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Title: Exploring the Role of MicroRNAs and Associated Proteins in Multiple Sclerosis

Authors: Melika Abrishami[1], Mostafa Rezaei-Tavirani[2,*]

1. *Student Research Committee, Medical Laboratory Sciences Department, School of Allied Medical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran.*
2. *Proteomics Research Center, Faculty of Paramedical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran.*

***Corresponding Author**: Mostafa Rezaei-Tavirani, Proteomics Research Center, Faculty of Paramedical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran. Email: Tavirany@yahoo.com

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Abstract

Introduction: Multiple sclerosis (MS) is a chronic autoimmune disease affecting the central nervous system. The diagnosis and monitoring of MS progression is challenging because of its complex pathogenesis and the lack of specific biomarkers. Introducing related microRANs and associated proteins with MS is aim of this study.

Methods: MS-related miRNA data of 4 peripheral blood profiles from relapsing-remitting MS (RRMS) patients and 8 healthy controls were extracted from the GEO database. Their related proteins were analyzed using bioinformatics methods, such as protein-protein interaction (PPI) network and action map.

Results: Numbers of 18 differentially microRNA were detected which discriminate the patient samples from controls. The 31 related proteins were identified and assessed via PPI network analysis and action map evaluation.

Conclusion: In conclusion, a protein panel of NCL, NOP58, SNRNP70, U2AF2, YBX1, PRPF8, BOP1, and PIK3K as the crucial individuals which are associated with MS was suggested for further investigation.

Keywords: Multiple sclerosis, MicroRNAs, Bioinformatics, Network analysis, Protein

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Introduction

Multiple sclerosis is a chronic autoimmune disease that affects the central nervous system. Due to complex pathogenesis and lack of specific biomarkers, diagnosis of MS is a challenge. Recent data show that the incidence of multiple sclerosis (MS) is increasing globally. Early diagnosis of MS reduces the burden of disability-adjusted life years and associated health care costs(1).

Extensive exploration has demonstrated that MS is thought to be caused by systemic immune activation of autoimmune mechanisms against components of the central nervous system. In MS, inflammation is regulated by interactions between several immune cells, such as T and B cells, macrophages, and central nervous system glial cells (microglia and astrocytes), as well as antigens that react against myelin, especially myelin basic protein (MBP) and myelin oligodendrocyte glycoprotein (MOG). Additionally, Th1 and Th17 cells can cross the blood brain barrier (BBB) and migrate to the central nervous system, then activate microglia and secretion of inflammatory cytokines, such as tumor necrosis factor-α (TNF-α), l IL-1β and IL-6 (2, 3). Diagnosis of MS is based on clinical and radiological assessment and current effective treatments is targeting of peripheral immune system (4-6). The diagnostic criteria for MS have limitations of sensitivity and specificity. This means that in lack of a specific test, some patients may be misdiagnosed or not diagnosed at all (7-10).

The Non-coding RNAs (ncRNAs) are RNA molecules that do not encode proteins but play important roles in gene expression regulation. Investigations indicates that ncRNAs are involved in the pathogenesis and progression of MS (۱۱،۱۲) . Roles of ncRNAs in the regulation of immune cells and pathways, and also involvement in the neurodegeneration process and MS progression are highlighted by researchers (13-15). In addition to their diagnostic potential, ncRNAs may also serve as therapeutic targets for MS treatment. It may be effective in development of personalize medicine (15-18). miRNAs are small non-coding RNA molecules that play a crucial role in regulating various cellular processes, including inflammation and immune response and they have emerged as potential biomarkers for MS, as they are stable, detectable, and quantifiable in various biological fluids, such as blood and CSF. Several studies have shown that the expression levels of miRNAs are altered in MS patients compared to healthy controls or other neurological diseases. Moreover, some miRNAs have been associated with clinical features, such as disease duration,

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disability score, relapse rate, lesion load, inflammation, neurodegeneration, and treatment response (19-22).

The application of bioinformatics in the interpretation of genomics outcomes has attracted the attention of researchers. Network analysis is a bioinformatics tool that appears as a suitable method to interpret genomics data (۲۳). There are several documents about the application of network analysis in exploring molecular aspects of MS (۲۴). Protein-protein interaction (PPI) network analysis is a computational method used to identify interactions between proteins and to understand the functional relationships between them. In a published study, researchers used PPI network analysis to identify hub long ncRNAs and potential drugs for multiple sclerosis. They constructed a PPI network using differentially expressed mRNAs and identified four modules enriched in immune-related pathways. They identified three keys long ncRNAs (LINC00649, TP73-AS1, and MALAT1) associated with MS (25–29). In the present study, the possible role of miRNAs and their related protein in the diagnosis of MS disorder is investigated via network analysis by using data from the GEO database.

Methods

Data collection: multiple sclerosis Micro RNAs data had been selected from the GEO database (GSE124900). data had been produced by using the NGS approach. The 16 peripheral blood-extracted miRNA profiles from relapsing-remitting MS (RRMS) patients and 8 healthy controls (HC) (۳۰) were selected for more analysis. UMAP analysis revealed the samples were not separated via the performed assessment. So, the suitable samples including 4 patients and 4 controls were candidates to be evaluated. In addition to the miRNA analysis, related proteins have also been investigated in the literature.

Pre-evaluation analysis: The GEO2R program was applied to evaluation data. To gain insights into potential miRNA expression signatures and identify differentially expressed miRNAs, visualization tools such as Boxplot, Venn diagram, and Volcano plot were employed. The use of these analytical tools is crucial in minimizing bias in miRNA expression levels and identifying potential diagnostic targets (microRNA) for future interventions. These visualization tools provide a visual representation of the data, enabling researchers to identify patterns and trends that may not be immediately apparent from raw data. The significant differentially expressed microRNAs with a p-adj less than 0.05 were selected for further analysis.

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PPI network analysis: Actions (activation, expression, catalysis, and post-translation) between the explored proteins were assessed via an action map by using Cytoscape software v 3.7.2. Furthermore, PPI network analysis has been employed to analyze the related proteins. The related proteins were included in an interactome via STRING database by Cytoscape software. The nodes were connected via undirected edges. The utilization of PPI network analysis has provided a comprehensive understanding of the binding relationship among proteins and has facilitated the identification of potential key proteins involved in the diagnosis of multiple sclerosis.

Overall, the integration of multiple analytical approaches, including miRNA profiling, related protein investigation, and PPI network analysis, has enabled a more comprehensive understanding of the molecular mechanisms underlying multiple sclerosis.

Statistical analysis: P adj<0.05 was applied to find the significant differentially expressed microRNAs. Confidence score = 0.4 was considered to form PPI network.

Results

The extracted significant differentially express microRNAs are presented in the table1. As it is shown in the table1 number of 18 microRNAs are eligible for more investigation. Box plot (figure 1) has been utilized for maintaining that our selected microRNAs are trustable for further investigation because the median line of them was in the same position. The Volcano plot was used to identify the micro RNAs that are significantly differentially expressed (DE) between the two groups. As it is shown in Figure 2, there are several significant DE microRNAs which discriminate patient samples from controls. Venn diagram visualizes the 18 significant DE microRNAs that differentiate the compare groups (see figure 3).

Furthermore, the number of 31 proteins related to 18 microRNAs including; FBL, NCL, NHP2L1, PTBP1, YBX1, NOP58, PRPF8, SNRNP70, U2AF2, STAT3, SP1, SNRPA, TLR4, PIK3CA, BOP1, DUSP1, TPD52, ZNF580, NECAP2, ELF3, GARS, BCL2, TRMT10A, N R2E3, KLF3, ZNF143, HCFC1, EEFA1, SF3B1, NHP2, and NPM1 were identified from literature. an action map was constructed to illustrate the connections among the 31 related proteins. Each color in the diagram represents a different protein-protein function: green, yellow, black, and purple consist of activation, expression, catalysis, and post-translation respectively. It should be noted that functional interactions for 12 proteins remained unknown. Following a thorough investigation, it

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was determined that NPM1 is the most highly connected protein in this protein-protein interaction (PPI) diagram. NPM1 is connected with: FBL, YBX1, NOP58, PRPF8, STAT3, BOP1, PIK3CA, and DUSP1.

The PPI network including 31 proteins is represented in Figure 5. As is detected in Figure 5 number of 27 proteins are connected in the subnetwork while the individuals that remain, are isolated nodes. Nodes of the network are visualized based on degree value.

Considering the results of the action map and PPI network analysis the carousal proteins were selected for more analysis. A list of the carousal proteins and their biological descriptions are presented in Table 2.

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Table 1. The significant non-coding RNAs related to the blood of MS patients. FC refers to fold change

GeneID	Padj	Log2FC	Symbol	Description
692206	0.049	2.734	SNORD90	small nucleolar RNA, C/D box 90
4550	0.049	2.532	RNR2	l-rRNA
26787	0.049	2.388	SNORD61	small nucleolar RNA, C/D box 61
26871	0.049	2.193	RNU1-1	RNA, U1 small nuclear 1
1E+08	0.049	-1.154	TRG-CCC2-1	tRNA-Gly (anticodon CCC) 2-1
494328	0.049	-1.962	MIR379	microRNA 379
26869	0.046	2.252	RNU1-3	RNA, U1 small nuclear 3
442913	0.046	-1.901	MIR376C	microRNA 376c
9304	0.040	2.328	SNORD22	small nucleolar RNA, C/D box 22
26829	0.040	2.537	RNU5E-1	RNA, U5E small nuclear 1
6060	0.040	2.166	RNU1-4	RNA, U1 small nuclear 4
442905	0.035	-1.882	MIR337	microRNA 337
692212	0.035	2.937	SNORD99	small nucleolar RNA, C/D box 99
26870	0.028	2.305	RNU1-2	RNA, U1 small nuclear 2
406954	0.006	1.054	MIR181A2	microRNA 181a-2
406995	0.004	1.063	MIR181A1	microRNA 181a-1
406956	0.002	1.296	MIR181B2	microRNA 181b-2
406955	0.000	1.107	MIR181B1	microRNA 181b-1

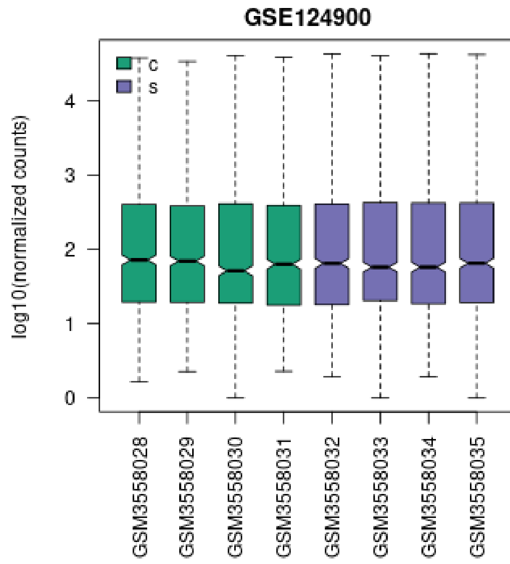

Figure 1. Boxplot of non-coding RNAs expression profiles. S and C refer to patients and control blood samples respectively.

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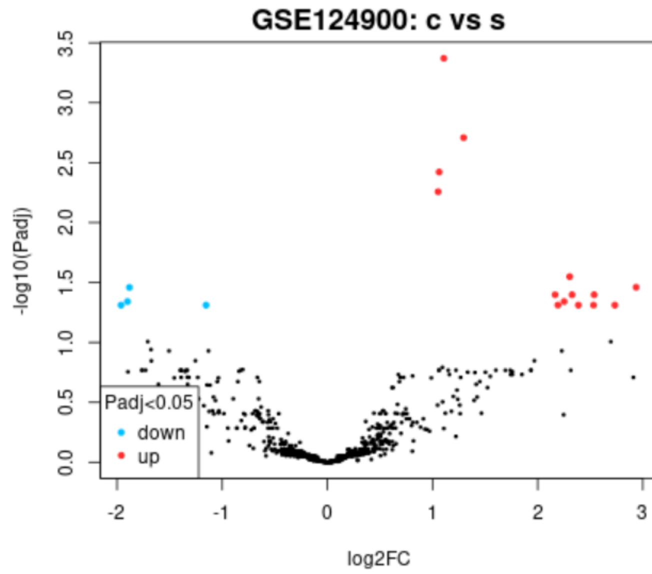

Figure 2. Volcano plot, Expression profiles of non-coding RNAs in patients (S) and control (C) blood samples.

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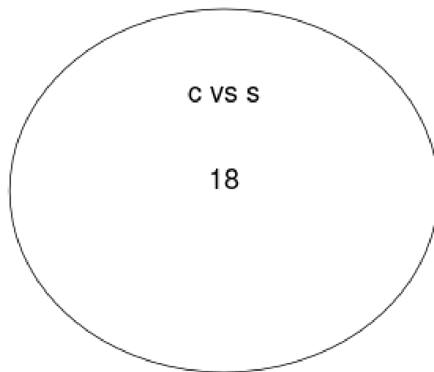

Figure 3. Venn diagram of non-coding RNAs expression profiles. S and C refer to patients and control blood samples respectively.

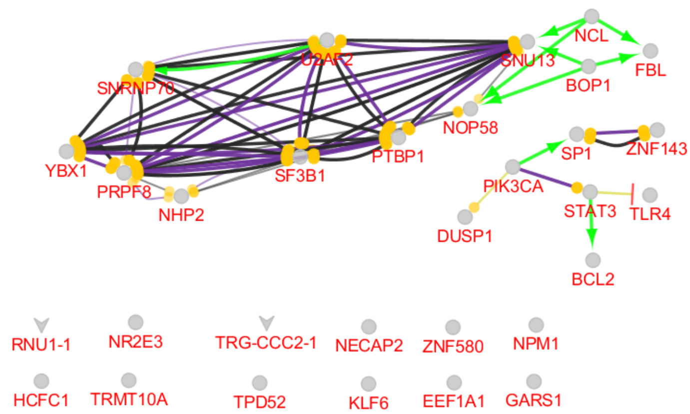

Figure 4. Action map diagram for the 31 related proteins. Colors: green (activation), yellow (expression), black (catalysis), purple (post-translational modification).

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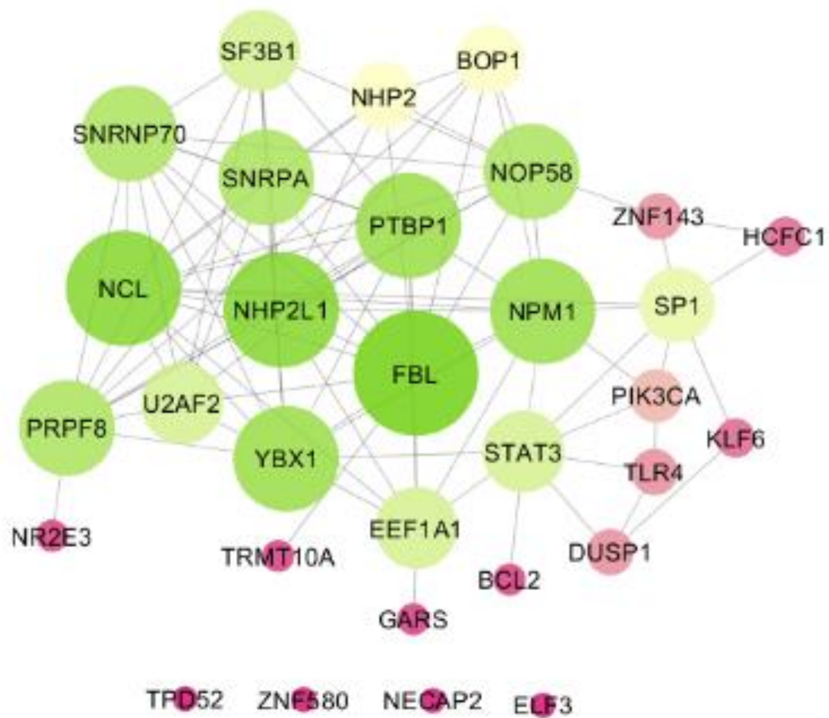

Figure 5. PPI network diagram of 31 proteins related to the selected microRNAs. Nodes are layout based on degree value; larger size and red to green refer to higher value of degree.

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Table 2. Prioritizing proteins in the PPI network based on their efficacy, functions, and potential.**No.**

	Protein	Function
1	FBL	Fibrillarin (FBL) is an essential nucleolar protein that participates in pre-rRNA methylation and processing (31). FBL plays a role in nucleolar organization and functions in innate immune response (32). Our diagram shows it can also Catalyzes reactions that are involved in the expression of NCL
2	NCL	Neuronal ceroid lipofuscinoses (NCLs) is a group of devastating neurological disorders that affect people of all ages and have a global distribution [1]. NCLs are caused by mutations in at least 13 genetically distinct genes, including the CLN1 and CLN3 genes(33,34)
3	NHP2L1	NHP2 non-histone chromosome protein 2-like 1, is a component of the H/ACA small nucleolar ribonucleoprotein (snoRNP) complex that catalyzes the pseudouridylation of rRNA and other RNA substrates. It also plays a role in pre-mRNA splicing and telomere maintenance (35).
4	NPM1	Nucleophosmin or B23 is a multifunctional protein that shuttles between the nucleus and the cytoplasm. It participates in ribosome biogenesis, mRNA processing, chromatin remodeling, DNA repair, apoptosis, and genome stability (36). NPM1 has been implicated in the regulation of microglia activation(37).
5	PTBP1	Polypyrimidine tract-binding protein 1 binds to the polypyrimidine tracts of pre-mRNA and regulates alternative splicing. It plays a role in neuronal development, differentiation, and function. It is involved in Alzheimer's disease, Parkinson's disease, schizophrenia, autism, and epilepsy and cancer, immune response and inflammation (38-42). PTB1 has been shown to regulate the expression of BDNF(43) BDNF has been shown to promote neuronal growth and survival in the central nervous system, synaptic plasticity, and mitochondrial biogenesis, making it a promising biomarker in neurodegenerative conditions (44,45).
6	YBX1	Y-box binding protein1 is also a component of messenger ribonucleoprotein (mRNP) complexes and may have a role in microRNA processing (46). YBX1 expression is upregulated in a variety of cancers, pointing towards its role as a potential oncogene (47). YBX1 is one of the mRNAs significantly enriched in oligodendrocyte progenitor cell (OPC) processes (48).
7	NOP58	NOP58 is involved in the biogenesis and function of small nucleolar ribonucleoproteins (snoRNPs), which are essential for the processing and modification of ribosomal RNA (rRNA) (46). NOP58 is also a component of the telomerase complex, which is responsible for maintaining the length and integrity of telomeres, the protective ends of chromosomes (47).

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8	PRPF8	PRPF8 is involved in the process of pre-mRNA splicing. It plays a critical role in the assembly, stability, and catalytic activity of the spliceosome(49,50).
9	SNRNP70	The SNRNP70 protein is a major component of the spliceosome. Recent research has identified a cytoplasmic pool of SNRNP70 that plays an important role in regulating motor axonal growth, nerve-dependent acetylcholine receptor clustering, and neuromuscular synaptogenesis. This cytoplasmic pool has a protective role for a limited number of axonal transcripts preventing them from degradation (51–53).
10	U2AF2	U2 small nuclear RNA auxiliary factor2 is a critical protein involved in pre-mRNA splicing, which is the process of removing non-coding regions (introns) and joining coding regions (exons) of mRNA molecules. As a component of the spliceosome (54,55).
11	STAT3	Signal transducer and activator of transcription3 is a transcription factor that is activated in response to various cytokines and growth factors and plays a crucial role in multiple cellular processes such as cell growth, survival, differentiation, and inflammation (56-61). It is associated with autoinflammation in myeloid, lymphoid, cancer and autoimmune diseases such as multiple sclerosis (62-64).
12	TLR4	Toll-like receptors (TLRs) recognize pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) and then initiate immune responses (65-66). It is involved in increment areas surrounding inflammatory vessels and the center of MS lesions, BBB integrity, and activation of T and B cells (67-69).
13	PIK3CA	phosphatidylinositol 3-kinase is an enzyme that plays a crucial role in various cellular processes. It plays a role in signaling pathways that regulate insulin responses, nutrient uptake, metabolism, immune responses, and inflammation (70,71). It also has a relationship with mir-21 in activating the PI3K/AKT pathway as a potential therapy for the treatment of MS (72-74).

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Discussion

Multiple sclerosis (MS) is a complex autoimmune disease that affects the central nervous system (CNS). The exact mechanisms underlying MS pathogenesis are still elusive. Micro RNAs (miRNAs), which are RNA molecules that do not encode proteins but regulate gene expression, have been suggested to play a role in MS. Moreover, miRNAs are stable biomarkers in peripheral blood that make them appropriate candidates for diagnosing MS. They can also reflect the status of immune cells and inflammatory pathways that are relevant to MS pathogenesis, distinguish MS patients from healthy controls and other disease controls, as well as different MS subtypes and stages, and predict the response to treatment and the risk of relapse or progression in MS patients (75–77). Therefore, by analyzing the related proteins of miRNAs, we can find critical pathways that can be the key to find new treatments and diagnosing methods for MS.

In the prsent study, data from the GEO database was used to identify microRNAs that were differentially expressed in MS patients compared to healthy controls. A total of 18 significantly dysregulated microRNAs were selected and 31 related proteins involved in MS disease were determined for analysis. Pre-evaluation of data indicates that the analysis is valid. An action map was used as a tool to screen data. Based on action map findings, NCL, U2AF2, SNRNP70, YBX1, and STAT3 participate in considerable relationships with others. Activation of SNRNP70 by U2AF2 is an important event because it has a role in neuromuscular synapses and U2AF2 also catalyzes and has an effect on post-translation of PTBP1 which has been investigated in literature as an MS biomarker (40). STAT3 can be a potential candidate because of its relation with IL-6 that were upregulated in MS patients (78).

SNRNP70 plays an important role in regulating motor axonal growth, nerve-dependent acetylcholine receptor clustering, and neuromuscular synaptogenesis and a protective role for a limited number of axonal transcripts preventing them from degradation. Moreover, non-nuclear SNRNP70 can locally regulate splice variants of transcripts such as agrin, thereby locally controlling the formation of synapses (51–53).

On the other hand, YBX1 effects both PTBP1 and U2AF2 and also expresses PRPF8 which has a role in the post-transcription of U2AF2. YBX1 has a role in oligodendrocyte differentiation, with special regard to process extension and ramification as well as myelin production (48). Relationship and effect of STAT3 (which has been investigated as an MS biomarker) and TLR4

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are remarkable; they both have positive regulation of each other. It's important to mention that TLR4 is a drug target because of its main role in inflammation and T cell and macrophage activities (65,79,80). TLR4 and BTK are interconnected in the context of immune signaling and inflammation, and both have been implicated in the pathogenesis of MS, TLR4 also has an impact on BTK which is an MS drug target (81,82). The other protein; PIK3K can be a potential biomarker as it has a relationship with mir-21 in activating the PI3K/AKT pathway as a potential therapy for the treatment of MS (72,73). It is connected with STAT3 by its post-translation modification and expression.

PPI network analysis was performed to identify the most relevant proteins that interacted with these microRNAs. Based on degree value, results indicated that several proteins such as FBL, NCL, PTBP1, SNRNP70, YBX1, PRPF8, and NPM1are important ones. Except NPM1, the other important nodes of PPI network are highlighted in action map. These proteins have also been reported in other studies. For instance, PTBP1 has been shown to regulate alternative splicing of genes involved in immune response and inflammation (42,83) which are key pathways in MS pathogenesis(40,84). NPM1 has been implicated in the regulation of microglia activation which is a hallmark of MS (37,85,86).

PTB1 has been shown to regulate the expression of BDNF (43). BDNF has been shown to promote neuronal growth and survival in the central nervous system, synaptic plasticity, and mitochondrial biogenesis, making it a promising biomarker in neurodegenerative conditions (44,45).

SNRNP70 has been shown to regulate alternative splicing of genes involved in neuronal function and synaptic plasticity which are disrupted in MS (51,53,87). YBX1 is identified as a common high central gene in protein-protein interaction networks corresponding to both type 1 diabetes and multiple sclerosis (88). NOP58 is another candidate that has a high degree and it is also activated by BOP1 and NCL as discussed before and NOP58 has a connection with PTBP1 and PRPF8.

Conclusion:

Moreover, as PTBP1, STAT3, and TLR4 have been suggested in the literature as potential biomarkers for multiple sclerosis (MS), based on our investigation with bioinformatics approach, they have roles in regulating the expression and function of NCL, NOP58, SNRNP70, U2AF2, YBX1, PRPF8, BOP1, and PIK3K proteins. These proteins are involved in various cellular

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processes that are relevant to MS pathogenesis, such as RNA splicing, transcription, inflammation, and apoptosis. Therefore, we propose a new biomarker panel consisting of PTBP1, STAT3, and TLR4, as well as SNRNP70, YBX1, PTB1, and PI3K as candidate drug targets. In addition, we suggest that monitoring the effect of targeting TLR4 on STAT3 activity could be a useful strategy for developing novel therapeutics for MS. To validate the proposed biomarker panel and drug targets, further experimental and theoretical investigations are suggested. Limited sources of databases and samples are limitations of this study.

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