„GEORGE EMIL PALADE" UNIVERSITY OF MEDICINE, PHARMACY, SCIENCE AND TECHNOLOGY FROM TÂRGU MUREȘ

SCHOOL OF DOCTORAL STUDIES

Abstract Of The Doctoral Thesis

An evaluation of the genetic factors involved in the susceptibility, prognosis, and treatment of acute myeloid leukemia

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Introduction

Acute myeloid leukemia (AML), a type of blood cancer, is a very heterogeneous disease in terms of clinical, phenotypic, and genetic characteristics. Understanding the pathogenesis, patient management, and targeted therapies are all ongoing processes. Unfortunately, the prognosis for AML patients remains bleak, with a low overall survival (OS). In consequence, more research is needed to improve the treatment of this terrible disease.

Objectives

The purpose of this study was to identify associations between certain mononucleotide polymorphisms (SNPs) and AML susceptibility, prognosis and treatment. We also wanted to see if there were any associations between the investigated SNPs, other genetic abnormalities, and clinical parameters in AML. Another objective was to assess the utility of various genetic techniques and to create a genetic testing protocol that could be used in Romanian laboratories with basic infrastructure.

Study 1. Association of *XPC* Val499Ala polymorphism with susceptibility to LAM

We enrolled 140 subjects in the control group and 149 patients in this study group. The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was used for genotyping. We identified an association between variant genotypes, AML susceptibility and smoking status. Other clinical variables, patient OS, or somatic mutations were not found to be associated with the investigated SNP.

Study 2. Evaluation of the effects of *MCM7* gene polymorphisms, somatic mutations, and clinical parameters on AML susceptibility and prognosis.

We included 405 people in the control group and 281 patients. We used real-time PCR method for genotyping. The variant allele of rs2070215 and the CAT haplotype (alleles of the *MCM7* SNPs rs2070215, rs1527423, and rs1534309) were associated with AML susceptibility. The somatic mutations studied [*FLT3*-ITD and D835, *DNMT3A* R882 and *NPM1* (type A-D mutations)] were associated with elevated lactate dehydrogenase (LDH) levels, a high percentage of blasts, and leukocytosis. Among the clinical and biological characteristics of patients [age at diagnosis over 65 years, European LeukemiaNet (ELN) unfavorable and intermediate risk score, the presence of *FLT3*, *DNMT3A* mutations, Eastern Oncological Cooperation (ECOG) performance score ≥ 2, leukocytes ≥ 10,000 cells / mm^3, LDH level ≥ 600 IU / L, all associated with low OS] only LDH level ≥ 600 IU / L was associated with an increased mortality rate, regardless of the presence or absence of *FLT3* (ITD, D835) gene mutations.

Study 3. The association of the *TERT* rs2853669 polymorphism with the OS of AML patients.

In this study we included 363 people in the control group and 146 patients. We used real-time PCR method for genotyping. *TERT* rs2853669 and rs2736100 SNPs do not increase the risk of developing AML. In the presence of other prognostic factors, the variant genotypes of *TERT* rs2853669 have a negative effect on the OS of AML patients.

Study 4. The analysis of the associations between *TP53* gene polymorphisms rs1042522, *MDM2* rs2279744, rs3730485, *MDM4* rs4245739 and AML susceptibility, risk stratification scores and clinical characteristics of patients

We included 406 people in the control group and 403 patients. For genotyping, we used both real-time PCR and Amplification Refractory Mutation System PCR (ARMS-PCR) techniques. The *TP53* rs1042522 and *MDM4* rs4245739 SNPs were associated with AML susceptibility. The *MDM2* rs2279744 and *TP53* rs1042522 SNPs interact and increase the risk of AML. Variant genotypes of *TP53* rs1042522 were associated with unfavorable molecular and cytogenetic risk scores, as well as with the presence of the *NPM1* mutation (type A-D).

Study 5. The role of rs361525, rs1800750, rs1800629, rs1800896, rs1800872, rs1800795, rs1800470 and rs2430561 SNPs in AML

We included 406 people in the control group and 226 patients. The genotyping was performed using real-time PCR and ARMS-PCR techniques. We identified that variant alleles of *TGF-β1* rs1800470 and *IFN-γ* rs2430561 SNPs were associated with AML susceptibility. Age over 65 years of AML patients, platelet count, variant genotype of *TNF-α* rs1800750, bone marrow blasts percentage > 70%, elevated LDH levels (≥ 600 IU / L) and unfavorable cytogenetic risk score may be used as independent risk factors for the assessment of mortality in AML patients.

Development of a genetic testing protocol tailored to Romanian laboratory infrastructure.

The gold standard method remains cytogenetic analysis, and array comparative genomic hybridization technique (arrayCGH) for DNA copy number variations (CNV) is recommended. In the absence of arrayCGH, the Multiplex ligation-dependent probe amplification (MLPA) technique is usefully in AML, identifying CNVs in 31.8% of patients in our studies. In 25% of our patients, the ligation-dependent reverse transcription chain polymerization reaction (LD-RT-PCR) detected gene fusions. MLPA and LD-RT-PCR are both inexpensive and can be completed in 24-48 hours. The next-generation sequencing (NGS) analysis performed on the patients included in our study identified an average of 22 gene variants per patient, but the costs remain high and the interpretation of the results takes a long time. All of these techniques are beneficial to patients; each technique overlaps the limitations of the others; however, based on costs for the NGS technique, national strategies such as selecting a single national laboratory would be required.

Conclusions

We found associations between susceptibility to AML and the following SNPs: *XPC* Ala499Val, the risk being higher in smokers; *TP53* rs1042522, the risk being 5.64 times higher in patients with homozygous variant genotypes for *TP53* rs1042522 and *MDM2* rs2279744; *MDM4* rs4245739; *TGF-β1* rs1800470; *IFN-γ* rs2430561 and the haplotype with CAT alleles of *MCM7* rs2070215, rs1527423 and rs1534309. *TERT* rs2853669 was associated with the OS, being a negative predictor in the presence of other known prognostic factors; *TP53* rs1042522, *IL-10* rs1800896, and *TNF-α* rs1800629 were associated with the unfavorable scores of ELN 2017 and cytogenetic risk assessment scores.

Regarding patient OS, we found significant associations between low OS and age greater than 65 years, leukocytosis ≥ 10,000 cells / mm^3, thrombocytopenia <40,000 cells / mm^3, high LDH level ≥ 600 IU / L, high percentage of blasts in bone marrow or peripheral blood > 70%, ECOG performance score ≥ 2 at diagnosis, intermediate and unfavorable cytogenetic risk and ELN 2017 scores, presence of *FLT3* ITD mutation, simultaneous presence of *FLT3* ITD / D835 mutations and *FLT3 / DNMT3A* mutations, low dose therapy and the variant allele of *TNF-α* rs1800750 and *TERT* rs2853669.

The thesis's originality

We believe that the following factors contribute to the thesis's originality: the studies published by our research team are among the very few in the literature that have managed to include over 400 patients with AML, and we are constantly increasing the number of people included; to the best of our knowledge, none of the published studies in the literature simultaneously investigated the mentioned SNPs, and they did not analyze the investigated SNPs taking into account the somatic mutations investigated in our studies; the patients who were included benefited from complex genetic analyses, the results of which were interpreted based on clinical and paraclinical data, prognostic scores, OS, treatment, etc. All of this information were statistically analyzed, using univariate and multivariate analyses, regressions, haplotype analyses, gene interactions, etc. The statistical methodology adds value and originality because many of the results of our studies were the first one published in the international literature in AML patients. The thesis's innovative idea was to adapt the testing protocol to the national infrastructure and funding resources, so that AML patients can be correctly classified and included in the risk stratification and prognosis scores.