Neurological Sciences (2019) 40:801–811
https://doi.org/10.1007/s10072-019-3720-3

ORIGINAL ARTICLE

Expression analysis of long non-coding RNAs and their target genes in multiple sclerosis patients

Maziar Ganji[1] · Arezou Sayad[1,2] · Mir Davood Omrani[1,3] · Shahram Arsang-Jang[4] · Mehrdokht Mazdeh[5] · Mohammad Taheri[2]

Received: 2 May 2018 / Accepted: 12 January 2019 / Published online: 24 January 2019

Abstract

Multiple sclerosis (MS) is a progressive chronic autoimmune-mediated disease. Recently, long non-coding RNAs (lncRNAs) are characterized to participate in the adjustment of immune responses. Here, we evaluated the expression levels of *GSTT1-AS1* and *IFNG-AS1* lncRNAs and their targets (*TNF* and *IFNG*, respectively) in Iranian MS patients.

In this case-control study, 50 relapsing-remitting MS patients and 50 healthy subjects were recruited. Expressions of *GSTT1-AS1* and *IFNG-AS1* lncRNAs, as well as *TNF* and *IFNG* genes, were assessed in their peripheral blood samples by SYBR Green-based Real-time quantitative PCR.

Expression levels of *GSTT1-AS1* and *IFNG-AS1* lncRNAs were both significantly downregulated (p values 0.032 and 0.013, respectively). On the other hand, the expression of *TNF* and *IFNG* showed increased levels, however, did not reach statistical significance after our analysis ($p > 0.05$). Spearman correlation analysis showed that *GSTT1-AS1* had a significant positive moderate correlation with *IFNG-AS1* ($r = 0.541$, $p < 0.0001$), *IFNG* ($r = 0.329$, $p = 0.001$), and *TNF* ($r = 0.204$, $p = 0.041$). Also, *IFNG-AS1* revealed the same correlation with *IFNG* ($r = 0.475$, $p < 0.0001$) as well as *TNF* ($r = 0.399$, $p < 0.0001$). Furthermore, *GSTT1-AS1* ($r = 0.313$, $p = 0.027$) and (IFNG $r = 0.478$, $p < 0.0001$) demonstrated a significant positive correlation with age at onset.

Briefly, the current study provided for the first time dysregulation of *GSTT1-AS1* and *IFNG-AS* lncRNAs network in MS, which highlights the significant role of epigenetic pathways in this autoimmune disorder. Larger sample size and further investigation assays could shed light on the underlying mechanisms in this area of science.

Keywords Multiple sclerosis · *IFNG* · *TNF* · *GSTT1-AS1* · *IFNG-AS*

✉ Arezou Sayad
ar.sayad@yahoo.com

✉ Mohammad Taheri
Mohammad_832@yahoo.com

[1] Department of Medical Genetics, Shahid Beheshti University of Medical Sciences, Tehran, Iran

[2] Department of Medical Genetics, School of Medicine, Shahid Beheshti University of Medical Sciences, PO Box 1985717443, Tehran, Iran

[3] Urogenital Stem Cell Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran

[4] Clinical Research Development Center (CRDU), Qom University of Medical Sciences, Qom, Iran

[5] Neurophysiology Research Center, Hamadan University of Medical Sciences, Hamadan, Iran

Introduction

Multiple sclerosis (MS) (OMIM 126200) is a progressive chronic autoimmune-mediated disease, characterized by the demyelination, chronic inflammation, and neurodegeneration processes in the central nervous system (CNS). The underlying immunopathogenic mechanisms of this disorder is yet unknown. However, there is a large body of literature supporting the role of perivascular infiltration of autoreactive T cells which recognize and react against autoantigens [1, 2]. There are reports which demonstrate the significant increase of MS rates especially in Iranian population [3].

Newly, accumulated evidence has displayed that defects in $CD4^+$ T helper cells may be involved in the MS process [4, 5]. $CD4^+$ T helper cells can be grouped into Th1, Th2, and Th17 as well as follicular helper T cells, based on the production of cytokines. The Th1 cell lineage is regarded as a subset of

inflammatory $CD4^+$ T cells mainly producing interferon gamma (IFN-γ), an inflammation-related cytokine that participates protectively against autoimmune diseases, delayed-type hypersensitivity, and intracellular microbes [6, 7]. Likewise, tumor necrosis factor (*TNF*) gene encodes for a multifunctional proinflammatory cytokine, which has been characterized to affect excitatory synaptic transmission and mediates immunological and neural cytological roles [8–10].

Long non-coding RNAs (LncRNAs) constitute a distinct class of non-coding RNAs with more than 200 nucleotides in length. LncRNAs are substantially appreciated as key regulators of genome expression, and so far, only some of their functions have been recognized [11, 12]. In some well-studied instances, their active involvement in many aspects of neuronal diseases such as neural degeneration or neurogenesis in both of central and peripheral nervous systems has been described [13, 14]. In addition, dysregulation of some lncRNAs has been recently shown in peripheral blood mononuclear cells of MS patients, suggesting their possible role in MS development [14].

GSTT1-AS1 (*lncRNA-CD244* in another words) is a lncRNA by which CD244 was found to mediate the inhibition of *IFNG* and *TNF* cytokine genes in tuberculosis pathogenesis [15]. It is speculated that *GSTT1-AS1* represses the transcription of *TNF* and *IFNG* genes, by recruiting EZH2 enzyme complex to their promoters, to facilitate H3K27 methylation that causes a repressive transcriptional environment [16]. *IFNG-AS1* (*IFNG Antisense RNA 1*), also named *Tmevpg1* (Theiler's murine encephalomyelitis virus persistence candidate gene 1), or *NeST* (nettoie Salmonella pas Theiler's) is another lncRNA initially characterized as a candidate gene to control the Theiler's virus persistence [17, 18]. *IFNG-AS1* is located adjacent to IFN-γ-encoding gene, *IFNG*, in human. Currently, studies have elucidated that *IFNG-AS1* is determined to be a pivotal checkpoint contributing *IFNG* expression [19, 20]. However, it is not yet known whether *IFNG-AS1* is involved in the pathogenesis of MS. Besides, the relationship between *GSTT1-AS1* and *IFNG-AS1* lncRNAs as well as *TNF* and *IFNG* is, to the best of our knowledge, not studied in MS disorder.

Altogether, here we aimed to study the relative expression levels of *GSTT1-AS1* and *IFNG-AS1* lncRNAs in addition to their target coding genes (*TNF* and *IFNG*, respectively) in Iranian MS patients.

Materials and methods

Subjects

The subjects of this case-control study included 50 unrelated sporadic relapsing-remitting MS (RR-MS) patients (38 women and 12 men), as diagnosed and identified by MRI (magnetic resonance imaging) and 2017 McDonald criteria [21] by specialized neurologists. All patients were treated with Interferon (IFN)-β therapy for at least 2 years (intramuscular injection of CinnoVex 30 mcg [CinnaGen Co, Tehran, Iran] once a week) and were recognized as IFN-β responders [22, 23]. Moreover, 50 sex- and age-matched healthy controls (37 women and 13 men) were involved. The blood samples were gathered from MS Society of Iran and, in addition, some hospitals in Tehran. All MS patients were, prior to this study, HLA-typed. Therefore, HLA-DRB1*15 was ruled out due to its major effect in MS predisposition[24].

Blood sampling

The present study was carried out on 5-ml peripheral blood samples collected from the participants (MS patients and controls). Blood samples of MS patients were obtained during stable phase of disease. Samples were collected in K3-EDTA tubes. All individuals gave their informed consent to incorporate into this study. Then, a complete history of patients was acquired. Study design and the entire measurements were approved by the Ethics Committee of Shahid Beheshti University of Medical Sciences (SBUMS).

Table 1 Sequences of specific primers designed for each gene

Gene name	Primer sequence	Primer length	Product length
B2M	F: AGATGAGTATGCCTGCCGTG R: CGGCATCTTCAAACCTCCA	20 19	104
IFNG	F: GGCAAGGCTATGTGATTACAAGG R:CATCAAGTGAAATAAACACA CAACCC	23 26	96
IFNG-AS1	F: AGGAAGCTGGGTAATTGAATGC R: CTTAGGAGGAGAATTTTGGGAGAG	22 24	94
TNF	F: TCCACCCATGTGCTCCTCAC R: TCTGGCAGGGGCTCTTGATG	20 20	97
GSTT1-AS1	F: CTTTTGCATAGAGACCATGACCAG R: TGGATAATAAACCTGGGCTCAGC	24 23	105

Table 2 Clinical and demographic information of RR-MS patients and healthy controls

Variables	MS patients	Controls
Female/male [no. (%)]	38 (76%)/12 (24%)	37 (74%)/13 (26%)
Age (mean ± SD, years)	36.2 ± 2.7	35.3 ± 2.4
Age range (years	17–55	22–60
Age of onset (mean ± SD, years)	31.41 ± 2.8	–
Relapsing-remitting course (no. %)	100 (100%)	–
Duration (mean ± SD, years)	4.58 ± 3.2	–
EDSS (mean ± SD)	3.07 ± 2.5	–

EDSS Expanded Disability Status Scale of Kurtzke

Quantitative real-time PCR assay

We extracted RNA from whole blood samples by using GeneAll Hybrid-R™ blood RNA extraction kit (cat No. 305-101). Then, 150 ng of RNA was used to synthesize the single strand cDNA through kit of Biosystems High-Capacity cDNA Reverse Transcription (PN 4375575), according to the manufacturer's instruction. To design the specific primers, Allele ID6 for × 64 windows software (Premier Biosoft, Palo Alto, USA) was used (primer sequences and PCR product length are shown in Table 1). *B2M* was applied as a housekeeping gene in order to normalize the expression level for all samples including lncRNAs and target coding genes.

SYBR Green-based Real-time quantitative PCR assay was carried out, by Corbett Rotor Gene 6000 machine (Corbett Life Science), in duplicates for all samples comprising housekeeping gene, lncRNAs, and target genes in both groups of MS patients and healthy controls. Afterwards, a mean value was reported for each one. Routinely, the NTC (No Template Control) sample was considered for each primer in each run for method quality control and detection of contamination.

Statistical analysis

To examine the data obtained from participants, independent *t* tests and one-way ANOVA test were both conducted. As well, Pearson correlation coefficient was used to identify the correlation level between the variables. The significance level was determined as less than 5% ($p \leq 0.05$). The analysis was accomplished via SPSS version 18 statistical package (Chicago, IL, USA). Spearman correlation test was performed to evaluate the correlations between relative expression levels of *GSTT1-AS1*, *IFNG-AS1*, *TNF*, and *IFNG*.

Results

Clinical information of all participants is given in Table 2. MS patients and healthy controls were evaluated in three separate ways: the results of total participants (regardless of their age and sex), sex-related results (male or female), and age-linked results (> 30, 30–40, and 40 < years). The whole group of patients was then compared with healthy subjects and independently analyzed for age and sex.

Relative expression level of *GSTT1-AS1* LncRNA

Statistical analysis revealed a significant downregulation in *GSTT1-AS1* lncRNA expression in total MS patients ($p = 0.032$) and total female subgroup as well as the subgroup of females 40 < years (*p* values 0.045 and

Table 3 The relative expression ratio of GSTT1-AS1 in age- and sex-based subgroups

GSTT1-AS1 expression		Control no.	MS patient no.	Expression ratio	Sd	p value	95% CrI
Total		50	50	0.7139	− 0.15	0.032	[− 1.7, − 0.34]
Male		13	12	1.1312	0.62	0.45	[− 1.7, 0.98]
Female		37	38	0.5482	− 0.4	0.045	[− 2.26, − 0.42]
< 30	Male	0	1	–	–	–	–
	Female	10	6	1.6638	− 4.9	0.892	[− 6.1, 6.1]
30–40	Male	2	5	1.1643	–	–	–
	Female	5	15	0.4913	1.32	0.12	[− 6.6, 1.7]
> 40	Male	11	6	1.1669	0.383	0.238	[− 9.04, 1.08]
	Female	22	17	0.4166	− 0.47	0.0005	[− 3.17, − 0.8]

Table 4 The relative expression ratio of IFNG-AS1 in age- and sex-based subgroups

IFNG-AS1 Expression		Control no.	MS patient no.	Expression ratio	Sd	p value	95% CrI
Total		50	50	0.4464	− 0.38	0.013	[− 1.73, − 0.43]
Male		13	12	0.4674	− 0.32	0.03	[− 2.6, − 0.02]
Female		37	38	0.4543	− 0.3	0.049	[− 1.7, − 0.25]
< 30	Male	0	1	–	–	–	–
	Female	10	6	0.6694	− 3.9	0.843	[− 5.04, 5.3]
30–40	Male	2	5	0.4049	–	–	–
	Female	5	15	1.2557	1.99	0.145	[− 5.8, 2.1]
> 40	Male	11	6	0.5344	− 0.51	0.06	[− 3.4, 0.56]
	Female	22	17	0.4546	− 0.45	0.037	[− 1.8, − 0.06]

0.0005, respectively) versus healthy controls (relative expression ratios are demonstrated in Table 3).

Relative expression level of *IFNG-AS1* LncRNA

Table 4 displays the results of total *IFNG-AS1* expression level in RR-MS patients in comparison with the healthy group as well as those based on the age and sex of the participants. *IFNG-AS1* lncRNA expression is significantly decreased in almost half of all categories, including total ($p = 0.0.13$), male ($p = 0.03$), female ($p = 0.049$), and women > 40 years ($p = 0.037$) MS patients compared to healthy subjects.

Relative expression level of *TNF* gene

TNF expression in MS patients in all categories was upregulated. Nonetheless, these discrepancies did not reach a significant level. The most considerable difference was for male patients > 40 years (sevenfold expression ratio compared with controls), while was not statistically significant. Table 5 indicates the *TNF* relative expression ratio in age- and sex-based subgroups.

Table 5 The relative expression ratio of *TNF* in age- and sex-based subgroups

TNF expression		Control no.	MS patient no.	Expression ratio	Sd	*p* value	95% CrI
Total		50	50	1.1349	− 0.26	0.303	[− 0.43, 1.4]
Male		13	12	1.9886	− 0.42	0.409	[− 1.5, 4.8]
Female		37	38	1.323	− 0.01	0.434	[− 0.61, 1.3]
< 30	Male	0	1	–	–	–	–
	Female	10	6	1.1887	− 3.7	0.68	[− 5.01, 6.06]
30–40	Male	2	5	1.4699			
	Female	5	15	1.8222	2.08	0.435	[− 5.5, 3.5]
> 40	Male	11	6	7.3023	− 1.7	0.51	[− 3.74, 7.4]
	Female	22	17	2.4438	0.4	0.293	[− 0.5, 1.8]

Table 6 The relative expression ratio of *IFNG* in age- and sex-based subgroups

IFNG expression		Control no.	MS patient no.	Expression ratio	Sd	*p* value	95% CrI
Total		50	50	1.3978	0.04	0.52	[− 0.33, 0.69]
Male		13	12	1.5149	1.02	0.962	[− 0.8, 0.92]
Female		37	38	1.3394	− 0.3	0.53	[− 0.33, 0.96]
< 30	Male	0	1	–	–	–	–
	Female	10	6	1.7006	− 2.59	0.792	[− 4.4, 5.1]
30–40	Male	2	5	2.0325	–	–	–
	Female	5	15	2.5461	1.87	0.819	[− 3.9, 2.9]
> 40	Male	11	6	1.4321	0.98	0.59	[− 1.3, 0.8]
	Female	22	17	1.5279	− 0.5	0.59	[− 0.69, 1.15]

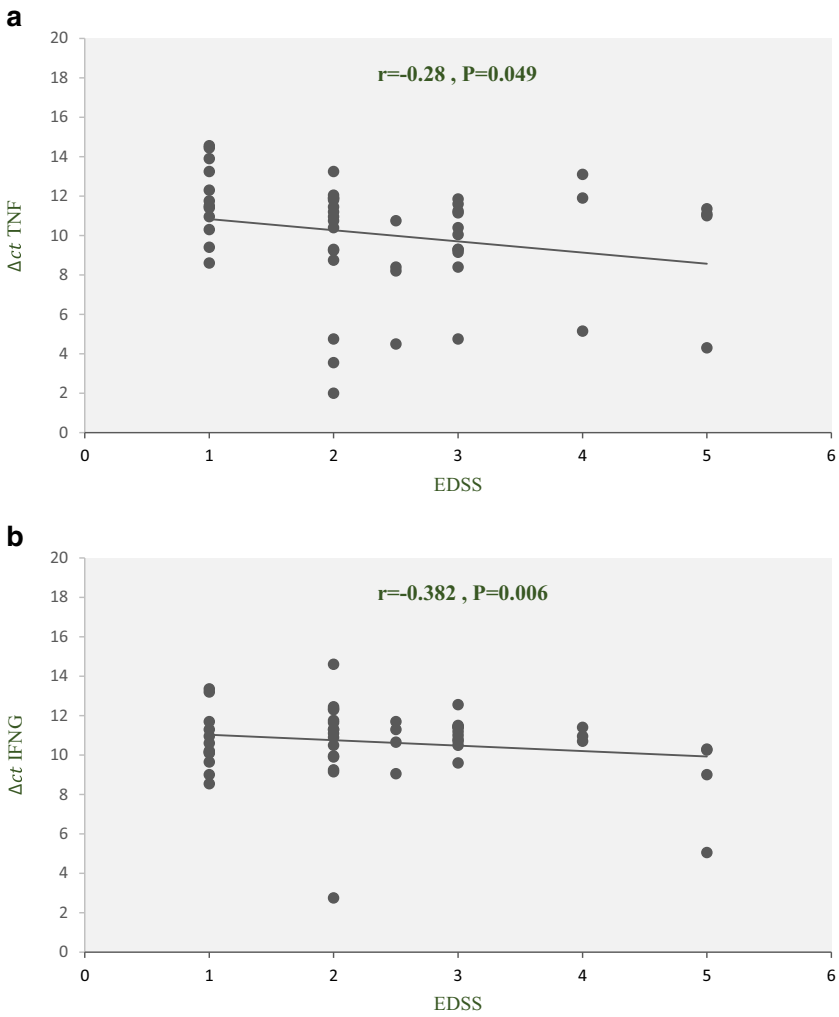

Fig. 1 Spearman correlation between TNF (**a**) and IFNG (**b**) relative expressions and EDSS in MS patients

Relative expression level of *IFNG* gene

Table 6 reveals the expression level of *IFNG* in patients and controls. All subgroups in relation to age- and sex-matched healthy subjects showed upregulated expression, but these changes were not statistically significant.

Correlation analysis between LncRNAs and target genes with expanded disability status scale (EDSS)

As seen in Fig. 1, *TNF* and *IFNG* expression levels both showed a significant and moderate correlation with EDSS ($r = -0.28$, $p = 0.049$; and $r = -0.382$, $p = 0.006$, respectively), while the correlations for GSTT1-AS1 and IFNG-AS1 with EDSS was not statistically significant (data not shown).

Correlation analysis between LncRNAs and target genes with disease duration

The correlation between all lncRNAs and target genes with disease duration did not reach a statistical significance (data not shown).

Correlation analysis between LncRNAs and target genes with age at onset

Correlation analysis results showed that there is a significant correlation between expression levels of *GSTT1-AS1* ($r = 0.313$, $p = 0.027$) and *IFNG* ($r = 0.478$, $p<0.0001$) with age at onset of MS disease (Fig. 2). The correlation of other lncRNAs and genes with age at onset was not significant (not shown).

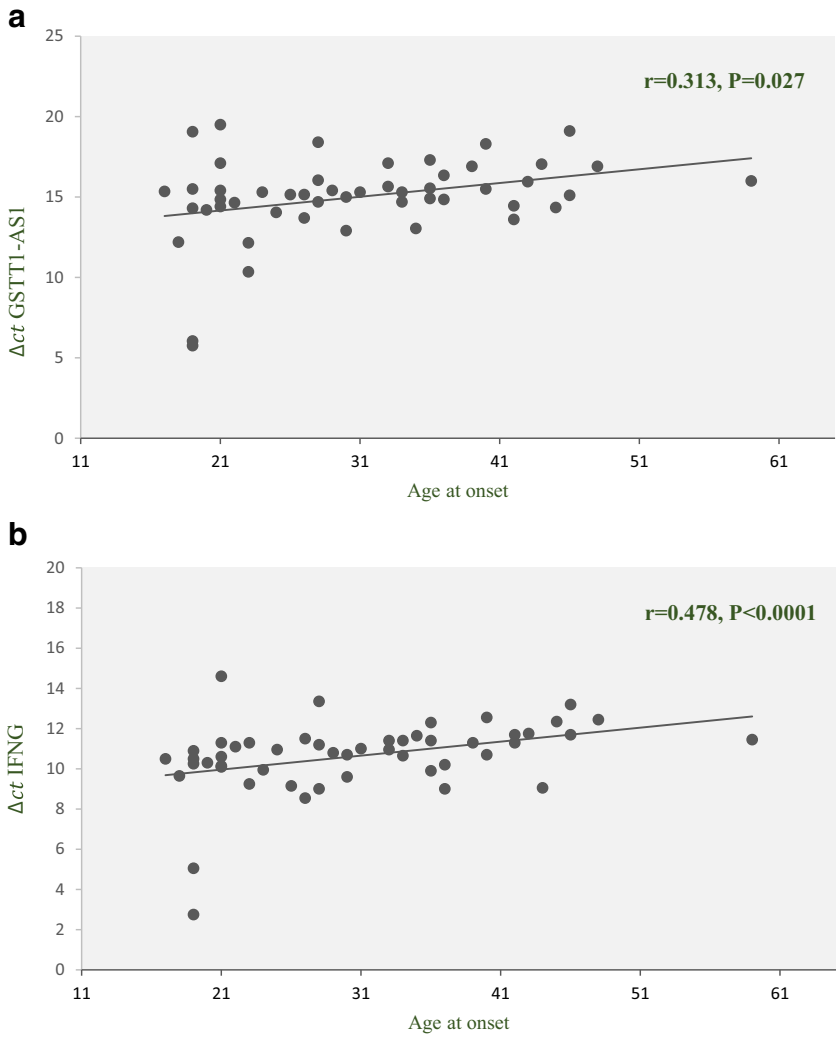

Fig. 2 Spearman correlation between GSTT1-AS1 (**a**) and IFNG (**b**) relative expressions and age at onset in patients

Correlations between expression levels of LncRNAs and target genes

Spearman correlation analysis between the expression levels of lncRNAs and target genes is illustrated in Fig. 3, as described below:

For GSTT1-AS1

There was a significant and positive weak correlation with *TNF* ($r = 0.204$, $p = 0.041$) in patients.

For IFNG-AS1

Significant and positive correlations were obtained with *GSTT1-AS1* ($r = 0.541$, $p < 0.0001$) and *TNF* ($r = 0.399$, $p < 0.0001$).

For IFNG

There was a significant and positive moderate correlation with two studied lncRNAs, *GSTT1-AS1* ($r = 0.329$, $p < 0.0001$) and *IFNG-AS1* ($r = 0.475$, $p < 0.0001$). Moreover, the correlation with *TNF* was not significant ($r = 0.19$, $p = 0.058$) (not shown).

Discussion

In the current study, we presented downregulation of *GSTT1-AS1* and *IFNG-AS1* lncRNAs and upregulation of their coding targets *TNF* and *IFNG* in whole blood of MS patients. The importance of such differential expressions and dysregulations would be embedded in the pathogenesis of MS and emphasizes their role as potential biomarkers for different stages of disease course or treatment response [11]. In addition, finding the interaction between lncRNAs and their

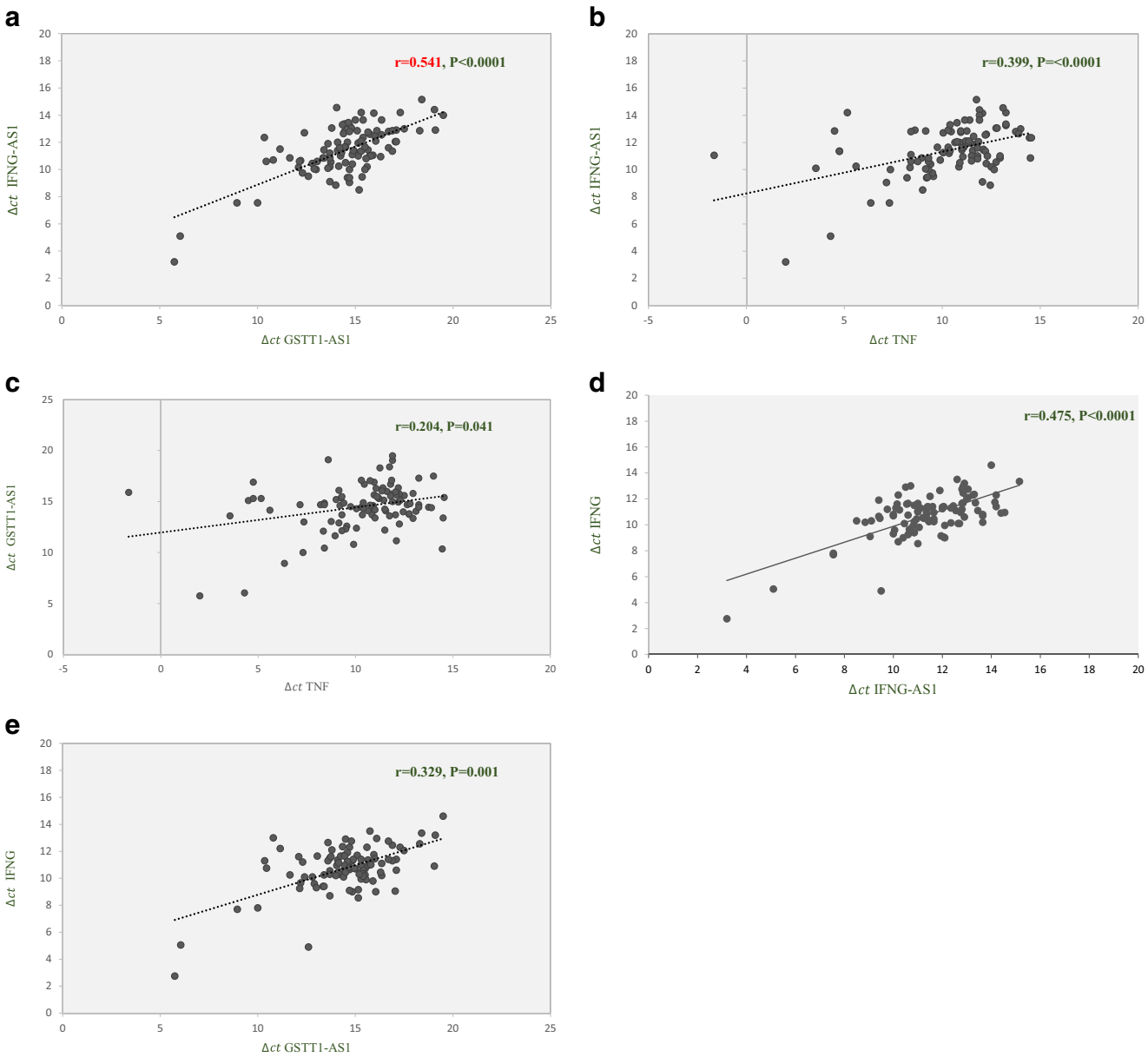

Fig. 3 Correlations between the expression levels of IFNG-AS1 and GSTT1-AS1 (**a**), IFNG-AS1 and TNF (**b**), GSTT1-AS1 and TNF (**c**), IFNG and IFNG-AS1 (**d**), and IFNG and GSTT1-AS1 (**e**) in RR-MS patients

targets can provide us with potential new therapeutic avenues for future measurements.

IFN-β therapy has been extensively used over decades as a first-line treatment for RR-MS. Its beneficial effects in MS are, in part, owing to the inhibition of $CD4^+$ T cell proliferation and modulation of proinflammatory cytokines expression, which are all necessary for the development of inflammation [25]. A large number of studies have examined the cytokines' production in MS, but results have been extremely inconsistent and most studies, even those with a longitudinal study design, have utilized a cross-sectional approach to evaluate remission samples with samples collected during or shortly before relapse and controls [26–31]. Expression of *IFNG* and *TNF* is regulated through various epigenetic elements, including methylation, histone acetylation, and non-coding RNAs [32, 33]. It was previously showed that CD244 signaling during active tuberculosis infection can exploit *GSTT1-AS1* lncRNA and histone-modifying enzymes to regulate effector functions of $CD244^+CD8^+$ T cells (Fig. 4) [15]. Indeed, CD244 provokes the expression of *GSTT1-AS1* via creating a permissive transcriptional environment at the *GSTT1-AS1* gene locus that results in loss of repressive histone mark, H3K27Me3. In turn, *GSTT1-AS1* interestingly appears to physically interact with a polycomb protein, EZH2. This interaction consequently mediates the EZH2 recruitment to *TNF* and *IFNG* loci. Moreover, it is thought that this could

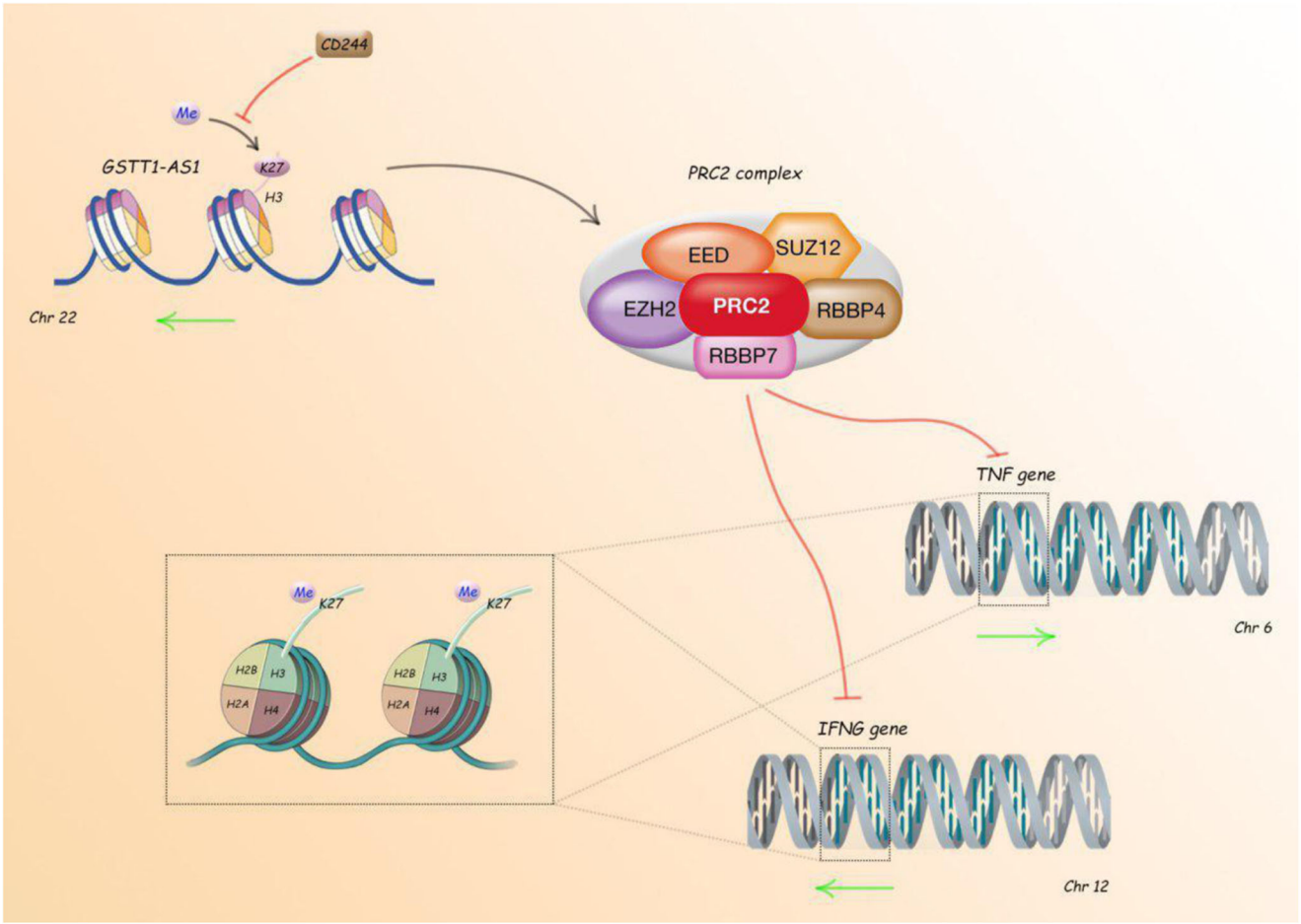

Fig. 4 Schematic model illustrating mechanism of action of *GSTT1-AS1* lncRNA [15, 16]. Orientation of transcription is indicated by green arrows bellow. *GSTT1-AS1* associates with EZH2 cargo and both connect to the target mRNAs. Locations of H3K27Me epigenetic marks which suppress the transcription of target genes are showed

trimethylate H3K27 at promoters of *TNF* and *IFNG* toward repressive chromatin status and suppression of *TNF* and *IFNG* expression [15, 34, 35]. Our findings support this hypothesis and suggest that *GSTT1-AS1* might have interaction with chromatin and mediate the targeted recruitment of repressive histone-modifying elements in epigenetic control of transcriptional silencing in MS patients. Therefore, the inverse relationship between expression levels of *GSTT1-AS1* lncRNA (downregulated) and the *TNF* and *IFNG* target genes (both upregulated) in MS patients of our study could be explained by this hypothesis, though for the two target genes, this increase was not significant after our analysis. Importantly, elevated expression levels of *TNF* and *IFNG* can contribute to the inflammatory responses and trigger cell death as well as tissue degeneration found in various autoimmune diseases [36, 37]. In harmony with our results, previous studies have shown higher IFN-γ production in stimulated cells of MS patients during or just prior to relapse [26, 29, 30, 38]. Particularly, in two of these studies, the difference in IFN-γ between MS patients and healthy controls did not reach statistical significance [29, 38]. These findings, however, are in contrast with Van Oosten work who showed no evidence of different stimulated production of IFN-γ in peripheral blood mononuclear cells (PBMCs) between relapse and remission samples [39]. Such differences might be, in part, due to the diversity of cross-sectional analysis method and sample size, which both may account for the failure to reach a significant difference.

IFNG-AS1 is characterized to cooperate with T-bet to stimulate the transcription of *IFNG* by effector Th1 cells [16]. This lncRNA associates with the WDR5 (Fig. 5), a component of histone H3K4 methyl transferase complex, and recruits this complex to create H3K4-methylation marks at the *IFNG* promoter as well as intronic regions to facilitate transcription [40]. While recently researchers have established that lncRNA *IFNG-AS1* could change the epigenetic marking of *IFNG* encoding chromatin and induce *IFNG* transcription [19, 20, 40], other researchers have obtained the contrary results [18]. We observed a significant decrease in *IFNG-AS1* expression in MS patients, but, to our surprise, the expression of *IFNG* was upregulated. In accordance with our data, a study performed by Li et al. [41] reported that *IFNG-AS1* expression in PBMCs of primary

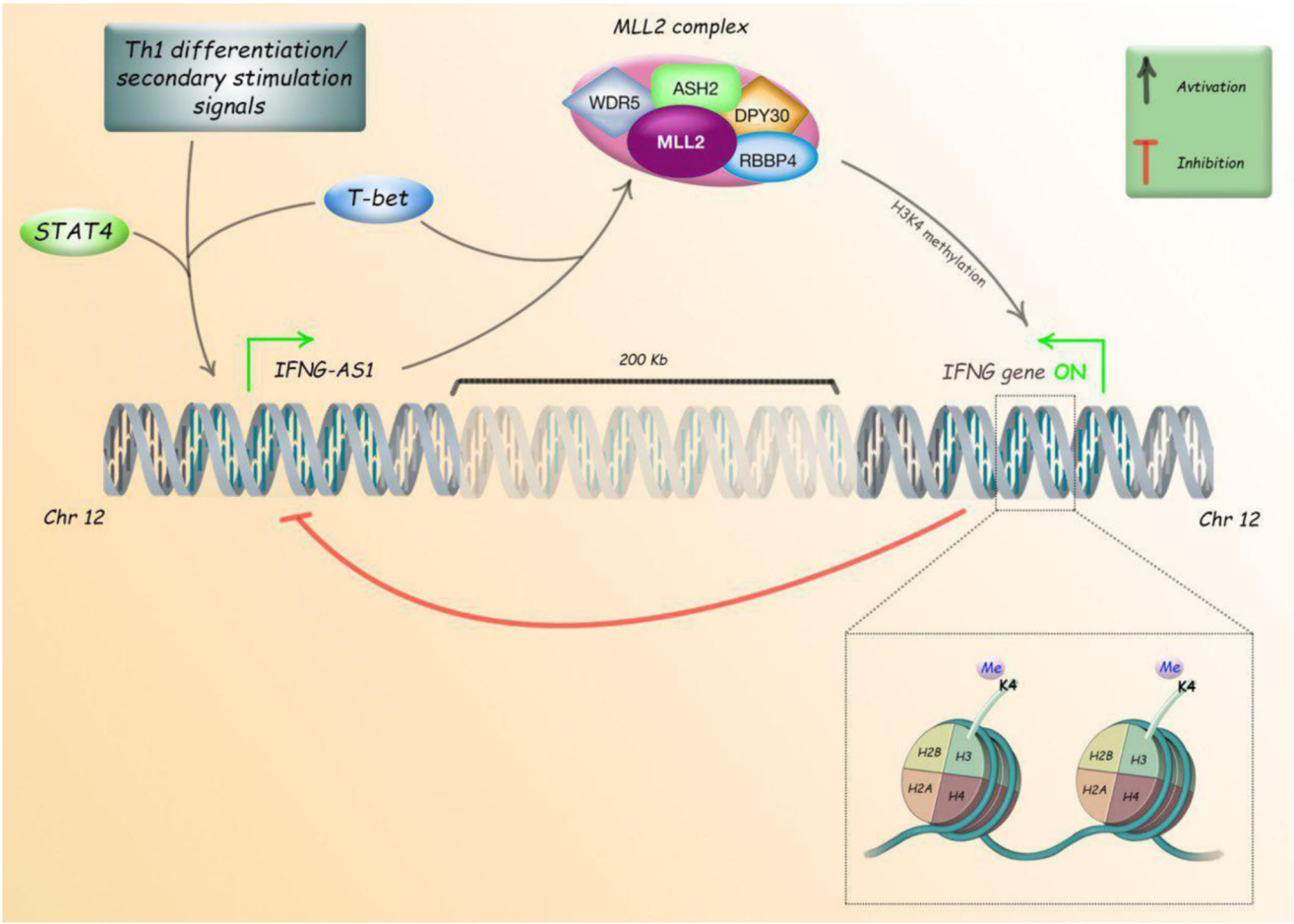

Fig. 5 Schematic model illustrating mechanism of action of *IFNG-AS1* lncRNA [16][16][16][16][16]. Orientation of transcription is indicated by green arrows. *IFNG-AS1* associates with WDR5 cargo and both connect to the target mRNA. Locations of H3K4Me epigenetic marks which favor transcriptional activation are displayed. By *IFNG* over-expression, *IFNG* can negatively feedback and suppress the expression of *IFNG-AS1* lncRNA

immune thrombocytopenia (ITP), as an autoimmune disease, was lower than in healthy controls. They speculated that *IFNG-AS1* can promote *IFNG* transcription, and that *IFNG* over-expression negatively regulates the expression of *IFNG-AS1*. It seems that the same applies to our data and we may suggest that significant decrease in *IFNG-AS1* and insignificant increase of *IFNG* could be mechanisms for avoiding excessive *IFNG* expression [41]. Again, in Li study, the upregulation of whole blood IFNG concentration in active ITP patients did not reach the statistical significance. It may be that findings of our work and other studies, related to lncRNAs and targets, can be explained, in part, by their sample size limitation and heterogeneity of autoimmune disorders such as MS and ITP.

Of note, we assessed the expression levels of the abovementioned lncRNAs and target genes in IFN-β responder MS patients to exclude the effects of numerous drugs and to assimilate the group of our patients and to unify the results; however, a limitation of our study can be that we did not include non-responder MS patients.

Briefly, the current study provided for the first time dysregulation of GSTT1-AS1 and IFNG-AS lncRNAs network in MS, which highlights the significant role of epigenetic pathways. Larger sample size and further investigation assays could shed light on the underlying mechanisms in this area of science.

Funding information The present article is financially supported by "Research Department of the School of Medicine Shahid Beheshti University of Medical Sciences."

Compliance with ethical standards

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

References

1. PILLI D, ZOU A, TEA F, DALE RC, BRILOT F (2017) Expanding role of T cells in human autoimmune diseases of the central nervous system. Front Immunol 8:652
2. RIEDHAMMER C, WEISSERT R (2015) Antigen presentation, autoantigens, and immune regulation in multiple sclerosis and other autoimmune diseases. Front Immunol 6
3. SAHRAIAN MA, SAHEBKAR M, DEHGHANI R, DERAKHSHAN-JAZARI M, KAZAMI-MOGHADDAM V, KOUCHAKI E (2017) Multiple sclerosis-a disease on a dramatically rising trend in Iran: review of possible reasons. Iran J Neurolt 16:34
4. DENDROU CA, FUGGER L, FRIESE MA (2015) Immunopathology of multiple sclerosis. Nat Rev Immunol 15: 545–558
5. JONES A, KERMODE A, LUCAS R, CARROLL W, NOLAN D, HART P (2017) Circulating immune cells in multiple sclerosis. Clin Exp Immunol 187:193–203
6. COSMI L, MAGGI L, SANTARLASCI V, LIOTTA F, ANNUNZIATO F (2014) T helper cells plasticity in inflammation. Cytometry Part A 85:36–42
7. RAPHAEL I, NALAWADE S, EAGAR TN, FORSTHUBER TG (2015) T cell subsets and their signature cytokines in autoimmune and inflammatory diseases. Cytokine 74:5–17
8. MARQUES-ROCHA JL, SAMBLAS M, MILAGRO FI, BRESSAN J, MARTÍNEZ JA, MARTI A (2015) Noncoding RNAs, cytokines, and inflammation-related diseases. FASEB J 29:3595–3611
9. OLMOS G, LLADÓ J (2014) Tumor necrosis factor alpha: a link between neuroinflammation and excitotoxicity. Mediat Inflamm 2014:1–12
10. TAHERI M, NEMATI S, MOVAFAGH A, SABERI M, MIRFAKHRAIE R, EFTEKHARIAN MM, ARSANG-JANG S, REZAGHOLIZADEH A, SAYAD A (2016) TRAIL gene expression analysis in multiple sclerosis patients. Hum Antibodies 24:33–38
11. EFTEKHARIAN MM, GHAFOURI-FARD S, SOUDYAB M, OMRANI MD, RAHIMI M, SAYAD A, KOMAKI A, MAZDEH M, TAHERI M (2017) Expression analysis of long non-coding RNAs in the blood of multiple sclerosis patients. J Mol Neurosci 63:333–341
12. SANTORO M, NOCITI V, LUCCHINI M, DE FINO C, LOSAVIO FA, MIRABELLA M (2016) Expression profile of long non-coding RNAs in serum of patients with multiple sclerosis. J Mol Neurosci 59:18–23
13. WANG A, WANG J, LIU Y, ZHOU Y (2017) Mechanisms of long non-coding RNAs in the assembly and plasticity of neural circuitry. Front Neural Circuits 11
14. ZHANG F, GAO C, MA XF, PENG XL, ZHANG RX, KONG DX, SIMARD AR, HAO JW (2016) Expression profile of long noncoding RNAs in peripheral blood mononuclear cells from multiple sclerosis patients. CNS Neurosci Ther 22:298–305
15. WANG Y, ZHONG H, XIE X, CHEN CY, HUANG D, SHEN L, ZHANG H, CHEN ZW, ZENG G (2015b) Long noncoding RNA derived from CD244 signaling epigenetically controls CD8+ T-cell immune responses in tuberculosis infection. Proc Natl Acad Sci 112:E3883–E3892
16. AUNE TM, CROOKE PS, SPURLOCK CF (2016) Long noncoding RNAs in T lymphocytes. J Leukoc Biol 99:31–44
17. PENG H, LIU Y, TIAN J, MA J, TANG X, RUI K, TIAN X, MAO C, LU L, XU H (2015) The long noncoding RNA IFNG-AS1 promotes T helper type 1 cells response in patients with Hashimoto's thyroiditis. Sci Rep 5
18. VIGNEAU S, ROHRLICH P-S, BRAHIC M, BUREAU J-F (2003) Tmevpg1, a candidate gene for the control of Theiler's virus persistence, could be implicated in the regulation of gamma interferon. J Virol 77:5632–5638
19. COLLIER SP, COLLINS PL, WILLIAMS CL, BOOTHBY MR, AUNE TM (2012) Cutting edge: influence of Tmevpg1, a long intergenic noncoding RNA, on the expression of Ifng by Th1 cells. J Immunol 189:2084–2088
20. COLLIER SP, HENDERSON MA, TOSSBERG JT, AUNE TM (2014) Regulation of the Th1 genomic locus from Ifng through Tmevpg1 by T-bet. J Immunol 193:3959–3965
21. CSEPANY T (2018) Diagnosis of multiple sclerosis: a review of the 2017 revisions of the McDonald criteria. Ideggyogy Sz 71:321–329
22. SAYAD A, GHAFOURI-FARD S, OMRANI MD, NOROOZI R, TAHERI M (2017) Myxovirus resistance protein A (MxA) polymorphism is associated with IFNβ response in Iranian multiple sclerosis patients. Neurol Sci 38:1093–1099
23. TAHERI M, GHAFOURI-FARD S, SOLGI G, SAYAD A, MAZDEH M, OMRANI MD (2017) Determination of cytokine levels in multiple sclerosis patients and their relevance with patients' response to Cinnovex. Cytokine 96:138–143
24. MAZDEH M, TAHERI M, SAYAD A, BAHRAM S, OMRANI MD, MOVAFAGH A, INOKO H, AKBARI MT, NOROOZI R, HAJILOOI M, SOLGI G (2016) HLA genes as modifiers of response to IFN-beta-1a therapy in relapsing-remitting multiple sclerosis. Pharmacogenomics 17:489–498
25. WANG Y, ZHONG H, XIE X, CHEN CY, HUANG D, SHEN L, ZHANG H, CHEN ZW, ZENG G (2015a) Long noncoding RNA derived from CD244 signaling epigenetically controls CD8+ T-cell immune responses in tuberculosis infection. Proc Natl Acad Sci U S A 112:E3883–E3892
26. BECK J, RONDOT P, CATINOT L, FALCOFF E, KIRCHNER H, WIETZERBIN J (1988) Increased production of interferon gamma and tumor necrosis factor precedes clinical manifestation in multiple sclerosis: do cytokines trigger off exacerbations? Acta Neurol Scand 78:318–323
27. CHOFFLON M, JUILLARD C, JUILLARD P, GAUTHIER G, GRAU GE (1992) Tumor necrosis factor alpha production as a possible predictor of relapse in patients with multiple sclerosis. Eur Cytokine Netw 3:523–531
28. CHOFFLON M, ROTH S, JUILLARD C, PAUNIER A, JUILLARD P, DEGROOTE D, GRAU G (1997) Tumor necrosis factor production capacity as a potentially useful parameter to monitor disease activity in multiple sclerosis. Eur Cytokine Netw 8: 253–257
29. DEBRUYNE J, PHILIPPÉ J, DEREUCK J, WILLEMS A, LEROUX-ROELS G (1998) Relapse markers in multiple sclerosis: are in vitro cytokine production changes reflected by circulatory T-cell phenotype alterations? Mult Scler J 4:193–197
30. HOLLIFIELD RD, HARBIGE LS, PHAM-DINH D, SHARIEF MK (2003) Evidence for cytokine dysregulation in multiple sclerosis: peripheral blood mononuclear cell production of pro-inflammatory and anti-inflammatory cytokines during relapse and remission. Autoimmunity 36:133–141
31. SIMPSON S, STEWART N, VAN DER MEI I, OTAHAL P, CHARLESWORTH J, PONSONBY A-L, BLIZZARD L, DWYER T, PITTAS F, GIES P (2015) Stimulated PBMC-produced IFN-γ and TNF-α are associated with altered relapse risk in multiple sclerosis: results from a prospective cohort study. J Neurol Neurosurg Psychiatry 86:200–207
32. KÜÇÜKALI Cİ, KÜRTÜNCÜ M, ÇOBAN A, ÇEBI M, TÜZÜN E (2015) Epigenetics of multiple sclerosis: an updated review. NeuroMolecular Med 17:83–96

33. KULAR L, CASTELO-BRANCO G, JAGODIC M (2017) Epigenetics and multiple sclerosis. In: Neuropsychiatric disorders and epigenetics. Elsevier
34. GUIL S, ESTELLER M (2012) Cis-acting noncoding RNAs: friends and foes. Nat Struct Mol Biol 19:1068–1075
35. NAGANO T, FRASER P (2011) No-nonsense functions for long noncoding RNAs. Cell 145:178–181
36. BECHER B, SPATH S, GOVERMAN J (2017) Cytokine networks in neuroinflammation. Nat Rev Immunol 17:49–59
37. GOVERMAN J (2009) Autoimmune T cell responses in the central nervous system. Nat Rev Immunol 9:393–407
38. PHILIPPÉ J, DEBRUYNE J, LEROUX-ROELS G, WILLEMS A, DEREUCK J (1996) In vitro TNF-α, IL-2 and IFN-γ production as markers of relapses in multiple sclerosis. Clin Neurol Neurosurg 98: 286–290
39. VAN OOSTEN BW, BARKHOF F, SCHOLTEN PE, VON BLOMBERG BME, ADÈR HJ, POLMAN CH (1998) Increased production of tumor necrosis factor α, and not of interferon γ, preceding disease activity in patients with multiple sclerosis. Arch Neurol 55:793–798
40. GOMEZ JA, WAPINSKI OL, YANG YW, BUREAU J-F, GOPINATH S, MONACK DM, CHANG HY, BRAHIC M, KIRKEGAARD K (2013) The NeST long ncRNA controls microbial susceptibility and epigenetic activation of the interferon-γ locus. Cell 152:743–754
41. LI H, HAO Y, ZHANG D, FU R, LIU W, ZHANG X, XUE F, YANG R (2016) Aberrant expression of long noncoding RNA TMEVPG1 in patients with primary immune thrombocytopenia. Autoimmunity 49:496–502