"Changes in cellular processes as a new aspect of mucopolysaccharidosis pathogenicity: transcriptomic studies"

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Currently, there are over a dozen thousand of genetic diseases known in the world, about 7,000 belonging to diseases caused by defects in single genes (monogenic diseases). Among them, it is estimated that about 70% cause serious disturbances in the functioning of the central nervous system. Despite the fact that their frequency is relatively low (from 1:2,000 to 1:200,000), if all of them are considered together, they already affect approximately 60 million people worldwide [1]. Patients struggle with the lack of prospects for effective therapy (limited availability of treatment), which generates huge economic and social costs. Both the patients and their relatives are excluded from normal life. The family is often forced to abandon functioning in society in favor of the daily care of relatives.

Mucopolysaccharidoses (MPS) belong to genetic disorders from the group of lysosomal storage diseases that perfectly reflect the above-described situation. They are caused by genetic defects, resulting in a lack or a significant deficiency in the activity of one of the lysosomal hydrolases that are involved in the process of glycosaminoglycan (GAG) degradation. Partially degraded GAGs accumulate in lysosomes, leading to the malfunction of cells, tissues and organs. There are 11 disease types and sub-types (distinguished on the basis of the nature of stored GAG(s) and kind of defective enzyme) with a cumulative prevalence of approximately 1 in 40,000 - 50,000 live births [2]. MPS are usually fatal (especially neuronopathic forms) with an average life span of one or two decades, although patients with milder forms may survive to adulthood [3]. They belong to progressive diseases, due to the continuing accumulation of GAGs. Condition of patients is constantly deteriorating, which is correlated with deterioration of cognitive abilities and behavior of patients suffering from neuronopathic forms. A brief description of one of MPS types, Sanfilippo disease (MPS type III), together with a summary of currently available therapeutic options (consisting in – contrary to majority of other MPS types – only symptomatic treatments) and a prognosis for development of novel therapies in the future, have been included in one of my review papers, published in *Metabolic Brain Disease* [**4**].

The reason for the lack of effective therapies is, on the one hand, the problem of mutations occurring in all the cells of the patient, and on the other hand, the extremely complicated pathogenesis of genetic diseases. Until recently, GAG accumulation was

considered not only key, but also the main cause of MPS [3]. Hence, the amazement at the failure of enzyme replacement therapy appeared, as after intravenous administration of a recombinant human enzyme, symptoms are only partially corrected, despite an efficient reduction in GAG levels [5]. However, our knowledge on the mechanisms leading to pathological changes within cells, tissues or the whole organism in MPS is only fragmentary. Even in monogenic diseases, it appears that a number of side effects arise from the lack of functionality of a single protein (potentially responsible for a single biochemical reaction). This results from the existence of an extremely complex network of dependencies within all cellular processes. Recent reports indicate that in MPS patients there is a much wider spectrum of cellular defects than previously estimated. These changes may be even more important for disease pathogenesis than the mere deposition of undegraded GAGs in the lysosomes. A summary of such changes, with a division into individual cellular processes and a proposal that they are underestimated aspects of MPS, was presented in my article published in *Cell Biology International* [**6**]. Undoubtedly, understanding the molecular mechanisms of genetic disorders is essential for understanding the pathogenesis of the disease and it is a starting point for the possible effective design of new therapeutic methods in the future.

In the light of the above facts, the aim of this doctoral dissertation was to determine changes in gene expression involved in individual cellular processes in MPS patients. Comprehensive analysis (consisting of investigation of expression of all genes in all MPS types), together with indication of the specificity of the detected changes (i.e. which of them are characteristic for all MPS types, and which are specific to one or several types or subtypes), was a novel approach, previously undescribed in the literature.

Transcriptomic analyzes carried out in the course of my work on this dissertation revealed changes in gene expression patterns for each MPS type, relative to healthy person's cells (ranging from 289 to 893 significantly altered transcripts). These results indicated a huge variation between the individual MPS types. On the other hand, by analyzing significantly altered transcripts in most types of this disease, I was able to identify the processes with the greatest number of miss-regulated genes. Among them, there are genes encoding proteins involved in different process such as homeostasis, cell division and vesicular transport. Importantly, expression of certain genes was particularly severely changed in MPS cells, suggesting a possibility to use them as targets for potential therapeutics in further studies on development of novel therapies. Results of these studies have been included in my article published in *International Journal of Molecular Sciences* [**7**].

My subsequent analyzes confirmed significant changes in the expression of genes encoding various regulators of cellular processes. These results have been published in my next paper, in *Metabolic Brain Disease* [**8**], indicating that dysregulation of gene expression may significantly contribute to the development of cellular dysfunction and further appearance of specific symptoms of MPS. Further studies indicated that these include neurodegeneration and behavioral changes [9].

There are some limitations of the experimental model used in my dissertation. They include: (i) the use of one cell line of each MPS type (it is necessary to take into consideration the genetic variability of patients within a given type); (ii) the use of the cellular, not organismal, model (thus, four biological replications and appropriately selected methods of statistical analysis were necessary to obtain reliable results); and (iii) the use of one cell type - fibroblasts (a convenient comprehensive model of pathomechanism for in vitro research). Nevertheless, the conducted transcriptomic studies allowed to detect global changes in expression of genes in MPS cells and provided the basis for experiments aimed at understanding the molecular mechanisms of cellular changes.

Due to the ongoing epidemiological outbreak, I decided to analyze the transcriptomic data in terms of potentially increased risk for SARS-CoV-2 infection in MPS patients. While the narrowing of the airways and the presence of thick mucus, characteristic of the disease, would indicate an additional factor for development of COVID-19, transcriptomic analyzes, supported by experimental studies, suggest that MPS patients may be less susceptible to infection. These studies have been described in my article published in *FEBS Letters* [**10**].

In conclusion, in the course of my dissertation, I provided information that there are significant changes in the expression levels of many genes whose products are involved in various cellular processes. Therefore, not only cell abnormalities appearing due to the physical storage of GAGs in lysosomes, but also cellular dysfunctions resulting from dysregulation of various genes, are responsible for the cellular disorders observed in MPS. Although the occurrence of secondary and tertiary cellular changes were postulated earlier, as summarized in one of my review articles [**6**], they were not associated with global changes in gene expression, and in previously reported studies, all types of MPS have not been investigated simultaneously. Moreover, the obtained results may contribute to the development of new therapeutic strategies. It is possible that only combination therapies, which should include drugs that correct specific cellular processes, while reducing GAG

storage, could lead to the development of an effective drug. Finally, as proposed in one of my publications [**7**], the pathomechanisms of diseases considered monogenic, such as MPS, may in fact involve dysregulation of the whole battery of genes, suggesting that such diseases might be considered "multigenic".

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