Epigenetic Mechanisms of Drug Resistance of Non-tuberculous Mycobacteria

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Abstract: High-performance pyrosequencing of the M. abscessus genome was carried out. The genome contains genes responsible for the functioning of the restriction-modifying system; the HPA2 gene encoding histone acetyltransferase HPA2 and related acetyltransferases; cda gene encoding deoxycytidine triphosphate deaminase that is, a complex of perfect epigenetic mechanisms that can protect mycobacteria from various influences, including drug attack.

Key words: Drug resistance, M. abscessus, epigenom, restriction and modification system, transferase.

1. Introduction

The treatment strategy of mycobacterios requires knowledge of the epigenetic mechanisms of drug resistance, where methylation of DNA using methyltransferases is prominent [1]. Methyl transferases are part of bacterial modification—restriction systems [2]. In addition to transcriptional regulation by methylation, there is also a method for chromatin remodeling [3] involving histone acetyltransferases [4] and histone-like HLP proteins [5]. Epitranscriptomics is actively studying these mechanisms, as well as RNA and DNA editing processes [6]. Epigenetic changes at the DNA level can be a response of a bacterial cell to constantly acting environmental factors, among which the anthropogenic factor—chemotherapy—is no exception. The knowledge of the epigenetic mechanisms of drug resistance of mycobacteria is relevant for phthysiatry, since the reaction of mycobacteria to antibiotics determines the treatment strategy.

The purpose of the work is to detect the epigenetic mechanisms of drug resistance in clinically significant isolates of non-tuberculous mycobacteria.

2. Materials and Methods

The new M. abscessus isolates were isolated from two patients at the clinic of the Federal State Budget TB research Institute of the Russian Ministry of Health. The first patient (medical record No. 113/22), B. A. T., 58 years old, lives in Novosibirsk. Diagnosis: cystic fibrosis pulmonary disease. In 2013, M. abscessus (IV gr. Runyon), resistant to the main anti-TB drugs, was isolated. The second patient—B. R. R., 28 years old, is living in the village Krasnoobsk, Novosibirsk Region, was diagnosed with Infiltrative C1C2 tuberculosis of the right lung in the decay phase. At the end of 2012 and the beginning of 2013, M. abscessus resistant to anti-TB chemotherapy was isolated.

The isolates were isolated by culture using a dense Levenshtein-Jensen nutrient medium.

Genotyping of isolates was carried out by strip-PCR hybridization (LAP test) using GenoType
Mycobacterium CM test systems (Hain Life science). A two-week pure culture of mycobacteria was used for DNA extraction; extraction was carried out using the CTAB/NaCl method (10% N-acetyl-N, N, N-trimethyl ammonium bromid, 0.73 M NaCl) [7]. The amount of bacterial DNA is 500 ng.

High-performance pyrosequencing of the *M. abscessus* genome was carried out on the 454 GS Junior (Roche) platform [8] and included three stages: (1) preparation of fast DNA libraries and ligation of adapters with qualitative and quantitative evaluation of the resulting library, (2) emulsion PCR with clonal amplification, (3) direct sequencing by means of chain synthesis with loading of particles with an amplified DNA library, then analysis of the obtained data, contig metric and targeted mapping [9].

### 3. Results and Analysis

The results of the study are presented in Table 1.

As a result of the sequencing of *M. abscessus*, we discovered the res and mod genes encoding the methylating subunit of type III restriction-modifying system. Unlike CP-M of the first type, they recognize two identical restriction sites located in the opposite orientation. The nuclease activity of the Res subunit appears only when it is in complex with Mod [9]. Full genomic sequencing data also showed that *M. abscessus* contains the genes responsible for the synthesis of DNA cytosine methyltransferase. In prokaryotes, similar enzymes with the same substrate specificity are usually included in restriction—modification systems. There is a 98% homology between human, mouse, plant, and prokaryotic methyltransferase homologs [10]. We consider it possible to carry out homology between the genes of methyltransferases in humans and the genes of *M. abscessus* that we found. In *M. abscessus*, we found an adenine-specific methyltransferase encoded by the *dam* gene.

The HPA2 gene encoding histone acetyltransferase HPA2 and related acetyltransferases was found in the genome of *M. abscessus*. No histones were detected in the cells of the vast majority of prokaryotes, but histone-like proteins were isolated [5]. In accordance with this, it would be more correct to call histone-acetyltransferase HPA2 as histone-like acetyltransferase HPA2. Histone acetyltransferase (NAT) Hpa2 is capable of acetylating not only histones, but also amino acids [11], as well as aminoglycosides. The Hpa2-Ab protein was isolated from *Acinetobacter baumannii* bacteria resistant to the aminoglycoside-carbapinem [12], which indicates the possibility of acetyltransferases to block the drug attack.

| Gene symbol | Decoding the gene symbol | Gene product | Gene product function |
|-------------|--------------------------|--------------|----------------------|
| Res and mod | Genes encoding the components of the R-M system are designated by a specific subunit. Restriction—res; Modification—mod | Type III restriction-modification system methylation subunit | Epigenetic DNA control. |
| Human chromosome 2p23 DNMT3A genes | DNA methylase genes (DNMT): DNMT1, DNMT3a and DNMT3b | DNA-cytosine methyltransferase | DNA methylation, the formation of modified nucleotides and proteins with different chemical structures. |
| dam | DNA adenine methylase-site-specific DNA methylase described in *E. coli* | Adenine-specific methyltransferase | DNA methylation at the exocyclic K6 amino group of adenine in the GATC palindromic sequences. Epigenetic mechanism for altering gene expression. DNA modification through histone-like proteins and chromatin decomactivation. Prevents the incorporation of hazardous modified nucleotides into DNA. |
| Hpa2 | *Aur.1* heterochromatin protein acetyltransferase described in *Saccharomyces cerevisiae* (strain ATCC 204508/S288c) | Histone-acetyltransferase HPA2 and related acetyltransferases | |
| cda | Cytidine deaminase | Deoxycytidine triphosphat deaminase | |
As part of the *M. abscessus* genome, we found the *cda* gene encoding deoxycytidine triphosphate deaminase, which can prevent the inclusion of dangerous modified nucleotides in the DNA of mycobacteria.

As it turns out, non-tuberculous mycobacteria of *M. abscessus* isolate possess perfect epigenetic mechanisms that protect bacteria from the effects of external influences. The most important factors affecting epigenes include nutrition, toxins, viruses [13], ionizing radiation [14, 15], non-ferrous [16] and heavy metals [17]. These are the factors that we call environmental factors that can cause epigenetic changes in DNA in microorganisms. Medicines should also be classified as anthropogenic epigenetic factors.

To understand the mechanisms of drug resistance of bacteria, it is advisable to consider the issues of ecological and genetic order. There is a need for a closer study of the role of anthropogenic factors of developed technogenesis in the creation of microorganisms with environmentally dependent heterosis.

4. Conclusions

*M. abscessus* contains the *res* and *mod* genes, the *dam* gene, responsible for the functioning of the restriction-modifying system; the HPA2 gene encoding histone acetyltransferase HPA2 and related acetyltransferases; *cda* gene encoding deoxycytidine triphosphate deaminase.

*M. abscessus* contains a complex of perfect epigenetic mechanisms that can protect mycobacteria from various influences, including drug attack.

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