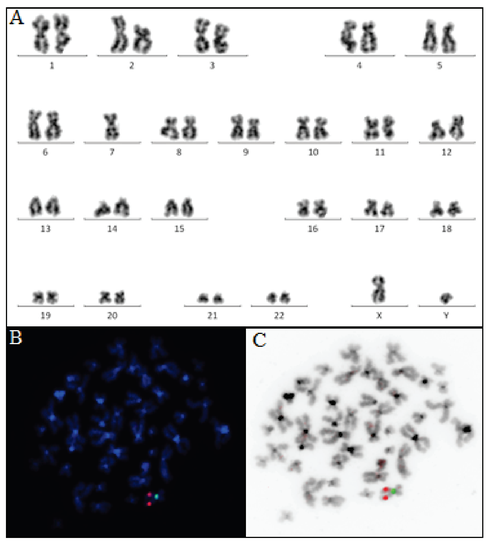


Figure 2: (A)G-banding analysis in bone marrow cell of a pediatric patient with RAEB showing monosomy 7; (B / C) FISH used Vysis's probe (D7s486 spectrum orange / CEP7 spectrum Green).

Analysis of Methylation Status of *p15*^{INK4B} Gene in Pediatric Primary MDS

Aberrant methylation of *p15*^{INK4B} gene was detected in 22 of 77 patients (29%). The frequency of *p15*^{INK4B} gene methylation was significantly higher in later stages of disease (RAEB and RAEB-t) compared with the initial stage (RC) of primary MDS ($p < 0,02$). The association of *p15*^{INK4B} methylation status with evolution of the disease was clearly significant ($p < 0,009$) (Table 1).

Table 1: Correlation of Methylation Status of *p15*^{INK4B} gene with clinical characteristics.

Clinical Parameters	Methylation of *p15*^{Ink4b} Methylated (n=22)	Methylation of *p15*^{Ink4b} Unmethylated (n=55)	*p-valor* *p15*^{Ink4b}
Age			
<12 years (n=53)	9	44	
≥12 years (n=24)	13	11	X^2 p<0,001
Sex			
male(n=42)	15	27	
Female (n=35)	7	28	X^2 p>0,1
Cytogenetics			
Normal (n=29)	2	27	
Abnormal (n=48)	20	28	X^2 p<0,001
Subtypes			
Initial subtype RC (n=51)	10	41	
Advanced Subtypes RAEB (n=16)/ RAEB-t (n=10)	12	14	X^2 p<0,02
Blasts (%)			
<5%	10	41	
5-19%	5	11	
20-29%	7	3	X^2 p<0,001
Evolução da doença (MDS to AML)			
Yes (n= 28)	13	15	
No (n= 49)	9	40	X^2 p<0,009

Quantitative Analysis of DNA methylation in *p15*^{INK4B} gene using Pyrosequencing

Bisulfite pyrosequencing is a sensitive and quantitative technique that assesses the average methylation at each individual CpG dinucleotide within a given amplicon. We used bisulfite pyrosequencing for quantificate the DNA methylation in *p15*^{INK4B} gene in bone marrow cells of pediatric patients with priamry MDS. The hypermethylated profile was observed in 36.23% (11/30) of the patients and was correlated with abnormal karyotypes, mainly those involving chromosome 7 (Figure 3).

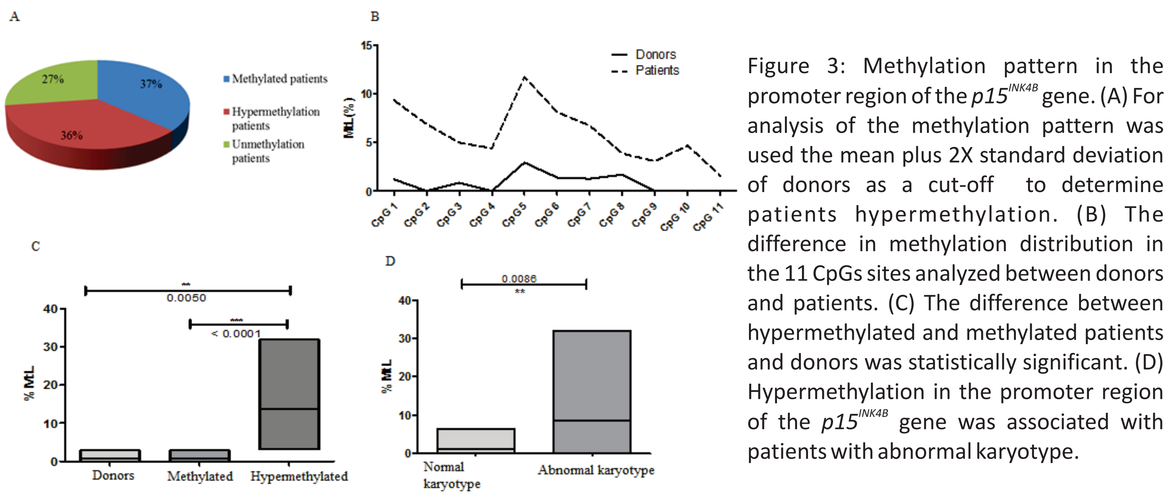


Figure 3: Methylation pattern in the promoter region of the *p15*^{INK4B} gene. (A) For analysis of the methylation pattern was used the mean plus 2X standard deviation of donors as a cut-off to determine patients hypermethylation. (B) The difference in methylation distribution in the 11 CpGs sites analyzed between donors and patients. (C) The difference between hypermethylated and methylated patients and donors was statistically significant. (D) Hypermethylation in the promoter region of the *p15*^{INK4B} gene was associated with patients with abnormal karyotype.

Expression Analysis of *DNMT1, DNMT3A*, and *DNMT3B* and *TET2* in Pediatric Primary MDS

Analysis of *DNMTs* expression showed that *DNMT1* and *DNMT3B* are significantly increased in relation to donors. Regarding the expression of *DNMT3A* and *TET2*, there was no significant difference in donor performance (Figure 4).

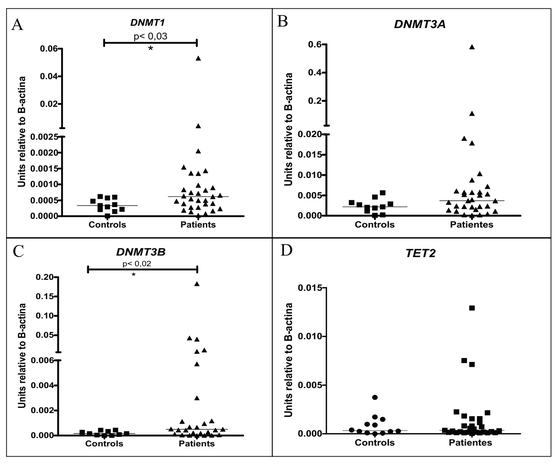


Figure 4: The median of relative expression levels of *DMNTs* and *TET2*. The graphics A, B, C and D show the relative expression levels of *DNMT1, DNMT3A, DNMT3B* and *TET2* genes compared with donors.

CONCLUSIONS

1-The association of *p15*^{INK4B} methylation with disease evolution was significant ($p < 0.009$). Methylation in *p15*^{INK4B} showed a strong association with chromosome 7 alterations, suggesting a probable pathway of disease evolution.

2-The relative expression levels of *DMNT1* and *DMNT3B* genes in MDS patients was higher than observed in donors ($p < 0.03$ and $p < 0.02$, respectively). However, the relative expression levels of *DNMT3A* and *TET2* genes did not show a statistical difference between patients and donors.

3-Our results suggest that -7/7q-, the presence of methylation in *p15*^{INK4B} gene and higher expression of *DMNT1* and *DMNT3B* genes are associated with poor prognosis, being possible biomarkers of disease evolution to AML.

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