Review

What do we know about the role of lncRNAs in multiple sclerosis?

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Abstract

Multiple sclerosis is a chronic, inflammatory and degenerative disease of the central nervous system of unknown aetiology although well-defined evidence supports an autoimmune pathogenesis. So far, the exact mechanisms leading to autoimmune diseases are still only partially understood. We know that genetic, epigenetic, molecular, and cellular factors resulting in pathogenic inflammatory responses are certainly involved. Long non-coding RNAs (lncRNAs) are non-protein coding transcripts longer than 200 nucleotides that play an important role in both innate and acquired immunity, so there is great interest in lncRNAs involved in autoimmune diseases. The research on multiple sclerosis has been enriched with many studies on the molecular role of lncRNAs in the pathogenesis of the disease and their potential application as diagnostic and prognostic biomarkers. In particular, many multiple sclerosis fields of research are based on the identification of lncRNAs as possible biomarkers able to predict the onset of the disease, its activity degree, its progression phase and the response to disease-modifying drugs. Last but not least, studies on lncRNAs can provide a new molecular target for new therapies, missing, so far, a cure for multiple sclerosis. While our knowledge on the role of lncRNA in multiple sclerosis has recently improved, further studies are required to better understand the specific role of lncRNAs in this neurological disease. In this review, we present the most recent studies on molecular characterization of lncRNAs in multiple sclerosis disorder discussing their clinical relevance as biomarkers for diagnosis and treatments.

Key Words: antisense IncRNAs; enhancer IncRNAs; epigenetics; immune system; intergenic IncRNA; intronic IncRNA; multiple sclerosis; sense IncRNAs; single nucleotide polymorphisms

Introduction

Multiple sclerosis (MS), the second leading cause of sustained neurological disability in young people after trauma (Ontaneda et al., 2017), is a chronic, inflammatory and degenerative disease of the central nervous system (CNS) (Naegle et al., 2014) of unknown aetiology. So far, there are two theories on its pathogenesis: the “outside-in” autoimmune hypothesis, for which MS is an exclusive autoimmune inflammatory disease caused by unregulated auto-reactive immune cells that from the periphery go into the CNS parenchyma, attacking various cell types, and the “inside-out” hypothesis, for which MS is a primary degenerative disease in which inflammation is secondary to a release of auto-antigens promoting autoimmunity (Stys and Tsutsui, 2019). Until now, it remains to be determined whether inflammation is primary or secondary to a degenerative process in the brain. Nevertheless, well-defined evidence (as well as the successful use of immunomodulatory drugs in reducing clinical relapses and/or neuroradiological ‘activity’) demonstrates that an uncontrolled inflammatory response in the CNS is destructive in MS (Thompson et al., 2018). Sustained disability, however, is due to a progressive neurodegenerative process, causing axonal loss and brain atrophy, primary or secondary to the peripheral and compartmentalized inflammation in the CNS (Machado-Santos et al., 2018). To date, no approved therapy has provided marked neuroprotective effects nor anti-inflammatory therapies, used in the treatment of the disease, showed efficacy in the progressive phase of MS.

Well-defined evidence showed that the MS pathophysiology is characterized by altered bidirectional interactions among several immune cell types in the periphery and resident cells of the CNS, such as microglia and astrocytes (Li et al., 2018). The MS relapses, occurring in the early phases of the disease, are characterized by the infiltration, into the CNS parenchyma, of pro-inflammatory, CNS-specific effector T, B and myeloid cells, which are activated and/or regulated in an aberrant way (Dendrou et al., 2015). The altered function of regulatory T (Treg) cells and resistance of CNS-specific effector T cells to Treg cell-mediated regulation could be one possible cause of the neuro-inflammation (Kaskow et al., 2018; Kitz et al., 2018). Furthermore, CNS-resident cells, that secrete many inflammatory mediators, recruit inflammatory cells into the CNS (Dendrou et al., 2015). Therefore, both peripheral and CNS-compartmentalized inflammatory mechanisms contribute to MS pathogenesis (Filippi et al., 2018). In the advanced stages of the disease, infiltrated immune cells into the CNS are few and ongoing CNS-compartmentalized inflammation seems to dominate progressive phases of MS. During this phase, the role of B cells in driving inflammation seems to be prominent, particularly within meningeal inflammation (Magliozzi et al., 2007; Correale et al., 2017).

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Until now, the exact mechanisms leading to autoimmune diseases are still only partially understood but we know that genetic, epigenetic, molecular, and cellular factors resulting in pathogenic inflammatory responses driven by self antigen-specific T-cells are certainly involved.

Whole-genome transcriptional analysis have recently shown that the genome, in eukaryotic cells, can be transcribed in numerous types of coding and non-coding RNAs, the latter accounting at least 90% of these RNAs (Djebali et al., 2012). Increasing evidence on non-coding RNAs (ncRNAs) demonstrated that, more than evolutionary "junk genes", they have an important role as regulators of different cellular processes also in many diseases (Sun et al., 2013). ncRNAs are grouped into small ncRNAs (< 200 nucleotides) and long ncRNAs (≥ 200 nucleotides).

Long-ncRNAs (Inc-RNA) have more extensive and complex regulatory mechanisms than small ncRNA (Schmitz et al., 2016). Among their several functions (cell proliferation and differentiation, immune responses, metabolism, and apoptosis), they are very important in human autoimmune diseases playing specialized roles in modulating immune cell differentiation and activation (Sigdel et al., 2015; Wu et al., 2015; Atianand et al., 2017).

Indeed, long non-coding RNAs (lncRNAs) have an important impact on both innate and acquired immunity (Sigdel et al., 2015; Atianand et al., 2017; Zhang et al., 2017).

Innate immune responses are the body’s non-specific first line defence against pathogenic microorganisms, by the action of macrophages, dendritic cells, and natural killer cells. LncRNAs may have a critical role in regulating this response to pathogens (Mao et al., 2015; Atianand et al., 2017; Ivanov et al., 2018). Many changes have been found in IncRNA expression in macrophages upon innate immunity activation (Hu et al., 2016; Tong et al., 2016; Yang et al., 2016; Fei et al., 2017; Ye et al., 2018) and in dendritic cells are antigen-presenting cells (Ahmad et al., 2020). LncRNAs also have an important role in regulating the acquired immune responses, a second line of defence against pathogens, producing antigen-specific responses and immunological “memory.” Lymphocytes T and B are the main immune cells of the adaptive immune system. T helper cells are also critical in the pathogenesis of several diseases, and overall in autoimmune diseases (Zhu et al., 2010). Important IncRNA expression patterns have been elucidated in T cell function (Atianand MK et al., 2017) and in distinct stages of B cell development (Petri et al., 2015; Brazao et al., 2016; Tayari et al., 2016). LncRNAs also have a key role in cytokine genes regulation but overall the antisense lncRNAs (Atianand et al., 2017; Wang et al., 2017).

However, recent studies identified same lncRNA with an open reading frame (ORF) and few exons suggesting with a high probability translational ability to encode proteins (Bazin et al., 2017; Wang et al., 2017).

LncRNAs showed a tissue and cell specific expression, so that their levels are influenced by developmental or physiologic and pathologic state (Darren et al., 2012; Ulltisky and Bartel, 2013).

Comparing with mRNA, IncRNAs presented a lower expression in all tissues so that they were initially considered transcriptional noise resulting from low RNA polymerase fidelity (Darren et al., 2012). With the advent of high-throughput sequencing such as RNA-sequencing (RNA-seq), several studies showed that IncRNAs are abundant in the human genome and they can regulate the gene expression by DNA, RNA and protein interaction affecting transcriptional, post-transcriptional and translational processes (Atianand and Fitzgerald, 2014).

LncRNAs biogenesis occurs in both the nucleus or the cytoplasm and their cellular localization can influence the regulation mechanism of IncRNAs (Figure 1).

In the nucleus the lncRNAs are mainly involved in transcriptional regulation, through epigenetic modulation including histone and chromatin structure alteration that limits DNA accessibility (Tang et al., 2017; Figure 1). Instead, in the cytoplasm the lncRNAs are mainly involved in post-transcriptional regulation and post-translational regulation through mechanisms of translation inhibition, alternative splicing regulation and degradation (Tang et al., 2017; Figure 1).

LncRNAs classification is based according to their genomic position including proximity to protein-coding genes although several lncRNAs do not fall into this classification but rather turn out to be a combination of these specific characteristics or they cover long genomic sequences (Kung et al., 2013).

The main IncRNA classes are:

1) Intergenic IncRNAs (lincRNAs) represent the largest and most representative group of lncRNAs located among protein coding genes (Xue et al., 2017). By RNA-seq several lncRNAs have been identified in the genome showing a low level of expression (Cabillic et al., 2011). They present few exons and can regulate gene transcription in a cell-specific manner by acting either in cis or in trans regulation (Cabillic et al., 2011) (Figure 1).

2) Intronic IncRNA (ilncRNA) are entirely derived from the introns of transcript gene in either sense or anti-sense
The lncRNA functions and their regulation mechanisms are unclear and relatively unexplored. However, these lncRNAs are transcribed together with their host coding gene, thus they could probably regulate the expression of the host gene (Boivin et al., 2018; Figure 1).

3) Enhancer IncRNAs (eRNAs) includes promoter-associated IncRNAs, untranslated region-associated IncRNAs and telomere-associated IncRNAs. Indeed, they are transcribed bidirectionally in both polyadenylated or non-polyadenylated forms from active enhancer genomic regions (Xu et al., 2019). These regions are located regulative sequences of the gene contributing to the transcription start by the binding of transcriptional factors.

In this way, eRNAs can modulate the promoter activity, enhancer interactions and chromatin conformation influencing the transcription of neighboring genes (Chen et al., 2017; Liu et al., 2017; Figure 1).

4) Sense IncRNAs are transcribed from the sense strand of protein-coding gene and their sequence overlap, partially or completely, with the entire sequence of a protein coding gene including intron and part of exons (Devaux et al., 2015; Figure 1).

5) Anti-sense IncRNAs or natural antisense transcripts are encoded from exons of protein-coding genes in the opposite strand with partial to complete overlapping (Devaux et al., 2015). Anti-sense IncRNAs can modulate neighbouring genes expression through a regulation in cis (Magistri et al., 2012). About 70% of coding genes have anti-sense counterparts (Villegas et al., 2015; Figure 1).

The IncRNAs have the ability to inhibit or promote the expression of coding genes in tissue or cell-type manner and stage-specific development, suggesting their involvement in the pathogenesis of several diseases such as autoimmune and neurological disorders (Ingwersen et al., 2015; Sigdel et al., 2015).

In the context of autoimmune diseases, the research on MS has been enriched with many studies on the molecular role of IncRNAs in pathogenesis of this disorder and their potential application in MS such as diagnostic and prognostic biomarkers (Yang et al., 2018; Li et al., 2020).

Expression Profile of Long Non-coding RNAs in Multiple Sclerosis

In the last years, many studies have provided evidence of how IncRNAs deregulation is involved in the pathogenesis of MS disease as shown in Table 1. Here, we show the most recent studies on IncRNAs expression in the MS disease considering the biological material [serum, plasma, peripheral blood mononuclear cells (PBMC) and blood] on which the analysis was conducted. Serum represents one of the most accessible biological samples and provides excellent materials for the study of possible disease biomarkers. Recently, Santoro et al., screened 84 IncRNAs, involved in autoimmunity and human inflammatory response, in serum from 8 healthy controls, 16 secondary progressive MS (SPMS), 12 primary progressive MS (PPMS) Italian patients (Santoro et al., 2020). The authors found the up-regulation of Taurine up-regulated 1 (TUG1) in SPMS patients, while PPMS patients showed a down-regulation of non-protein coding RNA 188 (LRRC75A-AS1) and a significant up-regulation of long intergenic non-protein coding RNA 293 (LINC00293) and RPL11-29G8.3 (Santoro et al., 2020). In-silico analysis with bio-informatics tools to predict the interaction of IncRNAs-miRNAs such as DIANA-LncBase v2 and HMDD v3.0 software (Paraskevopoulou et al., 2016; Huang et al., 2019), identified 21 miRNAs prediction targets of these four IncRNAs that are involved in MS disease (Santoro et al., 2020).

In previous papers, the same authors showed the specific over-expression of three circulating IncRNAs in the serum of 12 relapsing-remitting MS (RRMS) patients: nuclear paraspeckle assembly transcript 1 (NEAT1), 75K small nuclear RNA (RN75K RNA) and TUG1 (Santoro et al., 2016). These three IncRNAs are involved in specific regulatory functions: NEAT1 promotes the increase of CXCL8 expression of the gene encoding interleukin 8 via relocation of SFPQ splicing factor (Imamura et al., 2014), RN75K RNA is involved in regulation of CD4+ T lymphocytes (Sung et al., 2006) and TUG1 is a component of the p53 regulatory network (Rossi et al., 2014). Although both represent pilot studies given the limited number of samples and need confirmation in larger collection numbers.

Figure 1 | The biogenesis of IncRNAs involves both the nucleus and the cytoplasm with specific transcriptional and post-transcriptional functions. The IncRNA classification is based on their transcriptional origin: intergenic IncRNA (lincRNA) transcribed from the genomic region between two genes; intronic IncRNA (ilncRNA) transcribed from the introns; enhancer IncRNAs (eRNAs) transcribed from genomic regions that contain regulatory sequences of the gene; sense IncRNAs transcribed from the sense strand of protein-coding gene overlapping partially or completely with intron and exons; anti-sense IncRNAs or natural antisense transcripts (NATs) transcribed from the exons of the protein coding gene on the opposite strand with partial to complete overlapping. IncRNAs: Long non-coding RNAs.
of RRMS, SPMS and PPMS samples, data obtained suggest the potential role of these lncRNAs in progressive MS pathogenesis. Another recent study conducted on serum of 60 healthy controls, 42 RRMS and 18 SPMS Egyptian patients showed an up-regulation of Linc-R-Gng2-5′-AS and a down-regulation of Linc-R-Epas1-3′-AS, two anti-sense intergenic lncRNAs located in T helper 1 (TH1) and in T helper 2 (TH2) cells (Shaker et al., 2020). Furthermore, the unregulation of these two lncRNAs was more marked in SPMS than in RRMS patients with an opposite correlation regarding the Expanded Disability Status Scale (positive for Linc-R-Gng2-5′-AS and negative for Linc-R-Epas1-3′-AS) (Shaker et al., 2020).

Moreover, in a previous study, Shaker et al. (2019) analyzed the expression levels of metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) and Inc-Dicer IncRNAs in serum from 45 MS patients and 45 controls finding an up-regulation of MALAT1 in the SPMS subgroup with no significant differences in RRMS patients. Indeed, Inc-Dicer showed an opposed expression: up-regulated in RRMS patients and no changes in SPMS subgroup (Shaker et al., 2019). In both studies, the expression analysis was performed only on some specific lncRNAs making it impossible to know if other lncRNAs are also involved.

Regarding the lncRNAs expression analysis in plasma, we found only one paper by Ghaiad et al., (2019) that determined the expression levels of anti-sense (AS) lncRNAs APOA1-AS and IFNG-AS1 in the plasma of 72 RRMS Egyptian patients (37 during relapse and 35 in remission) and 28 healthy controls. The authors found a significant up-regulation of APOA1-AS during relapse and in remission comparing with healthy controls while ApoA1 and high-density lipoproteins-cholesterol levels were significantly lower in the same phase, together with higher low-density lipoprotein-cholesterol levels (Ghaiad et al., 2019).

Moreover, the expression levels of IFNG-AS1 were also significantly higher in RRMS patients during relapse and in remission comparing with healthy controls (Ghaiad et al., 2019).

This study provides evidence for diagnostic and prognostic role of APOA1-AS and IFNG-AS1 in RRMS patients although further analysis is needed for validation in a larger cohort of MS patients.

Whole blood is also often used to evaluate IncRNA expression levels. Moradi et al. (2020a) analyzed in the blood of 10 controls and 20 Iranian RRMS patients, the expression profile of MEG3a, AC000061.1_201, and AC007182.6, three lncRNAs involved in the pathogenesis of human autoimmune diseases. The authors found a down-regulation only of MEG3a that negatively correlate with Expanded Disability Status Scale. Additionally, analysis of receiver operating characteristic curve showed the ability of this lncRNA to discriminate between RRMS and healthy individuals suggesting MEG3a (Moradi et al., 2020a). Even if MEG3a is indicated as a potential diagnostic biomarker to distinguish MS patients, this study was conducted on a small cohort of samples (10 controls

### Table 1 | LncRNAs dysregulated in multiple sclerosis

| LncRNAs | Regulation | Patients | Sample | Function | References |
|---------|------------|----------|--------|----------|------------|
| TUG1    | ↑          | SPMS     | Serum  | Involvement in p53 pathway and cell cycle | Santoro et al. (2020) |
| LRRCT5A-AS1 | ↓        | PPMS     | Serum  | Not determined | Santoro et al. (2020) |
| LINC00293 | ↑          | PPMS     | Serum  | Not determined | Santoro et al. (2020) |
| RP11-29Q8.3 | ↑        | PPMS     | Serum  | Not determined | Santoro et al. (2020) |
| Linc-R-Gng2-5′-AS | ↑    | RRMS/SPMS | Serum  | Immune regulatory function | Shaker et al. (2020) |
| Linc-R-Epas1-3′-AS | ↓    | RRMS/SPMS | Serum  | Immune regulatory function | Shaker et al. (2020) |
| MALAT1  | ↑          | SPMS     | Serum  | Oncogenic role | Shaker et al. (2019) |
| lnDC    | ↑          | RRMS     | Serum  | Differentiation and maturation of dendritic cells | Shaker et al. (2019) |
| NEAT1   | ↑          | RRMS     | Serum/Blood | Regulation of CXCL8 expression | Santoro et al. (2016); Dastmalchi et al. (2018) |
| TUG1    | ↑          | RRMS     | Serum/Blood | Involvement in p53 pathway and cell cycle | Santoro et al. (2016); Dastmalchi et al. (2018) |
| RN75K RNA | ↑          | RRMS     | Serum  | Regulation of CD4+ T lymphocytes | Santoro et al. (2016) |
| APOA1-AS | ↑          | RRMS     | Plasma  | Negative transcriptional regulator of ApoA1 | Ghaiad et al. (2019) |
| IFNG-AS1 | ↑          | RRMS     | Plasma  | Transcription/expression of IFN-γ in Th1 cells | Ghaiad et al. (2019) |
| MEG3a   | ↓          | RRMS     | Blood   | Autoimmune diseases | Moradi et al. (2020a) |
| PVT1    | ↓          | RRMS     | Blood   | Control of IL-6 release | Eftekharian et al. (2017) |
| FAS-AS1  | ↓          | RRMS     | Blood   | Regulation of soluble Fas receptor | Eftekharian et al. (2017) |
| THRIL   | ↑          | RRMS     | Blood   | Regulative role in innate immunity | Eftekharian et al. (2017) |
| IFNG-AS1 | ↑          | RRMS     | Blood   | Transcription/expression of IFN-γ in Th1 cells | Ganji et al. (2019) |
| PANDA   | ↑          | RRMS     | Blood   | p53 protein stabilization | Dastmalchi et al. (2018) |
| GAS8-AS1 | ↑          | RRMS     | Blood   | Not determined | Patoughi et al. (2019) |
| OIP5-AS1 | ↑          | RRMS     | Blood   | Cell division | Gharesouran et al. (2019) |
| RP11-53OCS.1 | ↑   | RRMS     | PBMC    | Potential cis-regulatory | Ghoveud et al. (2020) |
| AL928742.12 | ↓     | RRMS     | PBMC    | Not determined | Ghoveud et al. (2020) |
| IFNG-AS1-001 | ↑    | RRMS     | PBMC    | Transcription/expression of IFN-γ in Th1 cells | Hosseini et al. (2019) |
| IFNG-AS1-003 | ↑    | RRMS     | PBMC    | Transcription/expression of IFN-γ in Th1 cells | Hosseini et al. (2019) |
| AC007278.2 | ↑    | RRMS     | PBMC    | Regulation of Th1 cell development | Hosseini et al. (2019) |
| NRON    | ↓          | RRMS/PPMS| PBMC    | Nuclear factor repressor of activated T cells | Fenoglio et al. (2018) |
| TUG1    | ↑          | RRMS     | PBMC    | Involvement in p53 pathway and cell cycle | Fenoglio et al. (2018) |
| Inc-DDT4 | ↑          | RRMS     | PBMC/CD4+ T cells | Th1 cell differentiation | Zhang et al. (2018) |
| Linc-MAPF-4 | ↑    | RRMS     | PBMC    | Th1/Th2 cell differentiation | Zhang et al. (2017) |
| HOXAIR  | ↑          | RRMS     | PBMC    | Vitamin D and inflammation regulation | Pahlevan Kahkhi et al. (2017) |

IFN-γ: Interferon gamma; IL-6: interleukin-6; lncRNAs: long non-coding RNAs; PBMCs: peripheral blood mononuclear cells; PPMS: primary progressive multiple sclerosis; RRMS: relapsing-remitting multiple sclerosis; SPMS: secondary progressive multiple sclerosis.

1. Santoro et al. (2016).
2. Ghaiad et al. (2019).
3. Hosseini et al. (2019).
4. Hosseini et al. (2019).
5. Fenoglio et al. (2018).
6. Fenoglio et al. (2018).
7. Zhang et al. (2018).
8. Zhang et al. (2017).
9. Pahlevan Kahkhi et al. (2017).
vs. 20 RRMS patients) and further experiments are needed to validate these results in a large number of individuals considering also the different stages of the MS disease.

Another blood study conducted on Iranian RRMS (n = 50) patients versus 50 healthy controls showed a down-regulation of Fas cell surface death receptor-antisense 1 (FAS-AS1) and plasmacytoma variant translocation 1 (PVPT1) with an up-regulation of TNF-α and heterogeneous nuclear ribonucleoprotein L (THRL) and (Eftekharian et al., 2017). Even if these three lncRNAs were involved in innate immunity apoptosis regulation during lymphocyte development and immune responses (Li et al., 2014; Aune and Spurlock, 2016; Austin et al., 2017), the authors do not find a significant correlation between the expression levels and clinical feature of RRMS thus the role of these lncRNAs in MS pathogenesis remains to be clarified.

Always in the blood of Iranian RRMS (n = 50) patients, Ganji et al. (2019) found a down-regulation of GSTT1-AS1 and IFNG-AS1 lncRNAs with an up-regulation of their coding targets TNF and IFNG. Indeed, GSTT1-AS1 (or IncRNA-CD244) is involved in the inhibition regulation of IFNG and tumor necrosis factor (TNF) genes (Wang et al., 2015), while IFNG-AS1 together with T-bet is able to regulate the transcription of IFNG (Aune et al., 2016). These data disagree with the results obtained by Ghaiad that found an up-regulation of IFNG-AS1 in RRMS patients.

This discordance is probably related to the different biological source (plasma versus blood) chosen for the analysis, ethnic group (Egyptian versus Iranian) or the phase of disease and drug treatment at the time of MS patient recruitment.

Furthermore, some studies conducted on whole blood have shown that the expression levels of some lncRNAs can be modulated by age and gender.

Indeed, Dastmalchi et al. (2018) detected an over-expression of NEAT1, TUG1 and P21 associated ncRNA DNA damage activated (PANDA) in blood samples of 50 RRMS patients. In particular, the expression of NEAT1 and TUG1 was inversely correlated with age at onset and disease duration in female patients while PANDA showed a more prominent over-expression in male patients (Dastmalchi et al., 2018). These data indicated a gender-dependent role of NEAT1 TUG1 and PANDA in the pathogenesis of MS suggesting that the presence of a sex-determined factor, hormones and drugs, can regulate the expression of these lncRNAs (Dastmalchi et al., 2018).

Patoughi et al. (2019) found in the blood of RRMS (n = 50) versus healthy controls (n = 50), an up-regulation of the antisense RNA 1 growth arrest specific 8 (GAS8-AS1) with higher levels of expression in male patients.

Moreover, Gharesouran et al. (2019) detected high levels of OIP5-AS1 expression in the blood of RRMS (n = 50) patients, and these data are particularly evident in men less than 30 years old. With the increasing age of patients, the expression of this IncRNA seems to influence the incidence and onset of the disease suggesting a regulatory role in the cell division process (Gharesouran et al., 2019).

Regarding the analysis of IncRNAs on cells, many studies have been carried out on PBMC isolated from blood.

Indeed, comparing 50 RRMS patients versus 25 healthy controls, Ghoveud et al. (2020) found in PBMC an up-regulation of RP11-530C5.1 and a down-regulation of AL928742.12 lncRNAs. Although the results obtained with the receiver operating characteristic analysis suggested potential biomarker roles of both these lncRNAs, the study needs validation in a larger sample of both healthy controls and MS patients at different stages of the disease.

Hosseini et al., found in the PBMC of 50 RRMS (25 in relapsing phase and 25 in the remitting phase treated with interferon beta) high expression of AC007278.2 and IFNG-AS1-001 lncRNAs in relapsing phase MS patients compared with the healthy controls while IFNG-AS1-003 IncRNA was elevated in MS patients in the remitting phase compared with those in relapsing phase (Hosseini et al., 2019). In association with MS disease, AC007278.2 turns out to be correlated with the expression of IL18R1 and IL1RAP genes that encode the α and β chains of the heterodimeric IL-18 receptor involved in Th1 cell development (Hosseini et al., 2019). Indeed, IFNG-AS1-001 is correlated with IFNG expression suggesting this IncRNA as a potential target for MS treatment (Hosseini et al., 2019).

Another study on PBMC from cohort of Italian population (27 RRMS, 13 PPMS and 31 healthy controls) showed a significant down-regulation of MALAT1, MEG9, NRON, ANRIL, TUG1, KIXT, SOX20T, GOMAFU, HULC, BACE-1AS lncRNAs (Fenoglio et al., 2018).

The validation analysis conducted on an independent Belgian cohort composed by 17 RRMS, 7 PPMS and 23 healthy controls showed a down-regulation of only NRON and TUG1 (Fenoglio et al., 2018). The data concerning the low levels of TUG1 expression were opposite to those of Dastmalchi et al. (2018) and Santoro et al. (2016) which instead showed an up-regulation. Probably, this discrepancy could be related to the biological sample used for the analysis (blood and serum versus PBMC), different phase of the disease and pharmacological treatment.

Besides analyzing the levels of expression, some lncRNAs have been characterized by their involvement in regulatory functions.

Indeed, transfection experiments for over-expression of Inc-DDIT4, that found up-regulated in both PBMCs and CD4+ T cells of RRMS patients, in naive CD4+ T cells showed an inhibition of T17 cell differentiation through the regulation of DNA-damage-inducible transcript 4 (DDIT4) expression resulting in the modulation of DDIT4/mTOR pathway (Zhang et al., 2018).

These data suggest a possible role of Inc-DDIT4 in the pathogenesis of MS through the regulation Th17 cell differentiation. Moreover, the over-expression of Linc-MAF-4, other IncRNA found up-regulated in PBMC from RRMS patients, into naive CD4+ T cells induced Th1-cell differentiation inhibiting Th2-cell differentiation by the action of transcription factor MAF (Zhang et al., 2017).

On the other hand, a down-regulation of linc-MAF-4 has an opposite effect with inhibition of Th1 cells differentiation and the development of Th2 cells (Zhang et al., 2017). These data suggest an important role of linc-MAF-4 in the pathogenesis of MS disease.

Finally, Pahlevan Kakhki et al. (2017) analyzed in the PBMC from 52 RRMS the expression levels of HOX transcript antisense intergenic RNA (HOTAIR) and anti-sense lncRNA of the INK4 locus (ANRIL), both lncRNAs are involved in the inflammatory disorders. The authors correlated the expression levels of these two lncRNAs with the serum levels of vitamin D considered that can regulate the expression of IncRNAs (Riege et al., 2017).

Their data showed that MS patients at base line (before vitamin D treatment) had higher expression levels of HOTAIR but not ANRIL lncRNAs compared with the healthy controls.

After vitamin D treatment, HOTAIR levels returned to those found in the healthy controls (Pahlevan Kakhki et al., 2017). These data suggest that vitamin D could modulate inflammation through the regulation of HOTAIR, however further studies are needed to clarify this issue.
In conclusion, a large number of studies have been carried out on the molecular analysis of IncRNAs and their role in the pathogenesis of MS. Unfortunately, the data produced are still limited and inconclusive. In fact, there are some important critical issues to consider: i) the number of samples with both MS patients and controls used for the IncRNAs analysis is limited and needs validation in a larger study sample; ii) the expression levels of IncRNAs is influenced by several factors such as the biological sample chosen for the analysis (serum, plasma, blood and PBMC), pharmacological treatment, stage of MS disease, age and sex; iii) an appropriate normalization method based on the stable expression of reference gene across different sample groups and tissue in order to avoid introducing a significant error in the quantification of IncRNA levels.

**Single Nucleotide Polymorphisms in Multiple Sclerosis-Related Long Non-coding RNAs**

IncRNAs perform their regulatory function through the secondary and tertiary structures they can adopt. The presence of single nucleotide polymorphisms (SNPs) inside the IncRNAs can alter these structures influencing their expression levels and functions (Hangauer et al., 2013). Indeed, several bio-informatic tools have been developed to predict IncRNA structures based on the presence of a specific SNP (Gong et al., 2015; Ren et al., 2018).

Moreover, some SNPs are involved in the modulation of IncRNA alternative splicing by regulating exon skipping and production of specific isoforms that could present a different binding affinity for their target modifying the downstream events (Aguilo et al., 2016; Chowdhury et al., 2017).

Based on these premises, several studies analyzed the association of some SNPs present within the IncRNAs sequence with the risk of MS.

Bahrami et al. (2020) analyzed the SNPs association with RRMS susceptibility on blood samples from Iranian population (300 RRMS patients vs. 300 healthy controls) of two IncRNAs involved in oxidative stress and autophagy: TRPM2-AS (rs933151) and HNF1A-AS1 (rs7953249). The authors found that SNP rs933151 was statistically associated with RRMS risk and T allele of this SNP was statistically poorly represented in RRMS patients compared with healthy controls. Instead, the rs7953249 was not associated with RRMS susceptibility (Bahrami et al., 2020).

The expression analysis on both IncRNAs has not been conducted in MS patients; therefore, it is not possible to understand whether this SNP may have a role in the modulation of TRPM2-AS and HNF1A-AS1 levels.

Other IncRNAs studied for a possible association between SNP and MS risk were HOTAIR and ANRIL. Previous data showed that only HOTAIR had higher expression levels in the MS patients, which can be modulated through vitamin D treatment (Pahlevan Kakikki et al., 2017).

Their data showed that MS patients at base line (before vitamin D treatment) but not ANRIL IncRNAs compared with the healthy controls.

Three SNPs (rs12826786, rs1899663 and rs4759314) of HOTAIR were genotyped by Taheri and colleagues in blood from 403 Iranian RRMS patients versus 420 healthy controls (Taheri et al., 2020). The authors found a significant association with MS risk only for rs4759314 SNP (Taheri et al., 2020). The high expression levels of HOTAIR found in MS patients suggest an important role of this SNP in the regulation of the HOTAIR expression.

On the other hand, Rezazadeh et al. (2018) also evaluated the association of rs1333045, rs4977574, rs1333048, and rs10757278 SNPs of ANRIL and MS risk in blood from 410 Iranian RRMS patients and 410 healthy controls. The authors found protective effect of CCGG and TAAA haplotypes against MS (rs1333045, rs1333048, rs4977574, and rs10757278 respectively), while TAGG and CCGA haplotypes were significantly associated with MS risk (Rezazadeh et al., 2018) even if the expression levels of ANRIL IncRNA are not altered in RRMS patients (Pahlevan Kakikki et al., 2017).

Moreover, MALAT1 was another IncRNA with an up-regulation in the SPMS and no significant differences in RRMS patients (Shaker et al., 2019). Indeed, also the analysis of two MALAT1 (rs619586 and rs3200401) SNPs conducted by Eftekharian et al. (2019) on blood from 428 Iranian RRMS patients versus 505 healthy controls showed a low association with MS risk only for rs619586 polymorphism.

In agreement with previous data, the up-regulation of GAS5 was found in whole blood of MS patients suggesting an important regulatory role of IncRNA GAS5 genetic variant in the pathogenesis of MS (Mayama et al., 2016).

All these data suggest a possible role of the SNPs in the regulation of IncRNAs even if the possible molecular mechanism that underlies the impact of SNPs on IncRNA structure and function remains unknown.

In general, the analysis of SNPs should also be extended to other ethnic groups as most of the published studies have been conducted on an Iranian population.

Furthermore, each SNP should be associated with the analysis of IncRNA expression, splicing and secondary structure in the same population and in the same patient.

It is necessary to adopt an approach that analyzes the functional differences of the IncRNA alleles and their ability to regulate expression of downstream genes.

**Concluding Remarks**

Until now, the exact mechanisms leading to diseases with an autoimmune pathogenesis, such as MS, are still not entirely understood, but we know that genetic, epigenetic, molecular, and cellular factors resulting in pathogenic inflammatory responses are certainly involved. IncRNAs have, among their several functions (cell proliferation and differentiation, metabolism, and apoptosis), an important impact on both innate and acquired immunity, so there is great interest on IncRNAs involved in autoimmune diseases. About MS, the research has been enriched with many studies on the molecular role of IncRNAs in pathogenesis of the disease and their potential application such as diagnostic and prognostic biomarkers. In particular, many MS fields of research are based on the identification of IncRNAs as possible biomarkers able to predict the onset of the disease, its activity degree, its progression phase and the response to disease modifying drugs. Last but not least, studies on IncRNAs in MS can provide new molecular target for novel therapies, missing so far, a cure for the disease.

Expert opinion: while our knowledge on the role of IncRNA in MS has recently improved, we know that knowledge is at the dawn and further studies are required to better understand the specific role of IncRNAs in MS and in other autoimmune diseases. Among the various biomarkers analyzed in MS, IncRNAs are of great importance because they are able to regulate the genome expression/stability and cellular functions especially in the activation of immune cells both in innate and in adaptive immune system. So far,
there are still several deep-rooted problems regarding the IncRNAs function in autoimmune diseases. Why are IncRNAs abnormally expressed in autoimmune diseases? What are the specific mechanisms underlying this abnormal expression? Have changes in IncRNAs got a causal role in disease activity and/or in disease progression? Assumed that IncRNAs play important roles as regulators of several biological processes, is it possible that this indirect unregulation is linked to autoimmune disease pathogenesis? Further studies are needed to answer the aforementioned and many other questions on this new and relevant topic for MS and other autoimmune diseases.

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