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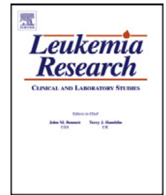

Brief communication

# MicroRNA-451 in chronic myeloid leukemia: miR-451–BCR-ABL regulatory loop?

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ABSTRACT

Chronic myeloid leukemia (CML) is caused by constituve activity of BCR-ABL tyrosine kinase. Despite of high efficiency of imatinib, selective BCR-ABL inhibitor, about 30% of patients develop resistance. Novel markers and targets for therapy are thus necessary. MicroRNAs are small intereference RNAs whose role in physiological and malignant hematopoiesis has been shown. This study is focused on miR-451 in CML. Following our observation of miR-451 downregulation in CML, we further show its relation to BCR-ABL activity. Our data together with current literature indicate a more complex relationship of miR-451 and BCR-ABL in CML.

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MicroRNAs are extremely short (21–23 nucleotides) single stranded RNA molecules which regulate gene expression via translational repression or mRNA cleavage. There is an increasing evidence in the literature that miRNAs belong among important regulators of both physiological hemopoiesis and abberant hemopoiesis of leukemias [1–3]. Abberant expression of miRNAs in chronic myeloid leukemia (CML) was reported previously [1–3], and in some of those miRNAs their relationship to BCR-ABL kinase was shown [1]. BCR-ABL tyrosin kinase constitutive activity was confirmed to be the main cause of CML and it has become a target for therapy. Imatinib (Glivec, imatinib mesylate, Novartis Oncology), selective BCR-ABL tyrosin kinase inhibitor is currently used as the first line therapy for all newly diagnosed CML patients. Non-negligible incidence of resistance to imatinib together with missing curative potential of tyrosine kinase inhibitors therapy call for novel biomarkers and molecules important in CML resistance, progression and pathogenesis in general.

In our previous study we tried to find possible novel biomarkers and/or players in CML pathogenesis among the miRNAs [4]. On the basis of literature data [1–3] and our own microarray data [4], we investigated miR-451 expression in CML patients by real-time PCR. We analyzed samples of total leukocytes in CML patients at the time of diagnosis (Dg, $n = 14$), in major molecular response (MMR; $n = 14$), in hematological relapse (Hr; $n = 17$) and in suboptimal response (SR/TF; $n = 7$). Response to therapy definitions and patients' samples characteristics are given in Table 1A. Total leukocytes of 11 healthy donors were used as controls. By this approach, we found miR-451 downregulated in most of the Dg and Hr in contrast to normal or slightly increased levels in MMR and SR/TF (Fig. 1).

The Ph positivity and BCR-ABL transcript levels correlated inversely with miR-451 expression at Dg, in Hr and MMR, but no such correlation was found in SR/TF (BCR-ABL as well as miR-451 levels were high in SR/TF). This discrepancy might be explained by results of our western blot analyses, which showed that there is a difference between Dg/Hr and SR/TF in BCR-ABL activity. While at Dg and Hr, BCR-ABL is not inhibited, BCR-ABL remains probably under partial control of imatinib in SR/TF (Fig. 1b). Our data thus indicated that the miR-451 downregulation might be related to BCR-ABL kinase activity.

To verify the hypothesized relation of miR-451 downregulation to BCR-ABL kinase activity we decreased BCR-ABL kinase activity by *in vitro* cultivations of cells with imatinib. We used leukocytes of imatinib-naïve CML patients, i.e., patients with the CML diagnosis but not yet treated with imatinib ($n = 6$, Table 1B); Ph+ (CML-T1, JURL-MK1) and Ph− cell lines (ML-2) and leukocytes of healthy donors as controls. Activity of BCR-ABL kinase was determined via phosphorylation of Crkl protein (western blots) [5]. Sensitivity of cells to imatinib was further checked by WT1 mRNA levels [6]. Based on literature data and our previous expe-

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**Table 1**
Characterization of patients samples.

| (A) Disease stage | Number of patients' samples | Months on imatinib, median (range) | WBC × $10^9$/l PB, median (range)<br>PLT × $10^9$/l PB median (range) | Blasts in PB (%) median (range) | Ph+ cells (%) | BCR-ABL (%), median (range) |
|---|---|---|---|---|---|---|
| Dg | 14 | 0 | 71,38 (22,32–457)<br>502 (130–824) | 0 | 100 | 132,1 (60,8–153,6) |
| MMR | 14 | 18,93 (7,4–26,89) | Physiological<br>Physiological | 0 | 0 | 0.015 (0.0005–0.11) |
| SR/TF | 7 | 21 (11–67) | Physiological<br>Physiological | 0 | 100 | 17,4 (11–69,83) |
| Hr | 17 | 21,25 (10–53,85) | 15,19 (5,83–28,13)<br>455,5 (71–2372) | 0 | 100 | 73,7 (14,1–256,8) |

| (B) Group | Patient No | WBC × $10^9$/l PB | PLT × $10^9$/l PB | Blasts in PB (%) | Ph+ cells (%) | BCR-ABL (%) |
|---|---|---|---|---|---|---|
| B | 1 | 81,17 | 606 | 2 | 100 | 179 |
| | 2 | 55,98 | 192 | <1% | 100 | 49 |
| A | 3 | 152 | 174 | 2 | 100 | 169 |
| | 4 | 188 | 206 | 1,5 | 100 | 41 |
| | 5 | 216 | 283 | 0,5 | 100 | 421 |
| | 6 | 86,57 | 683 | 2 | 100 | 139 |

(A) Characteristics of patients' samples of the course of CML Dg – diagnosis, samples prior to therapy; Hr – hematological relaps, increase in total leukocytes over the normal level of $10^*10^9$/L peripheral blood; SR/TF – suboptimal response/therapy failure, samples of complete hematological response of patients not achieving complete cytogenetic response within 12 or more month of imatinib therapy, MMR – major molecular response, decrease in BCR-ABL to less 0,1%; Scaling of responses to imatinib followed current criteria of European Leukemia Net. (B) Characteristic of patients used in cultivation analyses.

rience, 1 and 10 μM imatinib doses were applied on cells leading usually to partial and total inhibition of BCR-ABL activity, respectively [5]. Optimal time for *in vitro* cultivations was thoroughly tested [7]. It was necessary to find the time when changes of monitored parameters were not influenced by apoptotic/necrotic processes. We tested four different cultivation times: 2, 24, 48 and 72 h. Up to 48 h, number of apoptotic/necrotic cells (RNA degradation, Anexin V staining) was lower than 15% both in treated cells and non-treated controls. Changes of measured parameters at mRNA and protein levels were clearly detected in 48 h. At 72 h, high levels of RNA degradation and of apoptosis and necrosis (more than 60%, Anexin V staining) were found, which might distort results of our analyses. Therefore, 48 h were used in this study.

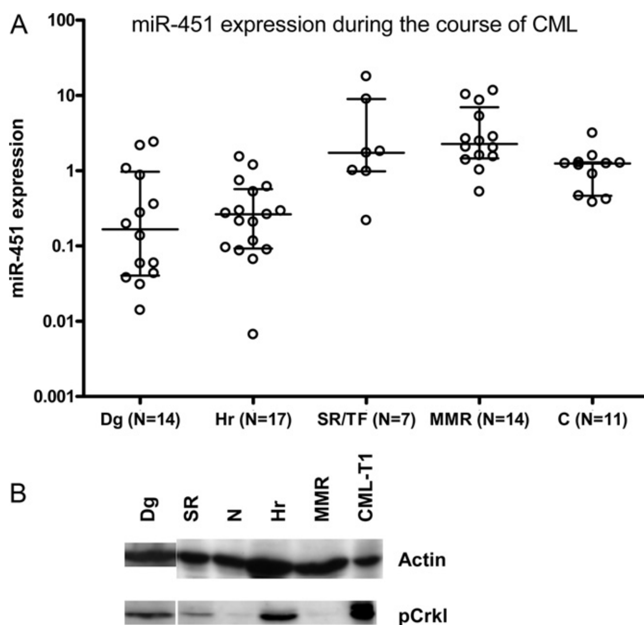


**Fig. 1.** MicroRNA-451 expression profile in the course of CML (A) MicroRNAs were quantified using comercially available assays (Life Technologies). miR-451 expression was evaluated with miR-30c as a control gene [4] and healthy control as a calibrator. (B) BCR-ABL activity during the course of CML was investigated by western blot analyses of pCrkl levels; results of representative samples of different responses to imatinib are shown.

We found BCR-ABL activity to be high in all Ph+ samples prior to cultivation and decreased or fully inhibited after imatinib treatment. No BCR-ABL activity was found in Ph− samples, which stayed unchanged after cultivation. Prior to cultivation, the miR-451 was downregulated in CML-T1 and JURL-MK1 cells in comparison to healthy leukocytes (0.00006 and 0.54, respectively). Interestingly enough, miR-451 was increased in both Ph+ cell lines after cultivation with imatinib (Fig. 2a, 2.8-fold in CML-T1 and 4.2-fold in JURL-MK1) suggesting relation of miR-451 downregulation to BCR-ABL activity in these cells. Control Ph− cell line ML-2 exhibited no changes in miR-451 levels after imatinib treatment which effectively excluded possible influence of imatinib via any other way then the BCR-ABL inhibition (Fig. 2a).

Concerning patient samples, samples of 4 out of 6 imatinib-naïve patients showed decreased miR-451 levels (group A) prior to cultivation (median of 0,04). Remaining 2 patients showed comparable levels of miR-451 to that of healthy leukocytes prior to cultivation (group B; median of 0.55). After *in vitro* cultivation with imatinib, miR-451 increased in samples from group A patients (Fig. 2c, 2.2 fold) and remained on almost the same level in group B (Fig. 2b). We detected no expression changes of miR-451 in healthy donor leukocytes after treating cells with imatinib (Fig. 2a). At present, we have no clear explanation of the differences in miR-451 expression between groups A and B. There were no significant differences in results of cytogenetic or BCR-ABL transcript and other analyses between patients of those two groups. Some other molecules may play a role, e.g. mediators between BCR-ABL and miR-451. To elucidate this, further studies are needed, preferentially on larger patient cohorts.

Nevertheless, the results of our *in vitro* experiments again confirmed our previous suggestion that expression changes of the miR-451 found in Ph+ cell lines and CML patients' samples are associated with BCR-ABL inhibition.

In conclusion, we showed that BCR-ABL activity plays certain role in downregulation of miR-451 in CML cells. On the other hand, Iraci et al. [8] showed previously that miR-451 had a potential to target BCR-ABL. Combination of those two findings suggests that there may exist a reciprocal regulatory loop between BCR-ABL and miR-451 (Fig. 3). BCR-ABL kinase activity

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