Abstract

Myeloid leukemias include malignant diseases characterized by clonal expansion of the myeloid cell lineage. While in case of chronic myeloid leukemia (CML), the main cause of the disease has already been identified – t(9;22) and the activity of the fusion product of the translocation BCR-ABL, acute myeloid leukemia (AML) has been associated with plenty of different translocations and mutations. The aim of this work was to contribute to the improvement of monitoring of patients with myeloid leukemias via detailed study of the panleukemic marker Wilms tumor gene 1 (wt1) expression. Prognostic value of wt1 expression has been proved for AML patients, however, it has not yet been confirmed for CML patients. Expression of different wt1 variants (more then 36 protein products) is known very poorly in both, AML and CML as well as in normal hematopoiesis. Most of the study is focused on CML, only limited parts are dedicated to AML.

In the first part of the work, we clearly proved prognostic value of total wt1 mRNA expression for CML patients. Statistical evaluations revealed critical wt1 values which enable to specify prognosis of patients responding non-optimally to imatinib. Bcr-abl looses much of its prognostic value in these patients. Further, we have designed and optimized PCRs for selected wt1 variants (combination of exon 5 and KTS sequence splicing, swt1 vs. full lenght wt1). We found out, that swt1 was expressed at very low levels in CML and AML patients and it thus could not be considered a candidate marker. On the other hand, -5/+KTS and +5/-KTS seem to be novel candidates on prognostic markers for CML and AML patients, respectively. Expression of wt1 variants might serve as a more specific markers for given diagnosis as compared to total wt1 expression. We have also confirmed a correlation between wt1 mRNA expression and sensitivity to treatment in vitro. We applied those in vitro cultivations with BCR-ABL inhibitors in a paralel study of miRNA expression in CML and we identified negative-feed back regulatory relationship between miR-451 and BCR-ABL. This mechanism is potentially highly important for maintenance of the leukemic cell phenotype.

In conclusion, total wt1 mRNA expression can be useful as an additional molecular marker to bcr-abl to predict further disease course in CML patients who do not respond optimally to imatinib. We identified selected wt1 variants as novel candidate markers specific for different leukemia types. Wt1 mRNA expression measured in vitro after treating patients cells with kinase inhibitors helps in characterization of cell status and sensitivity to the drug.