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Multiple sclerosis (MS) is a chronic neurological inflammatory demyelinating disorder of the central nervous system. While the etiology of MS is still not fully understood accumulating evidence suggests that it is a multifactorial entity with significant involvement of autoimmune processes. The working model of pathogenesis of MS suggests the interplay between genetic, environmental and epigenetic factors.

In recent years non-coding RNAs have received increasing attention as an important epigenetic mechanism, with particular role of microRNAs. Previous studies implicated miRNAs in regulation of autoimmune demyelination in MS. As the regulation of miRNA expression is highly dynamic and complex, growing evidence suggests the existence of another higher level of regulatory mechanism involved in miRNA activity. In this regard particular role has recently been attributed to circular RNAs (circRNAs). circRNAs represent novel, unique class of endogenous ncRNAs controlling the expression and function of miRNA. They are called natural miRNA “sponges” as the single circRNA molecule is able to interact and neutralize several miRNAs and thus might determine the availability of miRNAs and reduces their ability to control transcription. circRNA are characterized by exceptional high stability. Many of them are tissue-specific. Although accumulating evidence reveals circRNAs active role in physiological and pathological processes including CNS and immune regulation still little is known about their involvement in MS pathogenesis.

The research carried out as part of my dissertation was concerned with the assessment of circRNA expression in patients with MS, its correlation with the type and activity of the disease and determination of the effect of circRNA on the regulation of miRNA and protein transcripts.

A total of 104 participants have been enrolled in this study with 67 patients with RRMS and 37 healthy controls. All patients with MS fulfilled McDonald criteria 2017. Only MS patients free from disease immunomodulatory treatment (DMT) 6 months prior to sample collection were allowed for the study. The disability level for MS neurological status was assessed by expanded disability severity scale (EDSS). The relapse patients were sampled before administration of glucocorticosteroids treatment.

In the first exploratory stage of the investigation microarray hybridization analysis of RNA from PBMCs of 20 RRMS patients (10 in relapse and 10 in remission) and 10 healthy controls was performed. To determine global expression profile of circRNA from PBMC of RRMS patients microarrays containing probes for almost 14×10^3 human circRNAs were used. In the second, validation stage, RNA samples were isolated from a new cohort of 47 RRMS patients (19 in relapse, 28 in remission) and 27 controls and were subjected to quantitative polymerase chain reaction (qPCR).

Microarray results revealed that circRNAs were detected in high abundances in PBMCs in both RRMS groups and HC. Expression of individual circRNAs in RRMS relapse and remission groups significantly differed from controls. Unsupervised hierarchical clustering based on circRNA expression allowed for clear separation of both RRMS groups, relapse and remission, from controls. However, similar unsupervised hierarchical clustering based on circRNA expression in RRMS relapse vs. remission group did not provide a clear distinction between these two stages of RRMS. Thus, microarray data from the discovery set permitted for identification of a group of circRNAs that were specifically and differentially expressed in RRMS patients.

To validate the differentially upregulated circRNAs, top five circRNAs from the group of the highly differentially expressed circRNAs in RRMS versus control in

discovery cohort were tested. The results showed that expression levels of hsa_circRNA_101348, hsa_circRNA_102611 and hsa_circRNA_104361 were significantly higher in RRMS patients in relapse versus controls. All those three circRNA showed also significantly higher expression in RRMS relapse vs. remission.

These three circRNAs: hsa_circRNA_101348, has_circRNA_102611 and has_circRNA_104361 were found to be increased in patients during relapse with a gadolinium enhancement on brain magnetic resonance imaging. The differences for hsa_circRNA_101348 and hsa_circRNA104361 were statistically significant. The correlative analysis of all three circRNAs differentially expressed in MS patients did not associate with disease duration or the degree of disability.

To further characterize functions of the differentially expressed circRNAs the potential impact of these circRNAs on the ability to bind their correspondent microRNAs was further analysed. These microRNAs were deactivated, leading to the release of translation of transcripts of proteins controlled by microRNAs that were dependent on differentially expressed circRNAs in RRMS patients.

miRNA target database search revealed a number of mRNAs which might be targeted by miRNAs controlled by hsa_circRNA_101348, hsa_circRNA_102611 and hsa_circRNA104361. Subsequently shared annotated mRNAs for all three differentially expressed circRNA were analyzed. It was found that five mRNA overlapped between two circRNAs, hsa_circRNA_101348 and hsa_circRNA_104361. Group of these five transcripts consisted of AK2, CBX5, DGKH, IKZF2, RNF24. Among these five protein transcripts, there were two involved in the functions of B lymphocytes - AK2 and IKZF3.

To confirm the bioinformatic prediction of circRNA effect on the mRNA profile in RRMS relapse patients expression levels of those 5 mRNAs: AK2, CBX5, DGKH,

IKZF3 and RNF24 in PBMCs were directly measured. It was found that three transcripts have indeed shown significantly higher expression in PBMCs in RRMS relapse - AK2, IKZF3 and CBX5. Most interestingly, two of them, AK2 and IKZF3, have been implicated in B cell function.

In this study a distinct circRNA profile in PBMC of RRMS patients versus healthy controls was revealed. Overexpression of circRNAs hsa_circRNA_101348, hsa_circRNA_102611 and hsa_circRNA104361 in RRMS relapse group was validated in a new cohort. Further bioinformatics analyses revealed a group of miRNAs that showed specific binding sites for overexpressed circRNA, forming the basis for the biological action of circRNA as a miRNA "sponge". Subsequent bioinformatics analyses and direct measurements of protein transcripts showed that circRNA-miRNA complexes increased the expression of three mRNA. The most important observation of this work is that among these three protein coding transcripts there were two closely related to B cell function. AK2 gene mutations are associated with inhibition of B cell activation and antibody production. IKZF3 has been shown to play critical role in the function of mature B cells in the peripheral immune compartment and is required for the generation of high-affinity antibodies-secreting bone marrow plasma cells. Therefore, the results of this work indicate a possible hypothesis that the circRNA-miRNA system may have an impact on the observed in MS B-cell dysfunction. These data also point at new biomarkers of RRMS.