UNIWERSYTET MEDYCZNY W ŁODZI

WYDZIAŁ FARMACEUTYCZNY

ROZPRAWA DOKTORSKA

„OCENA EKSPRESJI I POLIMORFIZMÓW GENÓW, KODOWANYCH PRZEZ NIE CZYNNIKÓW TRANSKRYPCYJNYCH ORAZ TRANSKRYPTU GENU FUZYJNEGO *RUNX1-RUNX1T1* W OSTREJ BIAŁACZCE SZPIKOWEJ"

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Summary

Acute myeloid leukemia belongs to the group of heterogeneous neoplastic diseases of the white blood cell system. It is characterized by clonal proliferation and growth of cancer-transformed blast cells that originate from the precursor myeloid cell in the bone marrow and in the blood. The course of acute myeloid leukemia is extremely severe: if left untreated, it can lead to death of a patient within a few weeks. Among the factors that can lead to the AML development, more and more attention is paid to the role of molecular indicators of tumor transformation, which may contribute to the formation of a self-regenerating leukemia cell clone. Among genetic aberrations potentially related to the development of AML or prognosis assessment among patients, changes in genes coding for the so-called transcription factors regulating transcription processes as well as controlling the cell differentiation and formation seem singificant.

Transcription factors include, among others, proteins encoded by the RUNX family genes (RUNX1 and RUNX3) as well as the CEBPA and c-MYC genes.

The purpose of this dissertation was to evaluate polymorphisms, expression levels and the protein products presence of genes encoding transcription factors potentially involved in leukemogenesis and AML pathogenesis. The analysis was carried out at three molecular levels, including DNA, RNA and protein. Single nucleotide polymorphisms for the RUNX1 (rs2268277) and RUNX3 (rs6672420) genes were evaluated using PCR-RFLP method. The relative expression levels of the RUNX1, RUNX3, CEBPA and c-MYC genes were analyzed using RT-qPCR. The presence of RUNX3 and CEBPA gene protein products was assessed using the Western-Blot method. Additinally the expression presence of RUNX1-RUNX1T1 fusion gene in patients with acute myeloid leukemia using the RT-qPCR method with a TaqMan probe was evaluated. The study group consisted of 46 patients diagnosed with acute myeloid leukemia. Investigated material comprised blood collected on EDTA from which DNA and RNA were isolated, respectively. Blood samples came from the Institute of Hematology and Transfusion Medicine in Warsaw. The obtained genotyping results were compared to 60 DNA samples isolated from healthy people. All collected data was compared with demographic and clinical-pathological features of patients.

Analysis of the obtained results allowed to define the following conclusions. The presence of the RUNX1 gene rs2268277 polymorphism is not associated with an increased risk of developing AML, however GC heterozygotes and patients with the wild G allele have a higher age at diagnosis when compared to wild GG homozygotes and mutant CC homozygotes, which may indicate later occurrence of disease symptoms and subsequent clinical manifestation. The RUNX3 gene rs6672420 polymorphism does not affect its expression level nor is associated with etiopathogenesis and AML incidence.

Analysis of CEBPA and c-MYC transcript levels indicate that there is no association between the expression level of these genes and the development and course of AML. Significantly higher levels of RUNX1 gene expression in women compared to men have the potential to affect the incidence and different course of acute myeloid leukemia in both sexes. Significantly increased RUNX3 gene expression levels in the group of patients, who had a shorter survival time during the 3-year observation, may be associated with worse prognosis and a higher mortality rate in these patients. Increased relative expression of the RUNX3 gene may be a poor prognostic factor in AML patients. However, the obtained results should be confirmed on a larger group of patients. In addition, the presence of the RUNX1 / RUNX1T1 fusion gene in AML patients confirms its role as a favorable prognostic factor. The low levels of CEBPA and the qualitative presence of RUNX3 protein in only 3 patients confirm that the expression of these proteins in blood serum is extremely reduced. Confirmation of these results requires further analysis.

Understanding the function and full characteristics of genes from the RUNX family as well as CEBPA and c-MYC genes in the group of patients with acute myeloid leukemia can be particularly helpful in assessing prognosis and may be considered as a prognostic factors. This may translate into the development of new targeted therapeutic strategies and an increase in the effectiveness of treatment in patients with acute myeloid leukemia.