



A Novel Regulatory Function of Long Non-coding RNAs at Different Levels of Gene Expression in Multiple Sclerosis

Jalal Gharesouran¹ · Mohammad Taheri²  · Arezou Sayad¹ · Soudeh Ghafouri-Fard¹ · Mehrdokht Mazdeh³ · Mir Davood Omrani⁴

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Abstract

Long non-coding RNAs (lncRNAs) play critical roles in regulation of immunological pathways. Consequently, their expression profile represents new biomarkers for susceptibility and progression of immunological disorders. However, their role in chronic inflammatory diseases such as multiple sclerosis (MS) remained unknown. Here, we assessed the expression of lncRNAs *MALAT1* and *HOTAIRM1* as well as their target genes in peripheral blood of MS patients to show their possible roles in disease initiation and progression. In this study, 50 patients with relapsing-remitting MS and 50 healthy matched controls were enrolled. Comparative Ct method via TaqMan assay was used to quantify transcript levels of *MALAT1*, *HOTAIRM1*, *AGO2*, *CSTF2*, *CPSF7*, and *WDR33*. Our analysis depicted significant differences in lncRNAs and their target genes expression levels. *AGO2* expression was significantly elevated in MS patients ($P < 0.001$) whereas, *CSTF2* expression was considerably down-regulated ($P < 0.042$). These findings suggest that *AGO2* and *CSTF2* can be considered as potential theoretical biomarkers for MS and can be helpful for diagnosis and prognosis of responding patients to interferon.

Keywords Multiple sclerosis · *MALAT1* · *HOTAIRM1* · *AGO2* · *CSTF2* · *CPSF7* · *WDR33*

Introduction

Multiple sclerosis (MS) is a heterogeneous autoimmune disease with poor diagnosis and higher prevalence in women (Eftekharian et al. 2016). Previous studies on MS pathogenesis depict that the immune system dysregulation is associated with changes in transcriptome and gene expression. About 85% of affected patients have relapsing-remitting multiple sclerosis (RR-MS) (Hatami et al. 2018). Inflammatory reactions trigger

immune cells to cause localized demyelinating of nerve fibers. The unpredictable episodes of relapses or exacerbations lead to worsening of neurologic function or deterioration of existing symptoms (Compston and Coles 2008). Identification of genetic markers would facilitate prediction of disease prognosis during the early stages of the disease and monitoring both disease course and response to therapy.

Long non-coding RNAs (lncRNAs) and microRNAs (miRNAs) belong to non-coding RNAs (ncRNAs) family which is involved in many cellular processes (Eftekharian et al. 2017). miRNAs mediate regulation of other classes of ncRNAs and also harmonize the expression of protein coding genes by targeting their transcripts to AGO2-containing complexes in the cytoplasm. Mechanistically, it is proposed that lncRNAs perform “sponge-like” effect on various miRNAs, which subsequently inhibit miRNA-mediated functions. RNA-seq analyses have shown that the alteration in lncRNAs expression contribute to diverse neurological disorders (Wu et al. 2013). The lncRNA lung adenocarcinoma transcript 1 (*MALAT1*), also referred to as non-coding nuclear-enriched abundant transcript 2 (*NEAT2*), was initially reported to contribute in metastasis in lung cancer (Gutschner et al. 2013; Li et al. 2015). *MALAT1* is up-regulated in many solid tumors and is associated with cancer metastasis and

Jalal Gharesouran and Mohammad Taheri contributed equally to this work.

✉ Mir Davood Omrani
davood_omrani@yahoo.co.uk

¹ Department of Medical Genetics, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

² Student Research Committee, Shahid Beheshti University of Medical Sciences, Tehran, Iran

³ Neurophysiology Research Center, Hamadan University of Medical Sciences, Hamadan, Iran

⁴ Urogenital Stem Cell Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran

recurrence (Gutschner et al. 2013). *HOXA transcript antisense RNA, Myeloid-Specific 1 (HOTAIRM1)* as an lncRNAs is located between *HOXA1* and *HOXA2* genes, in the *HOX* gene cluster. The gene is expressed in myeloid lineage cells and is involved in myeloid transcriptional regulation. Transcription of *HOTAIRM1* is induced by retinoic acid and transcripts participating in myelopoiesis regulation. *HOTAIRM1* also plays an essential role in the regulation of autophagy pathways in myeloid cells differentiation through the degradation of PML-RARA oncoprotein. *HOTAIRM1* makes a miRNA sponge in a pathway which contains miR-20a/106b, miR-125b, and their targets AGO2, ULK1, E2F1, and DRAM2 (Chen et al. 2017). Recent studies confirmed the critical role of *HOTAIRM1* in controlling the development of other immune cell-types such as innate lymphoid cells (ILC), monocyte-macrophages, T cell subsets (Th1, Th2, Th17, and Treg), and B cells (Atianand and Fitzgerald 2014).

Functional studies have indicated that alteration in the expression of Cleavage Stimulatory Factor-64 kDa (CSTF2), which binds to a GU/U-rich cis-element downstream to the specific polyA site (PAS), affect pre-mRNA processing. The transcriptome-wide study has also demonstrated that the expression level of CSTF2 has a positive correlation with the tendency of global mRNA 3'-UTR shortening (Yao et al. 2012). It was revealed that the CSTF2 expression was up-regulated upon T cell receptor (TCR) stimulation. On the other hand, in native T cells, the expression level of CSTF2 is lower and thus proximal PASs are not efficiently exploited for gene regulation. However, when the TCR was stimulated, the CSTF2 level was overexpressed and accordingly the usage of the proximal PAS increased (Chuvpilo et al. 1999). The protein encoded by CPSF7 is one of the six necessary factors for correct end-termination in 3' UTRs in precursor mRNAs. This protein acts as a heterotetramer, each containing a dimer of 25 kDa subunits connected to 59 and 68 kDa subunits. The complex protein is composed of numerous factors which are involved in the editing and splicing of newly produced mRNAs like U2, and also in regulation of large complex activity associated with polyadenylation and its sub units like CSTF2.

CPSF7 and other factors such as CPSF73, CPSF100, Symplekin, and WDR33 cooperate in detection of poly(A) signal region and formation of the complex (Schönemann et al. 2014). WD repeats contain 40 amino acid regions typically include glycine, histidine, tryptophan, and asparagine

which may facilitate the formation of heterotrimeric or multi-protein compounds. WDR33 protein as a member of WD proteins is involved in a variety of cellular processes, including cell cycle progression, cell signal transduction, apoptosis, and nucleus protein regulation. This gene plays an important role in cell differentiation or DNA recombination and has several transcripts produced through alternative splicing.

In the present study, we evaluated the expression of *MALAT1*, *HOTAIRM1*, *AGO2*, *CSTF2*, *CPSF7*, and *WDR33* in patients with RR-MS compared to normal control.

Materials and Methods

A total of 50 unrelated patients in the relapsing stage of RR-MS disease and 50 healthy control subjects were recruited to participate in this study at Department of Medical Genetics, Shahid Beheshti University of Medical Sciences Tehran, Iran, during 2-year study period (2016–2018). (Table 1). Exclusion criteria for control group were pregnancy, steroid therapy, infection within 1 month prior to sample collection, existence of other autoimmune diseases, malignancies, and chronic infectious diseases. The selection criteria for RR-MS individuals included clinical or radiological relapse within 1 month from sample collection and no history of smoking. The patients were confirmed to have McDonald criteria for MS, as revised in 2010, and stable phase of the disease (Polman et al. 2011). The study groups were prospectively matched for the same geographical region, sex, age (mean age 35.3 ± 2.1), and body mass index (BMI) (Table 1). Individuals in case group were classified in three different groups based on their age ranges (< 30, 30–40, > 40). They were treated with interferon (IFN)- β therapy for at least 2 years (Cinno Vex had been injected subcutaneously at doses of 20 μ g three-times a week) (Taheri et al. 2017; Rezazadeh et al. 2018). Informed consent was obtained from each participant and the study was approved by the institutional ethics committee (No. 3275).

Total RNA was extracted from each blood sample by the Geneall Hybrid-RTM blood RNA extraction Kit (cat No. 305-101) according to the manufacturer's instructions. Total RNA was treated with DNase I for 30 min at 42 °C. The mRNA was reverse-transcribed into complementary DNA (cDNA) by using Geneall cDNA synthesis kit as described by the

Table 1 Characteristics of the study population

Variables	MS patient	Control
Female/male [no. (%)]	31 (62%)/19 (38%)	27 (54%)/23 (46%)
Age (mean \pm SD, year)	36.2 \pm 2.9	35.3 \pm 2.1
Age at onset (mean \pm SD, year)	31.41 \pm 2.8	–
Duration (mean \pm SD, year)	4.58 \pm 3.2	–
EDSS (mean \pm SD)	3.07 \pm 2.7	–

manufacturer's protocol. The expression level of lncRNAs was measured by qRT-PCR using TaqMan® assay in the Corbett Rotor gene 6000 machine (Corbett Life Science). The reaction system of PCR was performed in duplicate containing 2× concentrated Master Mix, 2 µL of template cDNA, and 100 nM of primers in a final volume of 25 µL. The sequence of probes and primers has been presented in Table 2. *Hypoxanthine guanine phosphoribosyl transferase (HPRT)* was applied as a reference gene based on its stable expression within different sample groups (Vandesompele et al. 2002). The initial denaturation step at 95 °C for 10 min was followed by 40 cycles at 95 °C for 15 s, and 60 °C for 45 s. Relative expressions of genes were calculated with $2^{-\Delta\Delta C_t}$ method, using HPRT as an internal control.

To identify the mRNAs as “cis-regulated target genes” in up- or downstream of the given lncRNAs and the correlation between lncRNA and mRNA expressions some databases were reviewed including LNCipedia, ChIPBASE, DIANA tools, GENECODE, ENCODE, lncRNA, disease databases, lncrnadb, FANTOM, lncCeDB, STRING, and HGNC.

The lncRNAs and co-expression genes such as chromatin regulators and transcription factors (TFs) were identified by using ENCODE and other online databases. The “TF–lncRNAs” two element networks were generated in the first step. After the primary evaluation merged network has been proposed.

Table 2 The sequence of probes and primers

Gene	Sequence(5' → 3')
HPRT1	Forward primer: AGTCTGGCTTATATCCAACA CTTCG Reverse primer: GACTTTGCTTTCCTTGGTCAGG Probe: 5 FAM-TTTCACCAGCAAGCTTGCGA CCTTGA-TAMRA3
MALAT1	Forward primer: CGCTAACGATTTGGTGGTGAAG Reverse primer: GGATCCTCTACGCACAACGC Probe:CTCGCCTCCTCCGTGTGGTTGCC
AGO2	Forward primer: ATGAGATGACAGACGTGACTGG Reverse primer: TTGATCTCGATGCCGTGTG Probe:TGTTCCGCATGTCCCAGACGCCCT
HOTAIRM1	Forward primer: GAAGAGCAAAAGCTGCGTTCTG Reverse primer: CTCTCGCCAGTTCATCTTTCATTG Probe:CCCGACTCCGTCGCCGCC
CSTF2	Forward primer: CTGAAAATTCTGCATCGCCAGAC Reverse primer: CTGCTGGTTCATTGACACATTGG Probe:CCCAACGCTGATTGCAGGCAACCC
CPSF7	Forward primer: CTCACAGCCATTGCGGTTATC Reverse primer: CCATGAAGACAGTCCTTAAGAGAG Probe:ACACGGCAACGCTCATCATTGGCA
WDR33	Forward primer: GGAAGACGCTTGGTCACTGG Reverse primer: GTCTGCTGTCAACATCCACATG Probe:ACCACGTCATGCCCTCACTGGG

SPSS software version 18 (statistical package for social sciences Inc., Chicago, IL, USA) was used to analyze the data. Independent *t* test was applied to compare expressions of genes between two groups. Pearson correlation coefficient was calculated for evaluation of the correlation between variables. *P* values less than 0.05 were considered as statistically significant. Spearman rank order correlation test was performed to evaluate the possible correlation between relative expression levels of genes and clinical variables.

Results

The Expression Levels of lncRNAs and Their Target Genes

The expression levels of *MALAT1*, *HOTAIRM1*, *AGO2*, *CSTF2*, *CPSF7*, and *WDR33* have been compared between MS patients and control group based on their age and sex. The results are presented in Table 3. As shown in Table 3, the expression of *MALAT1* gene was significantly different between two groups participants who were under the age of 30 ($p = 0.04$). The mRNA levels of *AGO2* was considerably up-regulated in MS patients compared to healthy subjects ($p < 0.001$) (Table 3). The results of statistical analysis showed no difference in the *HOTAIRM1* expression between participants (Table 3). There was significant decrease in expression level of *CSTF2* ($REx = 0.7533$, $p = 0.042$) between two groups especially when comparing female patients with female controls (Table 3). Compared with healthy controls, our patients demonstrated an increase in expression of *CPSF7* and *WDR33* levels ($REx = 3.2$, $p = 0.02$ and $REx = 2.05$, $p = 0.02$ respectively).

Co-expression Analysis

Co-expression analysis between expression of genes in healthy controls and MS patients revealed a significant correlation between *MALAT1* and *AGO2* ($r = 0.747$, $p < 0.0001$), whereas *HOTAIRM1* and *CSTF2* showed no remarkable correlation ($r = 0.158$, $p = 0.117$) (Table 3). We also detected direct and moderate correlation between *MALAT1* and *WDR33* expressions ($r = 0.489$, $p < 0.0001$).

Correlation Between Expression Levels and Clinical Characteristics of Patients

Data analysis showed a negative correlation between *MALAT1*, *HOTAIRM1*, and *AGO2* expressions and age at disease onset. The positive correlation between *AGO2* and *WDR33* expressions and disease duration, and between *HOTAIRM1* expression and age were significant.

Table 3 Relative expression of studied genes in MS patients compared with controls based on age and sex of the participants. *REx*, relative expression as calculated by LN (Efficiency^(deltaCt)) values in cases/LN (Efficiency^(deltaCt)) in controls; 95% *CrI*, 95% credible intervals; *SD* standard deviation; *p* value were calculated by frequentist method (median regression)

Total samples no.	<30						30–40						>40		
	Male			Female			Male			Female			Male	Female	
Control	50	23	27	6	3	5	8	5	10	10	9	19	14		
MS patient	50	19	31	4	7	10	5	5	10	10	10	14	14		
MALATI															
REx	1.0001	1.3504	0.6894	1.5844	3.6952	3.9974	3.3638	3.9974	3.9974	0.6549	0.6549	0.4611	0.4611		
Diff. of SDs	1.8	1.6	1.7	-0.13	2.03	2.73	0.508	2.73	2.73	2.7	2.7	0.963	0.963		
<i>p</i> value	0.74	0.356	0.411	0.04	0.071	0.075	0.17	0.075	0.075	0.384	0.384	0.05	0.05		
95% CrI	[-0.71, 1.02]	[-0.63, 2.01]	[-1.56, 0.63]	[-1.56, 0.63]	[-2.39, 6.02]	[-0.77, 5.86]	[-1.53, 5.7]	[-0.77, 5.86]	[-0.77, 5.86]	[-3.7, 1.76]	[-3.7, 1.76]	[-2.26, -0.02]	[-2.26, -0.02]		
AGO2															
REx	2.5435	2.9928	1.8837	2.4150	3.2986	6.9289	1.37224	6.9289	6.9289	1.1982	1.1982	1.3851	1.3851		
Diff. of SDs	1.01	0.726	1.16	-1.35	10.3	1.42	-0.06	1.42	1.42	0.76	0.76	0.81	0.81		
<i>p</i> value	0.001	0.03	0.043	0.257	0.267	0.001	0.006	0.001	0.001	0.842	0.842	0.226	0.226		
95% CrI	[0.787, 2.43]	[0.369, 3.34]	[0.297, 2.13]	[-3.38, 6.27]	[-19.9, 21.6]	[1.07, 5.75]	[0.43, 8.17]	[1.07, 5.75]	[1.07, 5.75]	[-2.37, 2.68]	[-2.37, 2.68]	[-0.14, 1.7]	[-0.14, 1.7]		
HOTAIRMI															
REx	0.6256	0.8402	0.4670	2.2161	0.5736	3.5259	1.4220	3.5259	3.5259	0.4020	0.4020	0.3658	0.3658		
Diff. of SDs	1.21	1.41	0.91	-4.5	3.39	0.93	-0.53	0.93	0.93	2.55	2.55	0.08	0.08		
<i>p</i> value	0.24	0.662	0.135	0.215	0.482	0.028	0.771	0.028	0.028	0.091	0.091	0.114	0.114		
95% CrI	[-1.92, 0.55]	[-2.6, 1.87]	[-2.55, 0.39]	[-7.01, 11.9]	[-9.51, 8.12]	[1.94, 7.51]	[-4.75, 5.58]	[1.94, 7.51]	[1.94, 7.51]	[-6.92, 0.89]	[-6.92, 0.89]	[-3.4, 0.19]	[-3.4, 0.19]		
CSTF2															
REx	0.7533	2.2595	0.3361	2.8763	0.2965	0.9342	1.3233	0.9342	0.9342	2.8226	2.8226	0.2425	0.2425		
Diff. of SDs	1.7	-0.95	2.21	-4.11	0.162	10.6	-2.5	10.6	10.6	-0.79	-0.79	0.952	0.952		
<i>p</i> value	0.042	0.495	<0.0001	0.762	0.117	0.594	>0.999	0.594	0.594	0.78	0.78	0.001	0.001		
95% CrI	[-2.3, -0.6]	[-1.05, 3.55]	[-3.38, -0.56]	[-8.9, 12.7]	[-4.62, 1.18]	[-11.8, 12.4]	[-4.8, 6.63]	[-11.8, 12.4]	[-11.8, 12.4]	[-2.63, 5.37]	[-2.63, 5.37]	[-3.38, -1.23]	[-3.38, -1.23]		
CPSF7															
REx	3.2106	5.7702	2.1160	10.0190	8.5433	2.9480	8.8989	2.9480	2.9480	3.7343	3.7343	1.6688	1.6688		
Diff. of SDs	0.727	-2.07	1.6	-4.84	1.58	5.37	-6.41	5.37	5.37	-1.21	-1.21	0.808	0.808		
<i>p</i> value	0.02	0.083	0.233	0.114	0.017	0.075	0.354	0.075	0.075	0.999	0.999	0.815	0.815		
95% CrI	[0.06, 1.66]	[0.22, 4.91]	[-0.17, 2.08]	[-6.7, 14.4]	[1.01, 4.6]	[-5.28, 8.53]	[-5.7, 13.5]	[-5.28, 8.53]	[-5.28, 8.53]	[-1.71, 3.71]	[-1.71, 3.71]	[-0.79, 1.53]	[-0.79, 1.53]		
WDR33															
REx	2.0523	2.1083	1.7442	3.8255	0.7795	3.6294	5.8831	3.6294	3.6294	1.0299	1.0299	1.7370	1.7370		
Diff. of SDs	1.3	0.04	2.1	-1.16	4.62	3.03	-0.172	3.03	3.03	0.45	0.45	2.39	2.39		
<i>p</i> value	0.02	0.177	0.06	0.35	0.06	0.054	0.036	0.054	0.054	0.963	0.963	0.19	0.19		
95% CrI	[0.063, 1.92]	[-0.87, 3.4]	[-0.22, 1.9]	[-7.21, 11.5]	[-8.69, 9.43]	[-2.13, 5.18]	[0.86, 7.1]	[-2.13, 5.18]	[-2.13, 5.18]	[-4.3, 3.8]	[-4.3, 3.8]	[-0.52, 2.47]	[-0.52, 2.47]		

Receiver Operating Characteristic (ROC) Curve Analysis

We assessed diagnostic power of all genes in MS through calculation of area under curve (AUC) in ROC curves. Based on the AUC values, *MALAT1* and *AGO2* genes had the highest diagnostic power (AUC = 0.882) (Fig. 1). The AUC values for *HOTAIRM1*, *CPSF7*, *WDR33*, and *CSTF2* were 0.8, 0.74, 0.639, and 0.62 respectively.

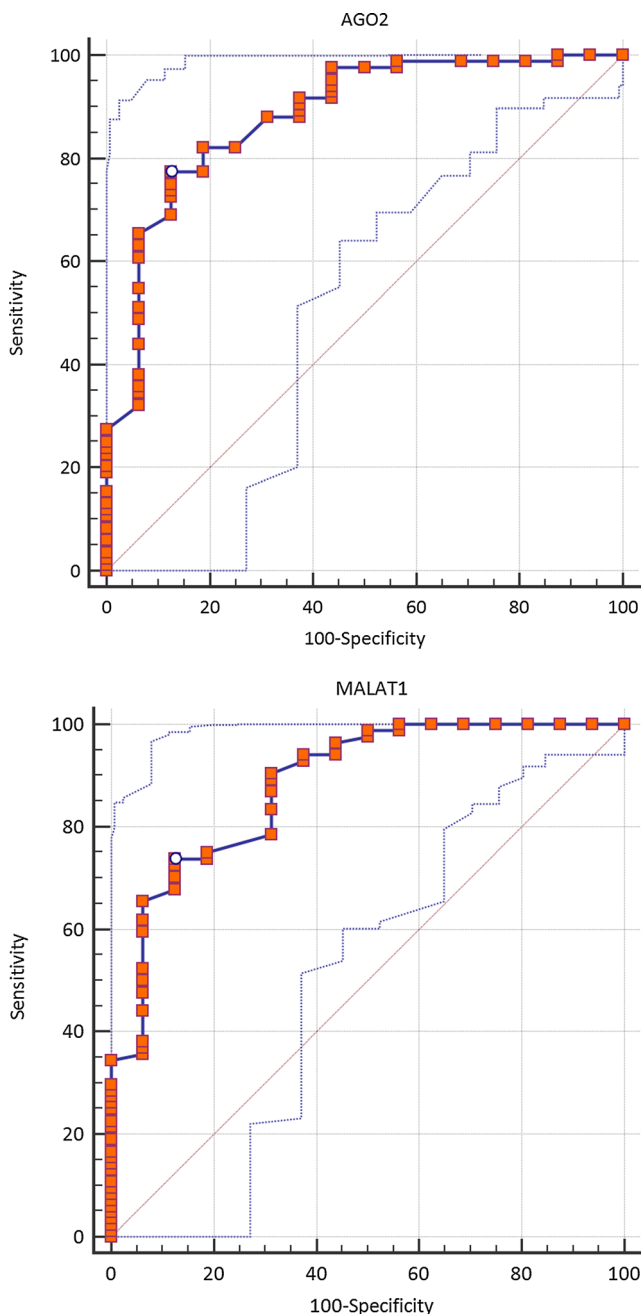


Fig. 1 ROC curve analysis for *AGO2* (a) and *MALAT1* (b)

Discussion

lncRNAs are a novel class of transcripts that are pervasively transcribed in the genome. Recent studies have approved that lncRNAs dysregulation are associated with human diseases including neurodegenerative disorders, but their expression have not been exhaustively investigated in MS. In recent years, many efforts have been done to identify genes which are involved in the MS initiation and severity. In this work, we highlighted the interaction of *MALAT1* and *HOTAIRM1* as lncRNAs and their target genes including *AGO2*, *CSTF2*, *CPSF7*, and *WDR33* with MS pathogenesis and progression.

lncRNAs act as competing endogenous RNAs against miRNAs via sponge mechanism. miRNAs could inhibit lncRNAs expression through AGO2-mediated pathway (Leucci et al. 2013). *MALAT1* as an active member of lncRNAs is mostly expressed in hippocampal neurons and particularly in RR-MS disease (Fenoglio et al. 2016). It seems that *MALAT1* promotes the inflammatory response in microglia via MyD88/IRAK1/TRAF6 signaling pathway (Wang and Zhou 2018). Also, it contributes in the inflammatory response through modulating miR-199b/IKK β /NF- κ B signaling and promotes the production of proinflammatory cytokines including TNF- α and IL-1 β by down-regulating of miR-199b (Zhou et al. 2016). Although *MALAT1* regulation mechanism by miRNAs has been remained unknown, it has been shown that miR-101 and miR-217 could suppress this lncRNA. This posttranscriptional regulation may lead to inhibition of cell growth, invasion, and metastasis (Wang et al. 2015). *MALAT1* also modulates expression of a subset of coding genes which are involved in regulation of synapsis. It is proposed that *MALAT1*-CREB complex conserve phosphorylated condition of CREB via inhibiting of PP2A-mediated dephosphorylation, which leads to continuous CREB signaling activation (Yao et al. 2016).

In the present study, the expression level of *MALAT1* in RR-MS patients was not significantly increased compared with the control group. The results of Spearman test revealed that there is a reverse correlation between the expression of *MALAT1* and age, whereas the expression of this gene decreased with disease duration. This gene may be considered as a trigger for MS disease and also may cooperate with some factors in disease development but further experiment needed to confirm this.

Previous studies showed that AGO2 has regulatory functions in inflammatory pathways and subsequently in inflammatory diseases such as rheumatoid arthritis and osteoarthritis (Pearson and Jones 2016; Yao et al. 2016). The role of *MALAT1* identified in abnormalities of capillary systems. However, its role in neurodegenerative abnormalities including MS has not been discovered yet. Our network analysis demonstrated that *AGO2* may have interaction with *MALAT1*. The data showed *AGO2* expression level was

increased up to 2.6-fold in RR-MS patients compared to controls. This increase was more than 3 times in male patients while in female patients it was 1.9 times. Interestingly, the increase in expression level of this gene was about 13.7 times in affected males with age of 30–40 years. At the same age range, in the female patients, this rate was increased to 7-fold. The expression of *AGO2* showed direct and moderate correlation with EDSS. These outcomes suggest that increasing in the expression level of *AGO2* may associate with worsening of clinical conditions. However, we found that inverse correlation between *AGO2* expression and disease duration. Evidences suggest that miRNAs regulate protein coding genes by leading their mRNAs to LARC complex. On the one hand, lncRNAs may compete with the miRNAs inside the cell. On the other hand, miRNAs can suppress the expression of lncRNAs via *AGO2*. It seems this process for *MALAT1* happens at the transcriptional level (Wang et al. 2015). In a very interesting way, miR-9 has critical role in degradation of *MALAT1* through the *AGO2* pathway in the nucleus (Leucci et al. 2013). These findings depict the existence of a new direct regulatory link between the two most important classes of non-coding RNAs, miRNAs, and lncRNAs. In line with these observations, our results indicate that *MALAT1* and *AGO2* have a very strong correlation ($r = 0.694$, $p < 0.0001$), suggesting that with increase in the expression level of *AGO2*, the possible inhibitory effect of miRNAs such as miR-9 on the *MALAT1* is compensated by higher expression of *MALAT1*.

HOTAIRM1 is expressed in myeloid cells and can play an important role in regulation of myeloid transplantation. Many effects of lncRNAs are done by employing histone modifying complexes, such as Polycomb suppressor complexes (PRC2), to induce silencing of target genes. Consequently, transcription may be active or suppressed depending on the three-dimensional complexes formed on the chromatin. Interestingly, some lncRNAs, such as *HOTAIRM1*, interact with a number of different chromatin proteins, such as CSTF2. This lncRNA seems to act like a molecular sponge to collect some targets such as CSTF2, *AGO2*, and miR-3960. In the current study, the results showed that *HOTAIRM1* expression in female patients aged between 30 and 40 years old was up-regulated compared with the corresponding control group. We also detected an increase in the expression of this gene by increasing the level of EDSS. Additionally, there was a remarkable correlation between the expression levels of *HOTAIRM1* and *AGO2* which proposes that *AGO2* expression level is upgraded to compensate the sponge activity of *HOTAIRM1*.

The *CSTF2* gene encodes a nuclear protein with RRM domain which is essential for exclusive binding to a specific position in target RNAs. The encoded protein is a cleavage stimulation factor which attaches to the GU-rich elements at the 3' UTR of the specific mRNAs. According to previous data, interaction between CSTF2 and TNF- α was anticipated

to be involved in the process of immunologic diseases. CSTF2 protein is also involved in the inflammatory processes in the development of neurodegenerative diseases (Curinha et al. 2014). It seems that the onset of neurodegenerative diseases is due to the collapse of the natural interactions between the inflammatory proteins and CSTF2. Transcripts of some lncRNAs, such as *HOTAIRM1*, are polyadenylated and also participate in the process of creating this poly-adenyl tail. The *CSTF2* gene also has interactions with GAS5, WDR33, and NR3C1. Expression profiling in rheumatoid arthritis has shown that changes in expression level of CSTF2 leads to autoimmune responses. The gene also has expression in both B and T cells and is involved in immune responses and expression regulation. Therefore, CSTF2 can be considered as an appropriate candidate for the evaluation of the pathogenesis of autoimmune disorders such as MS disease (Nunez-Iglesias et al. 2010). In this study, the *CSTF2* expression level in MS patients was significantly decreased. Such decrease was particularly prominent in female patient aged more than over 40 which is in line with the different pathways involved in the pathogenesis of MS in males and females.

Taken together, the expression pattern of these genes were distinct in females and males. Such finding is in line with the previously reported effects of gender in genetic, immunological, and clinical aspects of MS disease (Harbo et al. 2013).

The Spearman test showed statistically significant correlation between *HOTAIRM1* and *MALAT1*, but the correlation between *HOTAIRM1* and *CPSF7* as a second target for this lncRNA was not remarkable. Our study showed a 3.2-fold increase in expression of *CPSF7* in patients compared to control participants. The rate of increase in expression of *CPSF7* has gradually slowed down with increased age. Expression levels of *CSTF2* and *CPSF7* genes were directly correlated and showed a simultaneous increase in expression.

The *WDR33* expression in patients was 2-fold upper than controls. Relative expression of this gene in male patients aged between 30 and 40 was 5.9-fold higher compared to control group. The expression of this gene had reverse correlation with duration of the MS disease. The *WDR33* protein is directly connected to the AAUAAA signal, the branch point of the poly A, and the CPSF family factors interfere with this reaction (Chan et al. 2014). One of the factors within this complex is *CPSF7* which is involved in the polyadenylation process. The performed studies have shown that inhibiting the polyadenylation reaction leads to decrease in induction of genes involved in the inflammation process and one of the contributing factors is *CPSF7* (Kondrashov et al. 2012).

In conclusion, our findings propose a novel direct regulatory link between two important classes of ncRNAs, miRNAs, and lncRNAs, and coding genes. Besides, this work demonstrates robust dysregulation of several lncRNAs associated genes that they play significant roles in cellular mechanisms and regulation in MS patients compared with controls. Finally,

we demonstrated high diagnostic power of *MALAT1* and *AGO2* in MS disease. However, our study has a limitation regarding small sample size. The logic of this project might then be applied to set up future studies with the purpose of discovering potential biomarkers for MS and response to therapy.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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