Expression of Nuclear Pore Protein POM121 in Childhood Acute Leukemias and Its Relationship with Prognosis

Purpose: Nuclear pore complexes are a large group of proteins responsible for molecular passages between the cytoplasm and the nucleus. We aimed to investigate the status of POM121 gene expression, which is one of the nuclear pore proteins in childhood acute leukemias, compared with the normal population, and its relationship with prognosis and other clinical findings.

Methods: Fifty-nine patients with ALL and 21 patients with AML, followed up and treated between January 2008 and November 2013, and 36 control subjects were included in the study. A real-time PCR method was used to detect POM121 gene expressions.

Results: The mean value of POM121 expression was 3.75±2.91 in ALL patients, 5.79±7.04 in AML patients, and 3.32±3.76 in the control group. POM121 expression was markedly higher in AML patients, but there was no statistically significant difference compared with the control group and ALL patients. Overall survival (OS) results were better in patients with lower POM121 expression than the mean of the control group among ALL and AML patients. However, the results were not statistically significant. Among ALL patients, patients with a higher POM121 expression than the mean of the control group, patients who had relapse and central nervous system involvement, patients who were in the standard risk group and without thrombocytopenia had statistically significantly lower OS results in the 3rd and 10th years.

Conclusions: This is the first study in the literature to show the relationship between POM121 expression and prognosis in childhood leukemias, and this will be clarified further with more comprehensive studies.

KEYWORDS

Acute leukemia, POM121 expression, prognosis

1. INTRODUCTION

Nuclear pore complexes (NPC) are multiprotein structures found in all eukaryotic cells which are responsible for the passages between the cytoplasm and the nucleus.[1] NPCs also have many cellular functions such as differentiation, cell division, chromatin organization, and epigenetic modification.[2] NPCs are composed of approximately 30 different proteins called nucleoporins.[3] Nucleoporins are typically grouped in subcomplexes and are differentiated according to their different sequence and structural motifs.[4]

Many studies have been conducted in the literature on the oncogenic role of nucleoporins in human carcinogenesis in solid tumors.[5] In recent years, numerous studies have been published, which have emphasized the importance of nucleoporins in hematological malignancies.[6,7] Hematological malignancies are usually associated with chromosomal rearrangements, which lead to the expression of chimeric fusion proteins.[8] The proteins known to be part of such oncogenic fusions include the nucleoporins NUP98 and NUP214. The rearrangement of the genes encoding these two nucleoporins plays a role in the pathogenesis of, particularly acute myeloid leukemia, and sometimes myelodysplastic syndrome, and T-cell acute lymphoblastic leukemia (T-ALL).[9]

POM121, one of the nucleoporins, is called the transmembrane nucleoporin and is located in the nuclear envelope together with NUP210 and NDC14. POM121 is also one of the PAX5 fusion genes. PAX5, a transcription factor, encodes the B-cell lineage-specific activator protein and is the main regulator of B-cell development.[10] PAX5 fusion partners include not only transcription factors but also structural proteins such as ELN and POM121.[11] There is a limited number of studies in the literature showing that POM121 plays a role in leukemogenesis.[10,12] In this study, it was aimed to investigate the expression of POM121, one of the nuclear pore proteins, which is considered to be associated with the pathways related to cell proliferation in childhood acute leukemias, relative to the normal population, and its relationship with prognosis and other clinical findings.

2. METHODS

2.1. Patients

Patients who presented to the Pediatric Oncology Clinic of Çukurova University Hospital between January 2008 and November 2013 and were diagnosed with acute leukemia were included in this study. The number of patients diagnosed within this period was 117, and 24

of these patients continued their follow-up at other centers. In 80 out of 93 patients with acute leukemia who were followed up and treated in our clinic, RNA samples were isolated at the time of diagnosis, and these patients were included in the study. Of 80 leukemia patients whose RNA samples were isolated and POM121 gene expression was analyzed, 59 were diagnosed with acute lymphoblastic leukemia (ALL) and 21 were diagnosed with acute myeloblastic leukemia (AML). Patients with ALL L3 subtype were not included in the present study. The prognosis of the disease was evaluated in these patients until January 2020. Thirty-six children who presented to the Pediatrics Department of Çukurova University Faculty of Medicine General Children's Outpatient Clinic for check-up purposes, whose physical examination was normal, and who were found to be hematologically normal with complete blood count were included in the study as the control group for POM121 gene expression.

2.2. Treatments

Patients diagnosed with ALL and AML were subdivided according to the French–American–British classification and according to immunophenotyping. ALLs were immunophenotypically divided into the subgroups of B-ALL and T-ALL. The terminology of "Cluster of Differentiation, CD" was used to identify cell surface antigens. In immunophenotyping studies, those who scored above 20% in CD surface marker antigens were evaluated as positive.[13] Patients with ALL were treated with the chemotherapy protocol based on BFM TR-ALL 2000, and AML patients were treated with the chemotherapy protocol based on idarubicin+ARA-C.[14,15] Ethics committee approval was obtained from Çukurova University Faculty of Medicine, Non Interventional Clinical Research Ethics Committee with the decision number 28/15 dated February 14, 2014. Informed consent was obtained from the patients, control subjects, and/or legal guardians before enrollment in the study, which was conducted in accordance with local institutional regulations.

2.3. Study procedures

Blood samples of 3 ml were taken into a tube with ethylenediaminetetraacetic acid (EDTA) from the patients included in the study at the time of diagnosis and from the controls. First of all, the isolated RNA samples were stored in a freezer at −80°C until the day of the study. White blood cell values of the whole blood samples were detected before starting the study. RNA isolation from leukocyte cells was performed using the High Pure RNA isolation kit. RNAs obtained were translated to cDNA with Transcriptor First Strand cDNA Synthesis Kit

and stored at −20°C. A real-time polymerase chain reaction method was used to detect POM121 gene expressions. This study was conducted using a Light Cycler (Roche Applied Science). Housekeeping genes and target genes were analyzed with "Advanced Relative Quantification" calculations on LC 480 Software at a wavelength in accordance with the design.

2.4. Statistical analyses

All statistical analyses were performed using the statistical package for social sciences (SPSS v22) software package. Pearson correlation test was performed for correlation and Kaplan–Meier method was used for life analysis in the evaluation of the data. In addition, chi-squared, one-way analysis of variance, and Mann–Whitney U tests were used to compare the groups. $P<0.05$ was considered statistically significant.

3. RESULTS

Of the 80 acute leukemia patients, 28 (35%) were diagnosed with ALL L1, 31 (38.75%) with ALL L2, and 21 (26.25%) with AML. The number of controls was 36. ALL patients consisted of 39 boys (66.1%) and 20 girls (33.9%), and the boy/girl ratio was 1.95; AML patients consisted of 13 boys (61.9%) and 8 girls (38.1%), and the boy/girl ratio was 1.62. The control group consisted of 22 boys (61.1%) and 14 girls (38.9%), and the boy/girl ratio was 1.57. The age of the patients with ALL ranged from 5 months to 210 months, and the mean age was 91.22±63.39 years. The age of patients with AML ranged between 12 months and 172 months, and the mean age was 107±52.11 months. The ages of the children in the control group were between 5 months and 213 months, and the mean age was 87.78±55.80 months.

The mean value of POM121 expression was 3.75±2.91 in ALL patients; the mean value in the control group was 3.32±3.76, and no statistically significant difference was found between them ($p=0.539$). The mean value of POM121 expression was 5.79±7.04 in AML patients, and no statistically significant difference was found in the POM121 expression in AML patients compared with the control group ($p=0.089$). POM121 expression was significantly higher in AML patients than in ALL patients. However, when ALL and AML patients were compared with each other, the difference was not statistically significant ($p=0.069$).

When POM121 expression was evaluated with gender, relapse, survival, anemia, thrombocytopenia, neutropenia, hepatomegaly, splenomegaly, and lymphadenopathy in

patients with acute leukemia, there was a significant difference between POM121 expression and neutropenia only in ALL patients (p=0.016) (Table 1).

In ALL patients, there was a positive correlation between POM121 expression and urea (p=0.001; r=416), creatinine (p=0.001; r=422), uric acid (p=0.001; r=424), and CD117 (p=0.005; r=416). In AML patients, on the other hand, there was a positive correlation between POM121 expression and LDH (p=0.031; r=472). When POM121 expression was compared based on ALL risk groups, immunophenotypes, and ALL subtypes, there were no statistically significant results (Table 2).

In ALL and AML patients, POM121 expression was divided into two groups: high and low compared with the mean of the control group. Patients with POM121 expression higher than the mean of the control group were referred to as Group 1, and patients with POM121 expression lower than the mean of the control group were referred to as Group 2. ALL patients consisted of 24 patients in Group 1 and 35 patients in Group 2. AML patients consisted of 8 patients in Group 1 and 13 patients in Group 2. The effect of POM121 expression being higher and lower than the mean of the control group on overall survival (OS) was investigated in ALL and AML patients. In the 36th, 60th, and 120th months, OS was 68%, 58%, and 58%, respectively, in ALL patients in Group 1, and 77%, 70%, and 70%, respectively, in ALL patients in Group 2 (Figure 1). In AML patients, OS was 58% in Group 1 in the 36th, 60th, and 120th months, and it was 77%, 70%, and 70%, respectively in Group 2 (Figure 2). Overall, OS results of the patients in Group 2, whose POM121 expression was lower than that of the control group in both ALL and AML patients, were better. However, the results were not statistically significant (p=0.356 and p=0.504, respectively).

When OS results of patients were evaluated according to immunophenotyping, in B-ALL patients, there were 13 patients in Group 1 with a POM121 expression higher than the mean of the control group, and 25 patients in Group 2 with a lower POM121 expression than the mean of the control group. In B-ALL patients in Group 1, OS was 54%, 46%, and 46% in the 36th, 60th, and 120th months, respectively, and it was 84%, 75%, and 75%, respectively, in Group 2 patients (Figure 3). In T-ALL patients, in the 36th, 60th, and 120th months, 8 patients in Group 1 had an OS of 88% and 5 patients in Group 2 had an OS of 60% (Figure 4). In B-ALL patients, the OS results of patients in Group 2 with a lower POM121 expression were better compared with those of the control group, as in ALL and AML patients, but the results were not statistically significant. In T-ALL, in contrast, patients in Group 1 with a

higher POM121 expression than the mean of the control group had better OS results. However, the results were not statistically significant (p=0.065 and p=0.269, respectively).

The effects of some clinical and laboratory features and POM121 expression being higher or lower than the control average on the OS of patients in the 3rd and 10th year were compared in ALL patients. This comparison could not be made in AML patients because the number of patients in the groups was not sufficient. ALL patients in Group 1 with relapse and central nervous system (CNS) involvement had a lower OS in the 3rd and 10th years. However, the results of these patients were also lower compared with Group 2. Patients in standard risk group (SRG) and without thrombocytopenia in Group 1 had lower OS in the 3rd and 10th years. The results were statistically significant (Table 3).

4. DISCUSSION

Attempts have been made to define the role of NPCs in cancer in a detailed manner in several recent reviews.[16-18] For the first time in the literature, Blobel et. al. showed evidence that Nup214 had an oncogenic role in the development and progression of hematological malignancies in 1994.[19] After this study, it has been shown that some other nucleoporins (Nup62, Nup88, Nup98, Nup358/RanBP2, and Tpr) are also associated with tumorigenesis.[20]

There are numerous studies in the literature that analyze the relationship between NPCs and cancer; however, the number of studies that analyze the relationship between POM121, a nucleoporin, and leukemia is limited, and the relationship between POM121 and leukemogenesis could not be demonstrated.[10-12]

In the present study, POM121 expression was significantly higher in AML patients compared with ALL patients and the control group, but the difference was not statistically significant when ALL and AML patients were compared with each other (p=0.069). If there were more patients, a significant result could possibly be obtained.

In this study, when POM121 expression was assessed with gender, relapse, survival, anemia, thrombocytopenia, neutropenia, hepatomegaly, splenomegaly, and lymphadenopathy in patients with acute leukemia, there was a significant difference between POM121 expression and neutropenia in only ALL patients. In fact, POM121 expression was found to be lower in ALL patients with neutropenia (p=0.016). There was no correlation between POM121 expression and clinical and laboratory results in AML patients. This may also be because of the small number of cases.

There was no significant correlation between POM121 expression and ALL type (ALL L1 and ALL L2), immunophenotype (B-ALL and T-ALL), and risk group (SRG, MRG, and HRG) in the ALL patient group of this study.

A study by Nebral et al. showed that PAX-5 rearrangements were observed in B-cell precursor ALL at a rate of approximately 2.5% in 446 cases of childhood ALL. In this study, several new PAX5 common genes, such as DACH1, HIPK1, JAK2, and BRD1 including POM121, have been identified. The data of the study showed that they contain not only transcription factors but also structural proteins and genes such as ELN and POM121 are involved in signal transduction; however, these do not play a role in tumor formation.[10]

In the present study, the OS results of patients in Group 2, which had lower POM121 expression than the control group in both ALL and AML patients, were better. However, the results were not statistically significant. We also found that POM121 expression was significantly lower in the group with neutropenia in ALL patients. The occurrence of neutropenia in these patients can be considered to be associated with a high blast production rate. However, having neutropenia, thus an increased risk of infection, may have led to an inverse relationship. This will be clarified by expansion of the infection parameters and analyzing POM121 expression.

Denk et al. reviewed 12 cases with PAX5 fusion genes in t (7; 9) (q11.2; p13) leukemia and reported that two of them were cases containing the PAX5–POM121 fusion gene, but the relationship of these genes with the prognosis could not be demonstrated.[21] In a study with multiple PAX5 fusion proteins, including PAX5–POM121, it was shown that these proteins share some predominant features including nuclear localization and DNA binding, and this study demonstrated the possible functions of these proteins, as well as highlighted their effects on development of leukemia.[12]

When the OS results of patients were evaluated according to immunophenotyping, in B-ALL patients, the OS results of patients in Group 2, with lower POM121 expression compared with the control group, were better at 36, 60, and 120 months with 84%, 75%, and 75%, respectively. However, the results were not statistically significant (p=0.065). In contrast, in T-ALL, the OS results of patients in Group 1 were better with 88% at the 36th, 60th, and 120th months. However, the results were not statistically significant (p=0.269). This suggests that there is a different mechanism that we are not aware of in T-ALL patients.

When the effects of some clinical and laboratory features in ALL patients and POM121 expression being higher or lower than the mean of the control group on OS in the 3rd and 10th years of patients were compared, the OS results of ALL patients in Group 1 with relapse and CNS involvement in the 3rd and 10th years were lower than those in Group 2. Thus, it can be said that the higher POM121 expression compared with the control group adversely affects the prognosis. This assessment was not made in AML patients because there were not enough cases in the groups.

In a recent study of POM121 by Rampello et al., it has been reported that the nucleoporins, especially POM121 is involved in nuclear envelope herniations (blebs) and Torsin ATPase deficiency is observed in its defects, which may cause a predisposition to leukemia by reducing the sequestration of myeloid leukemia factor 2.[22]

In conclusion, in the present study, POM121 expression was found to be higher in AML patients compared with the control group and ALL patients. In ALL and AML patients, the OS results of patients in Group 2 with lower POM121 expression were better compared with mean of the control group. In T-ALL, in contrast, the OS results of patients in Group 2 with a higher POM121 expression were better compared with the mean of the control group. However, not all results were statistically significant. OS results of ALL patients in Group 1 with relapse and CNS involvement, those who were in SRG and, interestingly, those without thrombocytopenia, were statistically significantly lower compered with the group 2. Had there been a higher number of cases in this study, we would have definitely stated that high POM121 expression adversely affects the prognosis in patients with acute leukemia. This study is the first in the literature to demonstrate the relationship between POM121 expression and prognosis in childhood leukemias, and more comprehensive, multi-centered studies with a higher number of cases will further clarify these results and reveal the relationship of POM121 expression with the diagnosis and prognosis.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

ACKNOWLEDGMENTS

This study was supported by “Çukurova University Research Projects Funding Unit” with Project number TF2013 LTP30.

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Figure legends

Figure 1. Overall survival of acute lymphoblastic leukemia (ALL) patients

Figure 2. Overall survival of acute myeloblastic leukemia (AML) patients

Figure 3. Overall survival of B-cell acute lymphoblastic leukemia (B-ALL) patients

Figure 4. Overall survival of T-cell acute myeloblastic leukemia (T-ALL) patients