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The Relationship Between Bcr-Abl 1 Related MicroRNA's Expression Levels And Imatinib Resistance In Chronic Myeloid Leukemia Cases

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Abstract

Introduction: BCR-ABL fusion gene is targeted in the treatment of chronic myeloid leukemia and is essential in its follow-up. MicroRNAs are small RNA molecules with oncogenic and tumor suppressor properties. The aim of this study is to elucidate the roles of BCR-ABL1-related miRNAs in TKI resistance in CML.

Materials and Methods: This study was carried out on peripheral bloods collected from 25 imatinib-sensitive and 25 imatinib-resistant CML patients, and 50 healthy controls. miR-23a, miR-138, miR-181, miR-152-3p and miR-101 expression levels were determined by qRT-PCR.

Results: Our results showed that miR-23a, miR-101, miR-152 and miR-181 were downregulated in CML patients while miR-138 was downregulated in imatinib resistant group compared to sensitive group.

Conclusion: To the best of our knowledge, our study is the first study comparing miRNA expression levels in imatinib-sensitive and resistant CML cases. miR-138 may have a potential to be an indicator of imatinib resistance. This may provide evidence for potential therapeutic targets as well as novel prognostic biomarkers of imatinib resistanc[1]e in CML.

Key words: chronic myeloid leukemia; imatinib; microRNA

Introduction

Chronic myeloid leukemia (CML) is a clonal myeloproliferative malignancy that is based on the occurence of the BCR-ABL1 fusion that is created by a reciprocal translocation between chromosome 9 and 22, t (9;22) (1). Oncogene BCR-ABL1 is a structurally active tyrosine kinase leads to the pathophysiology of CML. BCR-ABL activates several signal transduction pathways like JAK, c-Myc, MAPK, and N-RAS pathways that are responsible for prevention of cellular differentiation, initiation of cellular proliferation, loss of adhesion, and inhibition of apoptozis (2).
Over decades, new therapies targeting BCR-ABL molecule were developed through focusing on CML genetics. As a result of these studies, the first drug discovered was imatinib. Imatinib inhibits the ATP-binding site of the fusion protein, thereby inhibiting the phosphorylation of proteins in further signal transduction. Patients develop resistance to imatinib over time, due to duplications or mutations in BCR-ABL1, which disrupts the suppression of kinase activity or unable imatinib to bind to its target. Resistance may also occur by a BCR-ABL1- independent mechanisms. This initiated the development of new generation tyrosine kinase inhibitors (TKIs) that would be effective on imatinib resistant cases (1). Imatinib, dasatinib, nilotinib, bosutinib, ponatinib, sciminib, and olverembatinib are the currently available TKIs for the treatment of CML. The European LeukemiaNet (ELN) endorse first four as options to begin the treatment of chronic phase in CML (CML-CP) (3). The last three TKIs are the drugs that are reported to be effective in the T315I mutation. The most studied and approved one is ponatinib (4). Treatment of CML is still a challenging practice, as its course and prognosis are difficult to predict, and patient survival and response to TKIs differs by case. The development of TKI resistance during the course of treatment is also a compelling factor. Resistance mechanisms are complex and targeting

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these pathways may be promising in terms of predicting and preventing the development of resistance. MicroRNAs (miRNAs) are short non-coding sequences (22–24 nucleotides) that regulate gene expressions via post-transcriptional mechanism. MiRNAs are expressed in biological processes such as cellular development, differentiation, apoptosis and tumor growth. MiRNAs are also involved in cancer by their oncogenic or tumor suppressor functions. MiRNAs have been studied in CML, and informative results regarding the clinical process have been obtained (5). There are various studies on the role of miRNAs in CML process and in the development of TKI resistance (6-8). In one Turkish study, significant miRNA profile alterations were seen in CML patients who take imatinib or ponatinib (9). Another study determined possible therapeutic miRNAs in CML cells (10). In CML, miRNAs at the BCR-ABL1 fusion gene level can be targeted, may have a supportive use in the treatment with TKIs. In our study, we expected possible associations between various miRNAs (miR-23a, miR138, miR-181, 152-3p and miR-101) which take part in BCR-ABL pathways and TKI resistance in CML. We evaluated the expression levels of miRNAs in CML patient groups who were either sensitive or resistant to imatinib and compared with a control group.

Materials and Methods

Sample collection: 50 individuals with a diagnosis of CML between the ages of 25-95 included in the patient group along with 50 healthy individuals as controls. Sample collections were performed between 2020-2021. The patient group divided into two-based on the criteria according to European LeukemiaNet 2020 recommendations (3) below; For the imatinib-sensitive group (S), patients having BCR-ABL1 ≤ %1 after 6 months of treatment with imatinib are selected. This group was sensitive (S) and consisted of 25 patients. For the imatinib-resistant group (R) patients having BCR-ABL1 > %10 after 6 months of treatment with imatinib and being responsive to either dasatinib or nilotinib were selected. This group included 13 dasatinib users and 12 nilotinib users.

RNA extraction and cDNA synthesis: Mononuclear cells isolateted using Ficoll-Hypaque density gradient centrifugation from collected blood and bone marrow samples. Total RNA is extracted by miRNeasy Mini Kit 50 (Qiagen GmbH, Hilden, Germany). Nanodrop is used for RNA concentration and purity measurement. cDNA was synthesized from the total RNA by reverse transcription method, using miScript II RT Kit (Qiagen GmbH) following the manufacturers instructions. From the 125 ng total RNA only small RNA's that also includes miRNA's were converted into cDNA by using miScript HiSpec (Qiagen GmbH).

Quantitative Real-Time PCR: For the measurement of miRNA expression levels, miScript Primary Assay Kit (Qiagen GmbH) was used for a total of 100 samples, 50 patients and 50 controls, following the manufacturer's instructions. The template cDNA concentration was 10 ng/µl for all samples. *SNORD61* was chosen as internal control for PCR data normalization for relative quantification. The selected miRNAs were analyzed on the Rotor Gene® using the 72 platform. After the RT-qPCR procedure *SNORD61*, miR-23a, miR-138, miR-181, miR-152-3p and miR-101 Ct (cycle thresold) values of case and control groups were determined by Rotor-Gene® Software. Using the obtained data, miRNA expression changes were calculated by the $2^{-\Delta\Delta Ct}$ (Livak) method. According to this method, the fold changes between the Ct values of the reference gene and the target gene were calculated using the formula $2^{-\Delta Ct}$ (ΔCt (delta Ct) = Ct Target Gene – Ct Reference Gene). The fold change between the patient and control groups was calculated using the formula $2^{-\Delta\Delta Ct}$ (ΔΔCt = Patient Group ΔCt – Control Group ΔCt).

Ethical Approval: We designed a case-control study and obtained informed consent of the participants. An approval from the Ethical Committee of the Ataturk University (decision number; 2019-7/39) recieved.

Statistical analysis: Multiple comparison analyses, calculation of mean and SD values were performed using the one-way ANOVA test in the GraphPad Prism 10 software, and values of $p<0.05$ were considered statistically significant.

Table 1: Descriptive characteristics of samples

Samples/Groups	S Group (n=25)	R Group (n=25)	K Group (n=50)	Total (n=100)
Female	14	13	28	55
Male	11	12	22	45
Mean±SD	54,64±16,98	52,32±18,07	52,2±14,94	52,84

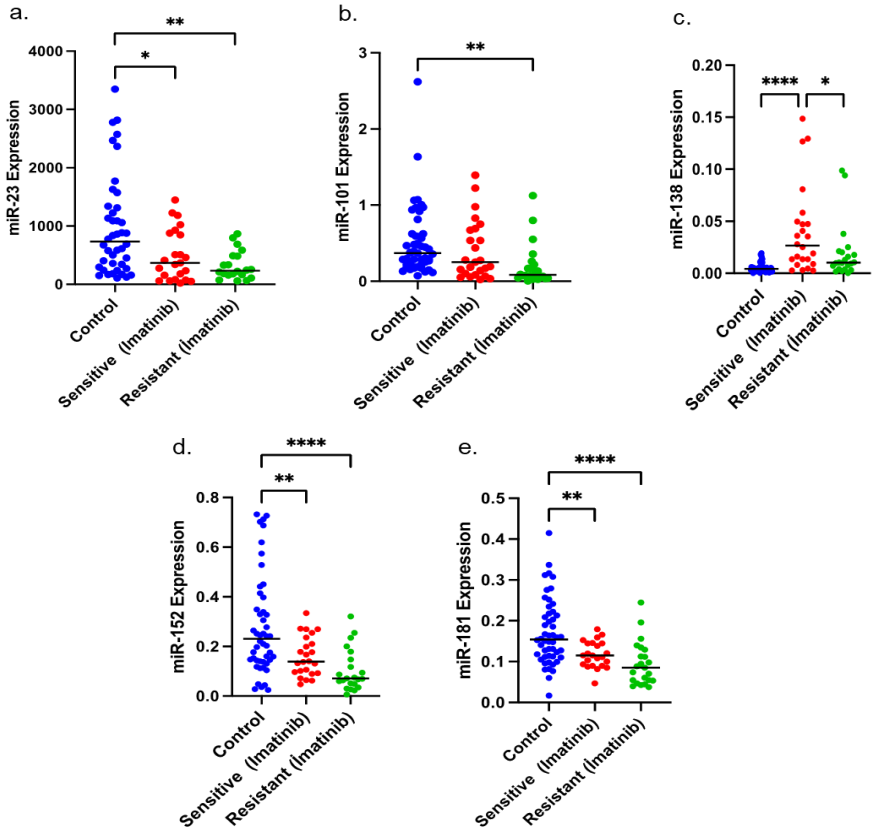

Figure 1. Expression profiles of (a) miR-23 (*=0.0145; **=0.0012), (b) miR-101 (**=0.007), (c) miR-138 (****0.0001; *=0.015), (d) miR-152 (**=0.006; ****0.0001), (e) miR-181 (**=0.0041; ****0.0001) in control, imatinib sensitive and imatinib resistant groups.

Results

Among all participants of our study 55 were females, and 45 were males, and the mean age was 52.84 (Std:18.75). There was no significant difference in the mean ages of females to males (p>0.05) and the patients to controls. In the patient group ages ranged from 26 to 94 years, 54% (n=27) were females 46% (n=23) were males. In the control group ages ranged from 28 to 91 years, 56% (n=28) were females 44% (n=22) were males (Table 1). Our analyses indicated that expression of hsa-miR-23 was three times lower in the Imatinib-resistant group and two times lower in the sensitive group compared to controls respectively (p=0.0012), (p=0.0145) (Figure 1a).

Hsa-miR-101 expression decreased 4-fold in the resistant group (0.0001) compared to controls. However, no significant differences were seen between the resistant and sensitive groups (Figure 1b). 5-fold increase in the expression of hsa-miR-138 was observed in the sensitive group compared to controls (p=0.0001). More importantly, hsa-miR-138 expression was lower in resistant group compared to sensitive group which was statistically significant (p=0.015) (Figure 1c). Expression levels of hsa-miR-152 were significantly decreased in imatinib sensitive (p=0.0036) and resistant groups (0.0001) compared to controls (Figure 1d). And levels of hsa-miR-181 also decreased in imatinib sensitive (p=0.0041) and resistant groups (0.0001) compared to controls (Figure 1e).

Discussion

BCR/ABL is solely sufficient to cause CML and is required for the oncogenic activity of the tyrosine kinase activity of this protein. For these reasons, a BCR/ABL tyrosine kinase inhibitors are an effective and selective therapy in CML (11). Tyrosine Kinase Inhibitors are first-line treatment options in CML, but some patients do not respond to TKI or develop drug resistance later on (3). Identifying those who may have TKI resistance among CML patients is important and may influence the choice of primary TKI. To select the exact TKI, clinical experience and individual patient characteristics should be well evaluated (12). miRNAs can take roles in cancer development and progression as they have oncogenic and tumor suppressor properties. There are studies on miRNAs, which are suspected to be associated with the pathogenesis of CML and resistance to TKIs and are thought to be potential biomarkers for the prognosis of the disease (5, 8, 13). In one Turkish study on newly diagnosed patients, treatment-response patients, treatment failure patients, and healthy controls, demonstrated that miR-150, miR-10a, and miR-148b were deregulated at different stages of CML (14). In our study, we measured the expressions of certain miRNAs (miR-23a, miR138, miR-181, 152-3p and miR-101) associated with BCR-ABL1 and the pathways it affects, since the BCR-ABL1 oncoprotein is the basis of the disease. We aimed to examine miRNA expressions in TKI resistant (R) and sensitive (S) groups and their relationship with resistance. miR-23a regulates the JAK1/Stat3 cascade. There are various studies on the diagnostic and prognostic values of miR-23a in cancers (15). miR-23a is dysregulated in various cancers and is downregulated in acute and chronic myelogenous leukemia, similar to our study. miR-23a targets the BCR/ABL 3'-UTR region. It has been studied on CML cell line (K562) by Xishan et al. They indicated that miR-23a had a role in regulation of BCR/ABL expression (11). In our study, mir-23a was downregulated in patient groups relative to controls and there was no significant expression difference between the imatinib-resistant and sensitive groups. Another study also supports the current results which decreased expression of miR-23a is associated with resistance of Imatinib in CML patients (16). miR-23a has the potential to be a target molecule in the resistance mechanism by targeting BCR/ABL. This suggests that it may be possible to reduce or overcome resistance with mir-23a mimics. Mir-101 is one of the miRNAs reported to be as a tumor suppressor miRNA. Wang et al. showed that miR-101 reduced tumor cell growth and supported apoptosis in breast cancer cells by targeting Jak2 (17). It is known that Bcr–Abl oncoprotein activates JAK2 tyrosine kinase. Regarding these data, it was hypothesized that Jak2 inhibition can increase apoptosis in imatinib resistant cell lines and overcome imatinib resistance (7). Farhadi et al. revealed that miRNA-101 regulates Jak2 expression and induces apoptosis in K562 cells. They also revealed that miR-101 upregulation increased miR-23a expression, and sensitized K562 cells to imatinib (7). These results were also consistent with our results. We showed downregulation of miR-101 in CML patients and also imatinib resistant group had lower miR-101 expression than control and sensitive group, although we did not observe statistically significant changes between sensitive and resistant group. Mir-101 mimics may become potential options to break imatinib resistance and increase its effectiveness. miR-152p is one of the miRNAs studied in CML. In the study of Wang et al, miR-152p expression was increased in bone marrow of CML patients, and transfection with miR-152p in K562 cells caused proliferation (18). p27 protein which is the target for miR-152p has tumor suppressor effects in nucleus, and oncogenic effects in cytoplasm (19). Agarwal et al. reported that inhibition of BCR-ABL with imatinib increased nuclear p27 and did not affect cytoplasmic p27. So nuclear p27 may translocate into cytoplasm and produce oncogenic effects and contribute to TKI resistance (19). We found downregulation of miR-152p in CML patients and the decrease was significant in imatinib resistant group. Since BCR/ABL1 levels remained high in the imatinib resistant group, we suggest that cytoplasmic p27 levels may have become dominant and contributed to the resistance. The downregulation of miR-152-3p we found may be associated with an increase in cytoplasmic p27. Comprehensive studies investigating the relationship between miR152p, p27 expression and TKI resistance may clarify this issue and predict the potential of p27 as a target molecule to overcome TKI resistance. In the study of Xu et al. miR-138 was down-regulated in CML patients and K562 cell line. miR-138 inhibits the proliferation of CML cells. After imatinib treatment, miR-138 was upregulated and apoptosis was induced. miR-138 inhibited the expression of BCR-ABL by targeting ABL. Imatinib increased miR-138 expression while inhibiting BCR-ABL tyrosine kinase (20) (16). Consistent with the literature, in our study miR-138 levels were significantly upregulated in sensitive group compared to

control group and this elevation may due to the responsiveness to imatinib. In contrary, we observed in resistance group, expression level of miR-138 significantly decreased compared to sensitive group and this downregulation was also supported to relationship between imatinib responsiveness and miR-138 effects. Studies on the effects of miR-181a in CML are limited. Fei et al., transfected miR-181a into K562 cell line and inhibited cell growth and increased apoptosis by targeting RalA which is a downstream molecule of RAS pathway of BCR-ABL (21). In the study of Wang et al., miR-181 was down-regulated in a patient with CML and in K562 cells. miR-181a transfection on K562 cells increased the imatinib response. miR-181a inhibited growth and promoted apoptosis in K562 cells which indicates miR-181a mimics may have the potential to increase imatinib sensitivity (22). In our study miR-181a was significantly downregulated in CML patients similar to the previous studies. Its levels were even lower in imatinib resistant patients although it was not statistically significant. In another study miR-181a acts as a tumor supressor via inhibition of CML CD34+ cells and sensitizes them to imatinib. miR-181a/SERPINE1 axis may also be a new approach to imatinib resistance (23). In paralel with these results, we detected gradual decrease in expression of miR-181a compared to controls and lowest miR-181a levels in the resistant group.

Study limitations: A larger number of samples would have helped us obtain more statistically significant results. A comprehensive miRNA profile analysis might have helped more conclusive results in the study groups.

Conclusion

miRNAs are being studied in many cancers because of their oncogenic or tumor suppressor effects. They are thought to be potential targets for diagnosis, prognosis, clinical follow-up and treatment. To the best of our knowledge, our study is the first study comparing miRNA expression levels in imatinib-sensitive and resistant CML cases. We concluded that miR-23a, miR-152p, miR-181a and miR-101 down regulation may have a place in CML diagnosis. and miR-138 upregulation may indicate imatinib resistance. Our findings may provide additional evidence for a potential therapeutic target as well as novel prognostic biomarkers about imatinib resistance in CML. Further studies should be performed to provide novel and effective prognostic and therapeutic agents.

Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Written consent was obtained from all the cases participating in our study. An approval from the Ethical Committee of the Ataturk University (decision number; 2019-7/39) recieved.

Conflict of interest: There is no conflict of interest of the authors for this study.

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Data availability statements: The data that support the findings of this study are available on request from the corresponding author.

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