XV MIĘDZYNARODOWY KONGRES POLSKIEGO TOWARZYSTWA ALERGOLOGICZNEGO

Next generation sequencing of genes with epigenetic alterations in mastocytotis

Aleksandra Górska[1], Thierry van De Wetering[2], Marta Sobalska-Kwapis[3], Bogusław Nedoszytko[4,5], Marek Niedoszytko[1]

[1] *Department of Allergology, Medical University of Gdansk*

[2] *Pomeranian Medical University in Szczecin*

[3] *Department of Molecular Biophysics, University of Lodz, Poland; Laboratory of Molecular Basis of Behavior, Nencki Institute of Experimental Biology, PAS*

[4] *Department of Dermatology, Venerology and Allergology, Medical University of Gdansk*

[5] *Invicta Fertility and Reproductive Center, Molecular Laboratory, Sopot*

Introduction

Mastocytosis is a neoplastic disease of the bone marrow with the risk of frequent and severe allergic reactions. However the genetic predisposition is not fully understood, the crucial element in pathogenesis is the presence of oncogenic KIT p. D816V gene somatic mutation. The epigenetic mechanism may play a role in mastocytosis.

Aim

Based on our previous epigenetical studies, we have selected 110 candidate genes which were analyzed using Next Generation Sequencing method.

Material and methods

The study group consists of 32 patients with mastocytosis (16 females and 16 males) and 16 control subjects (8 females and 8 males). The whole peripheral blood was taken from all subject. The Archer VariantPlex HS/HGC Protoocol for Illumina (released November 12, 2019) was used. 200 ng of DNA of each sample was used in a volume of 50 µl. The library preparation consists of several steps, starting with fragmentation of the DNA; end repair; first ligation; addition of MRC adapters; second ligation; first PCR and second PCR. The molarity was

established by using the NewEngland biolabs: NEBNext® Library Quant kit for Illumina® on a CFX BioRad PCR equipment. The results were imported into a NEBioCalculator.

Results

We have found 863 alterations on all autosomal chromosomes. On chromosome 3 were the most alterations found, while on chromosome 13 the least. Some of these alterations have been found only in one patient or control subject (n=276), while for 58 alterations have been fully genotyped for all subjects in both groups. 95 alterations have been genotyped for more then 40 subjects. For two alterations the p-value is below 5%. One alteration is a non-sense mutation and it might have an effect on the transcription process of the produced protein ABCA2. The other alteration is an missense alteration and it has been found in exon 11 of the CADH7 gene.

Conclusions

The results confirm our previous findings. The ABCA2 and CADH7 gene mutations are promising candidates for further analysis. Due to the limitation of the fact that study group was not large, these both mutations should be analysed in a bigger group in the future.