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Rozprawa na stopień doktora nauk farmaceutycznych.

Tytuł: Ocena metaloproteinaz w ostrej białaczce szpikowej.

Title: Evaluation of metalloproteinases in acute myeloid leukemia.

Rozprawa doktorska napisana pod kierunkiem

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Abstract

Acute myeloid leukemia is a malignant cancer of the hematopoietic stem cell which characterized by clonal proliferation of abnormally differentiated myeloid line cells. It is the most common hematological cancer in human population. Usually, people over 65 suffer from acute myeloid leukemia, but also acute myeloid leukemia occur in childhood. In spite of the pathogenesis of acute myeloid leukemia is not fully understood, it is considered to be due to the genetic transformation of the hematopoietic stem cell or progenitor cells that have acquired secondary self-renewal ability. The treatment of patients with acute myeloid leukemia mainly consists of polychemotherapy supplemented sometimes with new generation drugs or stem cell transplantation. The effectiveness of treatment of patients with acute myeloid leukemia is unsatisfactory and is associated with serious side effects including death.

Metalloproteinases are proteolytic enzymes which activity is dependent on the presence of zinc ion in the active site. They play a very important role in many physiological processes such as wound healing or organogenesis. The presence of 23 metalloproteinases was confirmed in human's organism, which were divided by the structure and substrate specificity. The overall plan of metalloproteinases structure consists of three domains: the catalytic domain, the hemopexin domain and the prodomain. Metalloproteinases are enzymes that are secreted outside the cell in an inactive form. Inactive form of metalloproteinase is maintained by the cover of active side on catalytic domain by the prodomain. Only after remove the prodomain due to the action of another protease or chemical compound reveals lytic activity. The regulation of metalloproteinase activity occurs at many levels from gene transcription by regulating transcript stability and the number of secreted enzymes and activity of tissue metalloproteinase inhibitors. Metalloproteinases show the ability to degrade the extracellular matrix and regulate various types of cellular processes, including proliferation, apoptosis, production of new blood vessels. For this reason, they have been widely studied in various types of carcinogenesis. A correlation was shown between the number of different types of metalloproteinases and the size of the cancer and its stage. Relatively few studies concerned the role of metalloproteinases in hematological malignances.

The aim of the study was to assess the role of MMP1, MMP2, MMP9 and MMP16 in acute myeloid leukemia.

The research material consisted of RNA samples isolated from peripheral blood leukocytes and serum taken from patients with acute myeloid leukemia and control group.

To compare the expression of the *MMP1*, *MMP2*, *MMP9* and *MMP16* genes, the real

time PCR method was used, an ELISA test was performed to compare MMP1 serum concentration, and zymography with gelatin as a protein substrate was performed to assess MMP2 and MMP9 in serum. People with acute myeloid leukemia showed lower expression of the *MMP1*, *MMP2* and *MMP16* genes than the control group. *MMP9* expression was similar in both groups. The expression level of all tested genes was not dependent on gender and age. There were no differences in expression of the examined genes due to the presence of cytogenetic changes in leukemic cells. The result of first induction therapy did not depend on the expression of *MMP1*, *MMP2*, *MMP9* and *MMP16* at the moment of diagnosis. Comparison of MMP1 serum levels in patients with acute myeloid leukemia and control group revealed that AML patients with the M2 morphological subtype had a higher MMP1 concentration than the control group and other patients with acute myeloid leukemia. Zymographic analysis did not reveal differences in the occurrence of different forms of MMP2 and MMP9 between the control group and patients with acute myeloid leukemia.

The results presented in this work show that the role of metalloproteinases in acute myeloid leukemia may be different than in solid tumors. Metalloproteinases may play an important role in the normal hematopoiesis and their impaired synthesis may be the result of abnormal differentiation of the hematopoietic stem cell. The obtained results also show that metalloproteinases are not only enzymes favoring the development of cancer, but their role depends to the type of cancer and its stage.