Research Article

Aberrant Expression of EZH2 in Pediatric Patients with Myelodysplastic Syndrome: A Potential Biomarker of Leukemic Evolution

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Pediatric myelodysplastic syndrome (MDS) is an uncommon disease and little is known about the molecular alterations of its development and evolution to acute myeloid leukemia (AML). The Enhancer of Zeste Homolog 2 (EZH2) is the catalytic subunit of Polycomb repressive complex 2 (PRC2). It is a histone methyltransferase, that targets lysine 27 of histone 3. This methylated H3–K27 is usually associated with the silencing of genes that are involved in fundamental cellular processes, such as cell proliferation and differentiation. There are only few studies showing the status of EZH2 expression in patients with MDS and they were performed in adult MDS patients. The aim of this study was to analyze the EZH2 expression in pediatric patients with MDS and its association with karyotypes and evolution to acute myeloid leukemia (AML). We conducted the first study of EZH2 expression in pediatric patients with MDS. Considering the EZH2 expression levels in 42 patients and 17 healthy pediatric donors, it was possible to define three groups of expression in patients: low, intermediate, and high. The intermediate level encompassed patients with normal karyotypes, low level included patients with monosomy 7/del(7q) and high level included patients with trisomy 8 and del(11q) (p < 0.0001). Comparing the leukemic evolution, the low expression group presented disease evolution in 100% (8/8) of the cases, the intermediate expression group showed disease evolution in 4.34% (1/23) and in the high expression group, 63.63% (7/11) patients showed evolution from MDS to AML (p < 0.0001). It is important to note that low and high EZH2 expression are associated with leukemic evolution, however low expression showed a stronger association with evolution from MDS to AML than the high expression. Our results suggest a scale of measure for EZH2 expression in pediatric MDS, where aberrant EZH2 expression may be a potential biomarker of disease evolution.

1. Introduction

Myelodysplastic syndrome (MDS) is a heterogeneous group of clonal hematological neoplasms with a variable clinical features and diverse genetic and epigenetic alterations. The major clinical MDS characteristics are ineffective hematopoiesis, dysplasias, peripheral cytopenias, and an increased risk of transformation to acute myeloid leukemia (AML) [1]. MDS is viewed as a disease of adults, particularly the elderly patients. Pediatric MDS is a rare disease, accounting for less than 5% of childhood hematologic malignancies [2–4].

In pediatric patients with MDS, the clonal cytogenetic alterations can be detected in 50–70% of the cases [5]. The most frequent chromosomal abnormalities are monosomy 7 and del(7q) [3]. The cytogenetic evaluation of a bone marrow sample from patients with MDS has become an integral part of
clinical care [5–7]. However, there is a percentage of patients with normal karyotypes. So, it is important a molecular characterization of genetic and epigenetic alterations associated with the evolution of the disease, which could help predict prognosis.

Most knowledge about molecular alterations in MDS was acquired from studies in adult patients and it has been observed the importance of epigenetic alterations in the MDS pathogenesis, since it is the most responsive disease to DNA methylation inhibitors drugs [8]. Epigenetic modifications play important biological roles because they regulate gene expression. There are two main epigenetic modifications: the DNA methylation and the chromatin modification, which are frequently associated to transcriptional gene repression. Among chromatin modifiers, Polycomb Group (PcG) proteins have been established as classical players of epigenetic regulation [9]. PcG proteins contain two core complexes: the maintenance polycomb repressive complex 1 (PCR1) and the initiation polycomb repressive complex 2 (PCR2). The Enhancer of Zeste Homolog 2 (EZH2) is a histone methyltransferase. It is the catalytic subunit of PCR2 for tri-methylation of histone 3 at lysine 27 (H3K27me) by SET domain in its C-terminus, which silences target genes involved in various biological functions as cell cycle, cell proliferation and differentiation. PcG proteins are important epigenetic regulators and critical factors of pluripotency and differentiation of stem cells as well as aberrant gene expression during the malignant transformation [10].

Overexpression of EZH2 is frequently observed in many cancer types, including prostate, breast, bladder, ovarian, lung, liver, gastric esophageal, pancreatic cancer, melanoma, and osteosarcoma. This overexpression in solid tumors is correlated with higher proliferation and poor prognosis. So, there are results suggesting that EZH2 has a critical role in cancer progression and an epigenetic therapy that pharmacologically targets EZH2 may constitute a novel approach to the treatment for some types of cancer [11, 12].

The biological functions of EZH2 in different tumor cells are under intense investigation. In MDS and AML, both overexpression and loss-of-function mutations of EZH2 gene suggest that it can function as an oncogene or as a tumor suppressor gene, respectively [10]. There are only few studies showing the status of EZH2 expression in patients with MDS. These studies were done in adult patients. Xu and colleagues studied for the first time the Polycomb expression genes including EZH2. In this study, it was observed that overexpression of the EZH2 gene is common in MDS and indicates poor prognosis [13]. However, Cabrera and colleagues observed underexpression of EZH2 and its association with chromosome 7 alterations [-7/del(7q)] and poor prognosis in MDS [14].

The functions of EZH2 and its mapping to the critical region for malignant myeloid disorders suggest that the EZH2 gene may be involved in the pathogenesis of 7q35-q36 alterations [15]. Until now, there are no studies showing the expression of EZH2 in pediatric patients with MDS. Therefore, the aim of this study was to analyze the EZH2 gene expression in pediatric patients with MDS, concerning their association with karyotypes, MDS subtypes and evolution from MDS to AML, giving new insights into pediatric MDS pathogenesis.

2. Materials and Methods

2.1. Patients and Controls. Bone marrow cells were obtained from 42 patients with pediatric MDS between 2007 and 2016. The patients included 27 boys and 15 girls, with ages between 5 months and 18 years (the mean age was 8 years). The patients were diagnosed at the Hematology/Oncology Units of hospitals in Rio de Janeiro, Brazil: National Cancer Institute (INCA-RJ) and Martagão Gesteira Institute of Pediatrics and Child Development (IPPMG-UFBJ). Diagnosis and classification were done according to the revised criteria of Hasle and colleagues, 2003 and 2016 [16, 17]. Twenty-two patients were classified as refractory childhood cytophenia (RCC), fifteen as refractory anemia with excess of blasts (RAEB), and five as refractory anemia with excess of blasts in transformation (RAEB-t). None of the patients had been previously treated for a malignancy. Healthy bone marrow samples were obtained from 17 pediatric donors of bone marrow transplant (the mean age was 12 years). This study was approved by the Ethics Committee of the National Cancer Institute (INCA, Rio de Janeiro, Brazil) and all procedures performed followed the bioethics standard, according to resolution 466/12 of Health National Committee.

2.2. Cytogenetic Analysis. Karyotypes of bone marrow cells from the 42 pediatric patients with MDS were obtained from cultures in RPMI 1640, with 20% fetal calf serum (GIBCO) at 37°C for 24 hours. Cell cultures were pulsed with colcemid to a final concentration of 0.05 µg/mL at the final hour of incubation. Cells were subsequently harvested by standard procedures (hypotonic shock: 0.075 M) and fixed in methanol:acetic acid (3:1). GTG-banding was performed. Chromosomes were identified and arranged according to the International System for Cytogenetic Nomenclature, 2016 [18]. Fluorescence “in situ” hybridization (FISH) analyses were performed using dual color probe for chromosome 11 (LSI MLL dual color break apart rearrangement probe) according to the manufacturer’s protocol, to confirm the del(11)(q23) with the allelic loss of MLL/KMT2A. We used the samples of cytogenetic cultures.

2.3. Analysis of EZH2 Gene Expression by Real-Time Quantitative PCR. Analyses of EZH2 mRNA level alterations in 42 pediatric patients with primary MDS and 17 healthy pediatric controls were carried out by real-time quantitative polymerase chain reaction (qRT–PCR). Total mRNA was obtained from the 42 pediatric patients with MDS and 17 healthy pediatric controls by real-time quantitative PCR. Total mRNA was obtained from the 42 pediatric patients with MDS and 17 healthy pediatric controls by real-time quantitative polymerase chain reaction (qRT–PCR). Total mRNA was obtained from the 42 pediatric patients with MDS and 17 healthy pediatric controls by real-time quantitative polymerase chain reaction (qRT–PCR). Total mRNA was obtained from the 42 pediatric patients with MDS and 17 healthy pediatric controls by real-time quantitative polymerase chain reaction (qRT–PCR). Total mRNA was obtained from the 42 pediatric patients with MDS and 17 healthy pediatric controls by real-time quantitative polymerase chain reaction (qRT–PCR). Total mRNA was obtained from the 42 pediatric patients with MDS and 17 healthy pediatric controls by real-time quantitative polymerase chain reaction (qRT–PCR). Total mRNA was obtained from the 42 pediatric patients with MDS and 17 healthy pediatric controls by real-time quantitative polymerase chain reaction (qRT–PCR).
of 20 seconds at 95°C, 30 seconds at 60°C, and 30 seconds at 72°C. The dissociation curve was used to determine the PCR efficiency, specificity of amplification and primer dimer formation. B-actin mRNA levels were used as a reference for normalization. The following primers sequence used were: EZH2 Forward 5′-TTGTGACAGTTCGTGCCCTTGT-3′ and EZH2 Reverse 5′-TGCTTGGTGTTGCACTGTTGCTT-3′; B-actin Forward 5′-TTGTGACAGTTCGTGCCCTTGT-3′ and B-actin Reverse 5′-TGCTTGGTGTTGCACTGTTGCTT-3′. The relative expression levels of EZH2 gene were calculated using the ∆∆CT method.

2.4. Statistical Analysis. The statistical difference between EZH2 expression in pediatric patients and donors (pediatric healthy individuals) was analyzed by Mann–Whitney test. This test was also used in the analyses of age groups (children versus adolescents), MDS subtypes (initial stage, RCC, versus advanced stages, RAEB and RAEB-t), karyotypes (normal karyotypes versus abnormal karyotypes) and EZH2 expression in donors and low EZH2 expression patients, donors and intermediate EZH2 expression patients and donors and high EZH2 expression patients. The Kruskal–Wallis test was used to verify the statistical difference between three groups defined according to the level of EZH2 expression (low, intermediate and high). We also analyzed the levels of EZH2 expression and the evolution of disease (from MDS to AML) through the chi-square test with Yates correction. The difference between low expression group and high expression group was analyzed by Fisher’s exact test. A value of \( p < 0.05 \) was considered significant in all analyses.

3. Results

3.1. Clonal Chromosomal Abnormalities in Pediatric Patients with Primary MDS. Among a total of 42 pediatric patients with primary MDS, clonal chromosomal abnormalities were detected in 24 patients (57%). The frequency of the chromosomal abnormalities in pediatric patients with primary MDS is shown in (Figure 1). The distribution of abnormal karyotypes according to MDS subtypes was: 23% (5/22) in RCC, 93% (14/15) in RAEB, and 100% (5/5) in RAEB-t. Cytogenetic results showed that patients with RCC presented normal karyotypes or single abnormalities as: del(4q), del(9p), del(11q), del(12p), and +mar. In the RAEB and RAEB-t subtypes, we observed single chromosomal abnormalities as +6, del(7q), −7, +8, del(11q), del(17p), +14, and complex karyotype. In the cases of del(11q), FISH analyses were performed showing the del(11) (q23) with allelic loss of MLL/KMT2A. The clinical and cytogenetic characteristics in pediatric patients with primary MDS is shown in Table 1.

3.2. Analysis of EZH-2 Gene Expression in Pediatric Patients with Primary MDS. The analysis of EZH-2 gene relative expression levels in 42 pediatric patients with primary MDS showed a higher expression when compared to the controls (donors), which led to a statistically significant analysis \( (p < 0.04) \) (Mann–Whitney test) (Figure 2(a)). Analyzing the EZH-2 relative expression levels according to the subtypes of MDS, being 22 patients classified at initial stage (RCC) and 20 patients classified at advanced stages (RAEB and RAEB-t), we
found no statistical significance (Figure 2(b)). The association of \( EZH2 \) relative expression levels between patients with normal karyotypes and abnormal karyotypes did not show a statistical significance (Mann–Whitney test) (Figure 2(c)).

However, it is very interesting to note that analyzing the distribution of the expression levels of \( EZH2 \) in patients and donors, it is clearly observed that there is a heterogeneous distribution in patients, while the distribution of expression levels in controls is more homogeneous. From this result, it was possible to define three distinct groups of \( EZH2 \) expression. This analysis was based mathematically on quartiles [19]. Since the distribution of the \( EZH2 \) expression in patients is skewed, we used the median as central measure and quartiles as dispersion measures, according to Zar, 2010 [19]. We found 1.8 for the median. The first quartile is 1 and the third quartile is 2.85. We divided the \( EZH2 \) expression in patients into three groups: low, intermediate, and high. In order to compare \( EZH2 \) expression of patients and donors, we also used the median and quartiles for the donors. In normal individuals (donors), the median was 1.15 and according to the classes of \( EZH2 \) expression, the median at low level was 0.2, for intermediate level the median was 1.61 and for high expression, the median was 4.81 (Figure 3(a)). We observed a significant difference between the \( EZH2 \) expression in donors and in low \( (p < 0.0001) \) (Figure 3(b)), intermediate \( (p < 0.0057) \) (Figure 3(c)) and high \( (p < 0.0001) \) (Figure 3(d)) \( EZH2 \) expression groups. It was possible to suggest a scale of \( EZH2 \) expression, where patients with \( EZH2 \) expression belonging to the real interval \([0,1)\) were classified in the low group, the real interval \([1,3)\) represented the intermediate group and the real interval \([3,13)\) represented the high group. We have \( n = 8 \), \( n = 23 \), and \( n = 11 \) as the number of patients in each group, respectively (Table 2).

Then, we analyzed the association of \( EZH2 \) expression levels with specific karyotypic patterns. Our results showed that intermediate \( EZH2 \) expression level encompassed patients with normal karyotypes \( (n = 18) \), low level of expression included patients with monosomy 7 and del(7q) \( (n = 8) \) and high level of expression included patients with trisomy 8 \( (n = 3) \) and del(11q) \( (n = 3) \). We observed a statistical difference between these groups \( (p < 0.0001, \text{Kruskal–Wallis test}) \) (Figure 4). It is important to notice that karyotypes can be categorized using the scale of \( EZH2 \) expression suggested in this work. More precisely, none patients with monosomy 7/del(7q) was in high expression group and none of patients with trisomy 8/del(11q) was in low expression group (Table 2). It is also important to observe that we found other karyotypes, as we can see in Figure 1, but we considered normal karyotypes, monosomy 7/del(7q) and trisomy 8/del(11q) for the association analysis with \( EZH2 \) expression, because of the number of patients in these cytogenetic groups (Table 2).

We also investigated the \( EZH2 \) relative expression levels and the evolution of the disease according to the three \( EZH2 \) expression groups. The low expression group presented evolution of the disease in 100% of cases (8/8 patients), the intermediate expression group showed disease evolution in 4.34% patients (1/23) and in the high expression group, 63.63%
patients (7/11) showed evolution from MDS to AML. We verified through the chi-square test with Yates correction a statistical significance in this result ($p < 0.001$). It is important to note that the difference between low expression group (8/8) and high expression group (7/11) is not significant statistically ($p = 0.085$, Fisher’s exact test). This result is very important, because it demonstrates that both groups, the low $EZH2$ expression group and the high $EZH2$ expression group, are associated with evolution from MDS to AML. Moreover, our result suggests that it seems that the low expression group presents a more elevated risk of progression to AML, since there was a 100% of disease evolution in the cases studied. Our results suggest that this scale of measure for $EZH2$ expression in pediatric MDS can give us a better understanding of the evolution from MDS to AML (Table 2).
Table 3: Association of EZH2 gene expression with clinical features, karyotypes and leukemic evolution in pediatric patients with primary MDS.

| Parameter                                      | EZH2 gene expression (median) | p value  |
|------------------------------------------------|-------------------------------|----------|
| Age of patients                                |                               |          |
| < 12 years old (children), n = 32              | 1.8                           | p < 0.4  |
| > 12 years old (adolescents), n = 10           | 2.7                           |          |
| Pediatric patients (n = 42)                    | 1.8                           | p < 0.04 |
| Pediatric healthy individuals (n = 17)         | 1.15                          |          |
| MDS subtypes                                   |                               |          |
| Initial stage (RCC) (n = 22)                   | 1.7                           | p < 0.9  |
| Advanced stages (RAEB/RAEB-t) (n = 20)         | 2.9                           |          |
| Karyotypes                                     |                               |          |
| Normal (n = 18)                                | 1.7                           | p < 0.8  |
| Abnormal (n = 24)                              | 1.8                           |          |
| Specific chromosomal pattern                   |                               |          |
| Normal karyotypes (n = 18)                     | 1.7                           |          |
| −7/del(7q) (n = 8)                             | 0.2                           | p < 0.0001|
| +8 (n = 3)                                     | 5.96                          |          |
| del(11q) (n = 3)                               | 6.35                          |          |
| Levels of EZH2 expression                      |                               |          |
| Low (n = 8)                                    | 0.2                           |          |
| Intermediate (n = 23)                          | 1.61                          | p < 0.0001|
| High (n = 11)                                  | 4.81                          |          |
| Donors versus levels of EZH2 expression (groups)|                               |          |
| Donors (n = 17) vs. Low expression patients (n = 8) | 1.15 | 0.2 | p < 0.0001 |
| Donors (n = 17) vs. Intermediate expression patients (n = 23) | 1.15 | 1.61 | p < 0.0057 |
| Donors (n = 17) vs. High expression patients (n = 11) | 1.15 | 4.81 | p < 0.0001 |
| Evolution of disease (MDS → AML) versus levels of EZH2 expression | Low (8/8) | Intermediate (1/23) | p < 0.0001 |
| Evolution of disease (MDS → AML) versus levels of EZH2 expression | Low (8/8) | High (7/11) | p = 0.085 |

4. Discussion

The molecular pathogenesis of pediatric MDS remains poorly understood due to its rarity, high heterogeneity and complexity of the disease. Until now, few studies have evaluated the expression of EZH2 in MDS. Xu and colleagues evaluated the expression of the EZH2 gene in bone marrow samples from 54 adult patients with MDS using the qRT-PCR methodology. The authors verified increased expression of EZH2 and two other Polycomb genes, BMI1 and RING1, in these patients. In this study, it is interesting to note that cytogenetic analysis showed, as main numerical chromosomal abnormality, the trisomy 8 and it was not detected the alterations in chromosome 7 (monosomy 7 and del 7q). The authors suggested that increased expression of Polycomb genes, including the EZH2, is an event related to poor prognosis in MDS [13]. On the other hand, Cabrero and colleagues studied the expression of EZH2 in 78 adult patients with MDS. According to this study, patients who presented alterations in chromosome 7, such as monosomy 7 and 7q deletion, showed a significantly lower level of expression when compared to controls, diploid patients and other chromosomal abnormalities [including del(5q), +9, +16, +8, del(11q), −Y, t(7;20), inv3, del(20q)]. In this study, the underexpression of EZH2 and alterations of chromosome 7 were associated with poor prognosis [14]. McGraw and colleagues studied EZH2 protein expression by immunohistochemistry in 33 MDS patients. It was observed that EZH2 expression was significant lower in −7/del(7q) compared to those patients without these chromosomal alterations [20]. Xu and colleagues showed that the genomic loss of EZH2 [−7 and del(7q)] leads to low EZH2 expression in MDS and it is associated with shorter survival and increased AML transformation [21]. However, Sashida and colleagues showed that EZH2 loss promotes development of MDS, but attenuates its predisposition to leukemic transformation [22]. Thus, it is possible to note that studies regarding the role of EZH2 in MDS in adult patients are still controversial.

We conducted the first study of EZH2 expression in pediatric patients with MDS. In our study, we observed a statistically significant difference in the expression of EZH2 between the group of patients and the group of controls. Patients showed an EZH2 expression distribution ranging from 0.03 to 12.8 and controls showed an EZH2 expression distribution ranging from 0.71 to 1.93. So, the EZH2 expression distribution observed in
patients is more heterogeneous than that in the controls. From this observation, it was possible to define three distinct groups of expression (low, intermediate, and high) using the median as central measure for the study. These results suggest a scale of measure for EZH2 expression in pediatric MDS.

Analyzing the expression levels of EZH2 in pediatric patients with MDS and the evolution of disease, we observed that the low expression group presented evolution of the disease in 100% of the cases, the intermediate expression group showed disease evolution in 4.34% patients, and in the high expression group, 63.63% patients showed evolution from MDS to AML. This result was significant ($p < 0.0001$). Thus, our results suggest that aberrant expression of EZH2 is associated with leukemic transformation, being a biomarker of disease evolution. It is important to note that low and high expression levels were associated with leukemic evolution; however, low expression showed a stronger association with evolution from MDS to AML than the high expression.

Regarding the evolution from MDS to AML, McGraw and colleagues suggested the EZH2 protein analysis by immunohistochemistry may be a molecular tool for discriminating disease outcome or transformation risk [20]. In our study, we also observed the importance of EZH2 expression results in MDS. So, studies based on EZH2 expression may have relevant clinical implications and may be incorporated as an additional laboratory test using also the real-time quantitative polymerase chain reaction (qRT-PCR).

Accumulating studies have demonstrated that EZH2 participates in various biological processes and displays different modes of action [10]. These results showed that the clinical impact of aberrant EZH2 expression on the progression of MDS is complex and appears to be associated with other events such as the presence of a specific chromosomal abnormality. This hypothesis may be re-inforced because in MDS there are different molecular evolution pathways associated with specific cytogenetic abnormalities [23, 24]. Based on these results, it is necessary new studies to clarify the signaling pathways in the three groups of EZH2 expression and studies involving a larger number of patients. Some studies have been reported the molecular pathways associated with EZH2 [10, 20, 25]. Xu and colleagues demonstrated that genomic loss of EZH2 contributes to overexpression of the HOX gene clusters in MDS by reducing H3K27me3 [21].

Overexpression of EZH2 has been found in different cancer types [26]. EZH2 was found to be overexpressed in MDS tumor cells associated with methylation of tumor suppressor gene cyclin dependent kinase inhibitor 2B (p15INK4B) compared with tumor cells that p15INK4B is not methylated. This association between EZH2 and p15INK4B was not observed in other PcG genes (EED, SUZ12, BMI-1 and RING1), suggesting that EZH2 is involved in the methylation of p15INK4B gene [13]. Methylation in p15INK4B and p16INKA were observed in pediatric MDS suggesting that these genes may play an important role during evolution from MDS to AML [2]. Recently, Ye and Li demonstrated in a systematic meta-analysis the important role of p15INK4B in the development, progression, and poor prognosis of MDS [27].

In prostate cancer, there is a positive feedback mechanism between Myc and EZH2, where Myc overexpression is associated with increased EZH2 and EZH2 could also induce Myc overexpression. Myc is a transcription factor that regulates cell proliferation. It has been considered as an important factor of the increase expression of EZH2 by repressing microRNAs expression (miR26a and miR26b). The initiation and evolution of prostate cancer is associated with overexpression of Myc and EZH2 [28]. In other tumors, like medulloblastoma, cooperation between Myc and EZH2 was also observed, where higher Myc levels were associated with increased EZH2 and pharmacological blockade of EZH2 is a potential therapeutic strategy for aggressive medulloblastoma [29]. Different mechanisms are involved in c-Myc deregulation expression in hematological neoplasms, such as chromosome rearrangements, amplification and epigenetic mechanisms. Increased expression of EZH2 by c-Myc has been described in AML. As observed in solid tumors, in hematological neoplasms, there is also an association between c-Myc deregulation and EZH2 overexpression [30]. In chromosome 8, region q24 is located the gene cMyc. In our study, we observed an association of trisomy 8 and an overexpression of EZH2, associated in evolution of disease. It was already observed that trisomy 8 cells in MDS have amplification intrachromosomally of c-Myc [31]. Therefore, it is possible, like in other types of cancer that c-Myc and EZH2 overexpression cooperate in the pathogenesis of MDS, mainly during evolution to AML.

It has been described that imbalance between Polycomb (PcG) and Trithorax (TrxG) expressions is involved in development of diseases, as cancer. The PcG proteins usually maintain the repression of gene expression and TrxG proteins act in the opposite way [32]. KMT2A (MLL) gene is located in region q23 in chromosome 11 and it is an important member of TrxG family, involved in pediatric acute leukemia pathogenesis [33]. Abnormalities in the balance between PcG and TrxG may be associated with leukemia pathogenesis [32]. In our study, we found an association of del(11q), involving the loss of KMT2A allele, and an overexpression of EZH2. Our results suggest that a possible mechanism involved in the evolution of disease in this case may be related with an imbalance between the expression of EZH2 and KMT2A genes.

The EZH2 aberrant expression in cancer cells may result from different mechanisms involved in poor prognosis with evolution of disease. Therefore, further investigation is necessary to a better understanding of the mechanisms associated with low and high expression of EZH2 during the pathogenesis of MDS.

EZH2 is a master regulator of chromatin and accumulated evidence suggests that it is involved in aberrant transcriptome in cancer cells. Thus, EZH2 is a potent target for cancer therapy and EZH2 inhibitors have been under pre-clinical and clinical investigations [25]. However, as demonstrated in other studies, the EZH2 may have tumor suppressive and oncogenic functions in MDS and in AML. It is also demonstrated that EZH2 has a dual role in the same disease, acting in different phases of the AML. This dual function has potential therapeutic implications [34, 35]. Analyzing our results of EZH2 expression in pediatric patients with MDS, it is also possible to suggest the dual role of EZH2 and this result has clinical implications, regarding the
evolution of disease and also to aid the choice of the treatment, reinforcing the importance of incorporate additional laboratory tests to analyze the EZH2 expression. Since this study was the first to investigate the association of aberrant expression of EZH2 with karyotypes and disease evolution in pediatric patients with MDS, a more number of studies are necessary to confirm our results and the relevant contribution of EZH2 in the pathogenesis of pediatric MDS.

5. Conclusions

We conducted the first study of EZH2 expression in pediatric patients with MDS. Considering the EZH2 expression levels in patients and healthy pediatric donors, it was possible to define three groups of expression in patients: low, intermediate, and high. The intermediate level encompassed patients with normal karyotypes, low level included patients with monosomy 7/del(7q) and high level included patients with trisomy 8 and del(11q) \((p < 0.0001)\). Comparing the leukemic evolution, it was important to note that low and high EZH2 expression were associated with leukemic evolution. However, the low expression showed a stronger association with evolution from MDS to AML than the high expression. Our results provides new insights about the pediatric MDS pathogenesis with the focus on the aberrant expression of EZH2, suggesting a scale of measure for EZH2 expression in pediatric MDS, where aberrant EZH2 expression may be a potential biomarker of disease evolution.

Data Availability

The data used to support the findings of this study are included within the article and the original data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interests regarding the publication of this paper.

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