Recent advances of long noncoding RNAs involved in the development of multiple sclerosis

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[ABSTRACT] Given the rapid increase of patients with autoimmune diseases and the lack of satisfactory therapies, the discovery of novel and effective therapeutic targets have been in an urgent demand. Recent studies have revealed that long noncoding RNAs (lncRNAs) play crucial roles in the development of multiple sclerosis (MS), which provides a new opportunity of uncovering novel mechanism associated with the progression of MS. This review highlights the dysregulation of lncRNAs in the development of MS in patients and animal models. Additionally, the potential clinical relevance of lncRNAs severed as therapeutic targets and diagnostic markers are discussed.

[KEY WORDS] Multiple sclerosis; Long noncoding RNA; Experimental autoimmune encephalomyelitis; Clinical relevance; Dysregulation


Introduction

Multiple sclerosis (MS) is a chronic autoimmune disease that is characterized by severe inflammation and demyelination of the central nervous system (CNS), causing relapsing and progressive neurological deterioration [1]. According to 3 Atlas of MS 2013 [2], 2–3 million people suffer from MS globally, with a prevalence of 33 per 100 000 people, but this may be underestimated given the relative lack of data onto large Asian countries. MS mainly affects young people with a female predominance, and the first symptoms of the disease usually appear between 20 and 40 years of age [3]. MS has varied clinical features, including sensory loss, gradual muscle weakness, cerebellar and autonomic spinal cord symptoms, etc [4]. The pharmacological approach to MS treatment includes symptomatic therapy and symptomatic/immune-modulatory therapy, which aimed to reduce relapse rate, delay the disability, maintain the function of body and improve the quality of life of patients [9]. The pharmacologic armamentarium for MS treatment is considerably improved in the last 20 years. As of June 2018, total 17 medications for modifying the course of MS have been approved by the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) (Table 1).

Despite numerous studies have been put forward to explain how progressive multiple sclerosis is triggered, the ultimate cause of MS still remains unknown [6-12]. It is progressively evident from recent studies that the hyperactivated adaptive immune system was the pivotal pathogenic factors of MS [13]. It has been proved that the migration of inflammatory immune cells like Th1 and Th17 into the CNS lead to the demyelination of the neuron sheath, thereby causing the neuroinflammation and neurodegeneration [14-15]. Long non-coding RNAs (lncRNAs) are non-protein coding transcripts longer than 200 nucleotides (nt), and it has been known as the key regulators of massive genes and pathways involved in the cell proliferation, differentiation, apoptosis and development, especially the immune cell differentiation and activation in adaptive immune system. Moreover, it has been demonstrated that dysregulated lncRNAs play key roles in the
The pathogenesis of inflammatory and autoimmune diseases, including systemic lupus erythematosus (SLE), rheumatoid arthritis (RA) and MS [16-17]. Although the detail mechanism still remains poorly understood for the vast majority of lncRNAs, there is growing evidence that lncRNAs interfere in the pathogenetic process of MS. Here, we review the essential roles of lncRNAs in MS and discuss their clinical relevance for better diagnosis and treatment of MS.

### LncRNAs in MS and Animal Models of MS

**LncRNAs**

LncRNAs are the major component of "regulatory" ncRNAs and have the following characteristics: being longer than 200nt...

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**Table 1 Therapeutic drug of MS approved by FDA and EMA (up to June 2018)**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Brand Name/ Manufacture</th>
<th>Indications</th>
<th>Dose/administration</th>
<th>Approval</th>
<th>Mechanism</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interferon beta-lb</td>
<td>Betaseron/Chiron</td>
<td>RRMS</td>
<td>250 mcg every other day/SC</td>
<td>FDA:23/06/1993; EMA:30/11/1995</td>
<td>Induce anti-inflammatory cytokines as well as decrease proinflammatory cytokines</td>
<td>[85-86]</td>
</tr>
<tr>
<td>Interferon beta-la</td>
<td>Avonex/Biogen</td>
<td>RRMS</td>
<td>30 mcg weekly/IM</td>
<td>FDA:17/05/1996; EMA:13/03/1997</td>
<td>Modulate inflammatory cells across the BBB</td>
<td></td>
</tr>
<tr>
<td>Interferon beta-la</td>
<td>Rebif/Merck</td>
<td>RRMS</td>
<td>22 or 44 mcg 3 times a week/SC</td>
<td>FDA:07/03/2002; EMA:04/05/1998</td>
<td>Increase the production of nerve growth factor and the number of natural killer cells</td>
<td></td>
</tr>
<tr>
<td>Interferon beta-lb</td>
<td>Extavia/Novartis</td>
<td>SPMS</td>
<td>250 mcg every other day/SC</td>
<td>FDA:14/08/2009; EMA:20/05/2008</td>
<td>Increase the production of nerve growth factor and the number of natural killer cells</td>
<td></td>
</tr>
<tr>
<td>Interferon beta-lb</td>
<td>Plegridy/Biogen</td>
<td>RRMS</td>
<td>125 mcg every 2 wk/SC</td>
<td>FDA:15/09/2014; EMA:18/07/2014</td>
<td>Increase the production of nerve growth factor and the number of natural killer cells</td>
<td></td>
</tr>
<tr>
<td>Peginterferon betalb</td>
<td>Betaferon/Bayer</td>
<td>RRMS</td>
<td>250 mg every 2 days/SC</td>
<td>FDA:14/08/2009; EMA:20/05/2008</td>
<td>Increase the production of nerve growth factor and the number of natural killer cells</td>
<td></td>
</tr>
<tr>
<td>Interferon beta-la</td>
<td>Copaxone/Teva</td>
<td>RRMS</td>
<td>20 mg once daily/SC or 40 mg three times weekly/SC</td>
<td>FDA:20/12/1996; EMA:04/05/1998</td>
<td>Resemble myelin basic protein and may block myelin-damaging T cells</td>
<td>[87-88]</td>
</tr>
<tr>
<td>Interferon beta-la</td>
<td>Glatopa/Sandoz</td>
<td>RRMS</td>
<td>20 mg once a day/SC</td>
<td>FDA:16/04/2015; EMA:16/04/2015</td>
<td>Exert neuroprotective effect via brain-derived neurotrophic factor</td>
<td></td>
</tr>
<tr>
<td>Mitoxantrone</td>
<td>Novantrone/Immunex</td>
<td>RRMSPMSIV</td>
<td>12 mg/m² every 3 months/ IV (a lifetime cumulative dose of no more than 140 mg/m²)</td>
<td>FDA:13/10/2000; EMA:27/06/2006</td>
<td>Inhibit activation of T cells and proliferation of B and T cells</td>
<td>[89-91]</td>
</tr>
<tr>
<td>Natalizumab</td>
<td>Tysabri/Biogen</td>
<td>RRMS</td>
<td>300 mg every 28 d/IV</td>
<td>FDA:23/11/2004; EMA:17/03/2011</td>
<td>Bind to α4β1-integrin to block the interaction of integrin with ligands; Inhibit migration of inflammatory cells across the BBB</td>
<td>[91-92]</td>
</tr>
<tr>
<td>Fingolimod</td>
<td>Gilenya/Novartis</td>
<td>RRMS</td>
<td>0.5 mg once a day/PO</td>
<td>FDA:21/09/2010; EMA:17/03/2011</td>
<td>Block lymphocytic invasion of the brain</td>
<td>[93]</td>
</tr>
<tr>
<td>Teriflunomide</td>
<td>Aubagio/Sanofi</td>
<td>RRMS</td>
<td>7 or 14 mg once a day/PO</td>
<td>FDA:12/09/2012; EMA:26/08/2013</td>
<td>Reduce proliferation of B and T cells through inhibition of dihydroorotate dehydrogenase</td>
<td>[94-95]</td>
</tr>
<tr>
<td>Dinethyl Fumarate</td>
<td>Tecfidera/Biogen</td>
<td>RRMS</td>
<td>120 mg twice a day for 7 d/PO; then 240 mg twice a day/PO</td>
<td>FDA:27/03/2013; EMA:30/01/2014</td>
<td>Activate the Nrf2-dependent antioxidative pathways</td>
<td>[96-98]</td>
</tr>
<tr>
<td>Alemtuzumab Lemtrada/Genzyme</td>
<td>RRMS</td>
<td>12 mg once a day for 5 d/IV; after 1 year 12 mg for 3 days/IV</td>
<td>FDA:07/05/2001; EMA:12/09/2013</td>
<td>Against CD52 and mediate the depletion of lymphocytes</td>
<td>[99]</td>
<td></td>
</tr>
<tr>
<td>Daclizumab</td>
<td>Zinbyra/Biogen</td>
<td>RRMS</td>
<td>150 mg once every 28 days/SC</td>
<td>FDA:27/05/2016; EMA:01/07/2016</td>
<td>Against CD25 and increase the number of regulatory CD56+ natural killer cells</td>
<td>[100-102]</td>
</tr>
<tr>
<td>Ocrelizumab</td>
<td>Ocrevus/Roche</td>
<td>PPMS RRMS</td>
<td>300 mg/IV; after 2 weeks 300 mg /IV; then 600 mg every 6 months/IV</td>
<td>FDA:28/03/2017; EMA:08/01/2018</td>
<td>Against CD20 and deplete B lymphocytes</td>
<td>[103-104]</td>
</tr>
<tr>
<td>Cladribine</td>
<td>Mavenclad/Merck</td>
<td>RRMS</td>
<td>20 mg once a day/PO</td>
<td>EMA:22/08/2017</td>
<td>Preferentially affect lymphocytes through the disruption of cellular metabolism, the inhibition of DNA synthesis and repair, and subsequent apoptosis</td>
<td>[105-106]</td>
</tr>
</tbody>
</table>
in length, generally transcribed by RNA Pol II and exhibit mRNA-like features and without protein coding capacity. According to the NONCODE database (version v5.0), there are 96,308 and 87,774 lncRNA genes for human and mouse, respectively. And they can generate 172 216 (human) and 131 697 (mouse) lncRNA transcripts. In comparison, the current number of human and mouse protein coding genes and transcripts is much fewer. Based on the GENCODE (version 28), there are 19 901 protein coding genes and 82 335 protein coding transcripts in human, while 21 978 protein coding genes and 57 388 protein coding transcripts in mouse. According to their genomic proximity to protein-coding genes, lncRNAs can be classified into six categories (Fig. 1): bidirectional lncRNA, intergenic lncRNA, intronic-antisense lncRNA, natural-antisense lncRNA, intron sense-overlapping lncRNA, and exon sense-overlapping lncRNA.

**Fig. 1** Classification of lncRNA. (i) Bidirectional lncRNA: transcribed from the promoter region of the protein coding gene with transcription proceeding in the opposite direction; (ii) Intergenic lncRNA: transcribed from the genomic interval between two protein coding genes; (iii) Intronic-antisense lncRNA: transcribed from the introns of the protein coding gene on the opposite strand; (iv) Natural-antisense lncRNA: transcribed from the exons of the protein coding gene on the opposite strand; (v) Intron sense-overlapping lncRNA: transcribed from the intron of the protein; (vi) Exon sense-overlapping lncRNA: transcribed from the exon of the protein.

The research of lncRNA dates back to at least 1990, when Brannan CI et al firstly identified a lncRNA, named H19, in mammalian cell. With the speedy progress in technology of gene research, more and more functional lncRNAs have been identified and the mechanisms of action of lncRNAs have become clear gradually. A fast-growing number of studies have shown that lncRNAs can regulate gene expression by interacting with protein, RNA or DNA via complementary base-pairing and/or structural interaction. LncRNAs are localized in the nucleus or the cytoplasm and their cellular localization has great influence on the mechanisms of action of lncRNAs. The lncRNAs that specifically expressed in the nucleus mainly play a role in transcriptional regulation and epigenetic modification; while cytoplasmic lncRNAs primarily take part in post-transcriptional regulation and post-translational regulation.

**LncRNAs associated with MS**

Studies of lncRNAs demonstrated that lncRNAs are usually lower expressed and more tissue-, cell-type- and development-stage-specific compared to protein coding genes, suggesting that their possible involvement in a particular process of diseases. In the pathogenesis of various autoimmune diseases (AID), an increasing number of studies have reported that lncRNAs dysregulation plays a key role. Besides in SLE, RA and so on, deregulated lncRNAs are also identified in MS patients in recent years, as shown in table 2.

**Linc-MAF-4**

Linc-MAF-4 was firstly reported that regulated CD4+ T cell differentiation through regulating MAF transcription by recruitment of chromatin modifiers in 2015. In 2016, by microarray analysis of lncRNAs in peripheral blood mononuclear cells (PBMCs) compared six MS patients with five healthy controls, Zhang et al found that linc-MAF-4 was significantly up-regulated in MS patients. Subsequently, they overexpression and knockdown of linc-MAF-4 in CD4+ T cells from the additional 28 MS patients to track changes in CD4+ T subsets and their function. Their experimental data indicated that linc-MAF-4 elevated Th1 cell differentiation but inhibited Th2 cell differentiation and was a necessary element in the differentiation of Th1 cells and production of IFN-γ. Moreover, linc-MAF-4 also promoted activation of CD4+ T cells in MS patients, but didn’t affect the prolifera-
HOTAIR HOX transcript antisense intergenic RNA (HOTAIR) is a 2148-nucleotide-long lncRNA transcribed in an antisense manner from HoxC gene cluster on chromosome 12 [28]. Many evidences have identified HOTAIR exerted diverse functions in pathogenesis of various malignancies and inflammatory diseases [29-31]. Recently, Majid Pahlevan Kakhki et al [32] examined the role of HOTAIR in MS pathogenesis in relation to vitamin D levels suing in vivo, in vitro, and in silico investigations. Their data revealed that MS patients had higher expression levels of HOTAIR compared with controls before vitamin D treatment. With vitamin D treatment, there was a tendency toward lower HOTAIR compared with controls before vitamin D treatment. With vitamin D treatment, there was a tendency toward lower HOTAIR.

HHMT and vitamin D is affected, which need to be further studied.

**Lnc-DDIT4**

DNA-damage-inducible transcript 4 (DDIT4), a cytoplasmic protein, was found to play an important role in proliferation and survival of T cells via mTOR pathway [33]. Previous studies found that DDI4 was highly expressed and mTOR pathway was among the top most enrichment pathways in MS patients [34-35]. Lnc-DDIT4 were detected that increased in both in PBMCs and CD4+ T cells of MS patients and regulated DDI4/mTOR signal pathway via cis-acting mechanism [36]. The expression of Lnc-DDIT4 was much higher during Th17 cell differentiation than other CD4+ T cells. Th17 has been recognized as pivotal etiological factors in the development of MS. Overexpression of Lnc-DDIT4 in naïve CD4+ T cells inhibited Th17 cell differentiation through increased DDI4 expression and decreased activation of the DDI4/mTOR pathway, and vice versa. Concretely, Lnc-DDIT4 inhibited the phosphorylation of mTOR, p70S6k and 4EBP1 associated with differentiation of Th17 cell via directly targeting DDI4. In conclusion, Lnc-DDIT4 could directly regulate Th17 cell differentiation and participate in the pathogenesis of MS.

**ANRIL**

Antisense non-coding RNA in the INK4 locus (ANRIL), located within the CDKN2B-CDKN2A gene cluster spanning across a ~30–40 kb region at chromosome 9p21, was reported that altered chromatin and transcription of target genes through the chromatin-remodeling complexes [37-38]. And the regulated roles of ANRIL in cell proliferation and inflammatory response have been highlighted in many studies [39-40]. A recent study has shown the association between ANRIL genetic variants and MS risk (41). Totally, four variants of ANRIL [rs1333045 (GRCh38.p7:chr9:22119196), rs4977574 (GRCh38.p7:chr9:22098575), rs1333048 (GRCh38.p7:chr9:22125348), and rs10757278 (GRCh38.p7:chr9:22124478)] were evaluated in a population of 410 Iranian MS patients and 410 healthy subjects. Statistical analysis revealed that

**Table 2 LncRNAs in MS and EAE**

<table>
<thead>
<tr>
<th>LncRNA in MS patients</th>
<th>LncRNA</th>
<th>Genome location</th>
<th>Sample</th>
<th>Known target(s)</th>
<th>Function</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linc-MAF-4</td>
<td>chromosome 16q23.2</td>
<td>PBMC</td>
<td>chromatin modifiers LSD1 and EZH2</td>
<td>Differentiation of Th1 cells</td>
<td>[26-27]</td>
<td></td>
</tr>
<tr>
<td>HOTAIR</td>
<td>chromosome 12q13</td>
<td>PBMC</td>
<td></td>
<td>Regulation of inflammation and vitamin D</td>
<td>[32]</td>
<td></td>
</tr>
<tr>
<td>Lnc-DDIT4</td>
<td>chromosome 10q22.1</td>
<td>PBMC, CD4+ T cells</td>
<td>mTOR pathway</td>
<td>Differentiation of Th17 cells</td>
<td>[36]</td>
<td></td>
</tr>
<tr>
<td>ANRIL</td>
<td>chromosome 9p21</td>
<td>PBMC</td>
<td></td>
<td>Not determined</td>
<td>[41]</td>
<td></td>
</tr>
<tr>
<td>Lnc-IL-7R</td>
<td>chromosome 5p13</td>
<td>PBMC</td>
<td>IL-7Ra</td>
<td>Regulation the human T cells homeostasis</td>
<td>[44-46]</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2 LncRNAs in MS and EAE**

<table>
<thead>
<tr>
<th>LncRNA in EAE</th>
<th>LncRNA</th>
<th>Genome location</th>
<th>Sample</th>
<th>Known target(s)</th>
<th>Function</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tmepg1</td>
<td>chromosome 12q15</td>
<td>CD4+ T and CD8+ T splenocytes</td>
<td>T-bet</td>
<td>Regulation IFN-γ expression</td>
<td>[52-53]</td>
<td></td>
</tr>
<tr>
<td>LncRNA Dleu2</td>
<td>chromosome 13q14.2</td>
<td>CD4+ T cells</td>
<td>Foxo1</td>
<td>Regulation the Foxp3 expression</td>
<td>[54-55]</td>
<td></td>
</tr>
<tr>
<td>Hotair</td>
<td>chromosome 12q13</td>
<td>microglia</td>
<td>AKT2-NF-κB signal pathway</td>
<td>Regulation the remyelination</td>
<td>[57-58]</td>
<td></td>
</tr>
<tr>
<td>1700046D17Rik</td>
<td>chromosom3; 3 F2.1</td>
<td>CD4+ T splenocytes</td>
<td>RORγt</td>
<td>Differentiation of Th17 cells</td>
<td>[61]</td>
<td></td>
</tr>
<tr>
<td>GAS5</td>
<td>chromosome 1q25.1</td>
<td>microglial</td>
<td>TRF4</td>
<td>Suppression microglial M2 polarization</td>
<td>[63]</td>
<td></td>
</tr>
</tbody>
</table>
there was no significant difference in allele and genotype frequencies between MS patients and healthy subjects in any of inheritance models. Haplotype analysis demonstrated protective effect of CGCG and TAAA haplotypes against MS, and it also found the significant association between TAGG and CGCA haplotypes and MS risk in the studied population. These results provide the new evidences for participation of ANRIL in MS pathogenesis, but the further functional studies are needed to do in future.

**Lnc-IL-7R**

Lnc-IL-7Ra locates on 5p13 human chromosome and overlaps with the sense strand of Interleukin 7 receptor (IL-7R) gene at the 3'UTR \[42\]. IL-7R gene codes Interleukin 7 receptor alpha (IL-7Ra) which has two isoforms resulted from rs6897932 SNP of IL-7R gene (membrane-bound (IL-7RB) and soluble (IL-7RS) isoforms of IL-7R gene, respectively) \[43\]. Because IL-7Ra is essential for the development, maturation, and homeostasis of human T cells, IL-7R gene is considered as one of candidate genes for susceptibility to MS \[44-45\]. Recently, the expression level and role of lnc-IL-7R in regulating the expression of two variants of IL-7Ra in MS patients was evaluated by Bina et al \[46\]. Their results revealed that there were no significant differences between the expression levels of IL-7RB, IL-7RS and Lnc-IL-7Ra genes in MS patients compared with controls, but some significant correlations were observed. There were a significant correlation between IL-7Ra and Lnc-IL-7Ra, IL-7RB and Lnc-IL-7R, IL-7RS and Lnc-IL-7R as well as IL-7RB and IL-7RS in MS and control groups. And the correlation between IL-7RB and disease duration in MS patients as well as IL-7RS and disease duration in male MS patients also was observed. These data may reveal new evidence regarding the controlling role of Lnc-IL-7Ra in the expression level of genes and their roles in MS, but it is necessary to replicate in a larger sample size of more reliable data.

In addition, three LncRNAs including THRIL, FAS-AS1, and PVT1 were shown to be dysregulated in PBMCs of 50 Iranian relapsing–remitting multiple sclerosis (RRMS) patients compared with healthy person \[47\]. Real-time PCR results indicated that PVT1 and FAS-AS1 were significantly down-regulated while THRIL significantly up-regulated in RRMS patients. Three other LncRNAs, NEAT1, PANDA and TUG1, were found to be abnormal in PBMCs from 50 Iranian RRMS patients as well \[48\]. All three LncRNAs were significantly overexpressed in MS patients compared with healthy subjects. Moreover, significant correlations were found between these six LncRNA expression levels and clinical data. These data implied that they may participate in an interaction network with important roles in the pathogenesis of RRMS in Iranian population, but the exact mechanisms need to be further studied. Furthermore, a series of inflammation-related LncRNAs were shown a generalized dysregulation in PBMC from the Italian MS patients \[49\], including MALAT1, MEG9, NRON, ANRIL, TUG1, XIST, SOX2OT, GOMAFU, HULC and BACE-1AS. Further, a validation analysis in an independent cohort of Belgian origin was performed. And the data showed that NRON and TUG1 were significantly down-regulated in MS patients compared with controls.

**LncRNA in the animal models of MS**

EAE is a useful animal model of MS that shares many similarity features with the MS patients. And indeed a significant number of the major discoveries on the pathogenesis of MS were made in the EAE model \[50-51\]. One suggests that the over-activated immune cells like Th17 and γδT cells play key pathogenic roles in CNS inflammation in EAE, so did in MS. Thus learning the roles of LncRNAs in the pathogenesis of EAE would help to understand the pathogenesis of MS. Here, we summarized some pathogenic LncRNAs in EAE and the potential functions and mechanisms of these LncRNAs are revealed, some was shown in table 2.

**Tmevpg1**

Tmevpg1 is adjacent to Ifng gene in mouse genomes and encodes a non-coding RNA, which is transcribed in response to the program of Th1 cell differentiation and cooperates with T-bet to promote Ifng expression \[52\]. In 2003, S. Vigneau et al reported that Tmevpg1 is expressed in spleens, thymuses and CNS-infiltrating immune cells of B10.S mice after inoculation with Thélier’s virus \[53\]. After the stimulation of murine CD4 and CD8 splenocytes, the expression level of Tmevpg1 is down-regulated, whereas Ifng is up regulated. According to the results, they proposed that the silence of Ifng gene might be regulated by a noncoding RNA encoded by the Tmevpg1 gene, and this noncoding RNA might play a role in susceptibility to persistent infection by Thélier’s virus. As previously stated, Thélier’s virus infection is often used in establishing an experimental murine model for MS. Therefore, Tmevpg1 is considered to be one of LncRNA involved in MS \[53\].

**LncRNA Dileu2**

The differential expression profiles of LncRNAs and mRNAs in CD4+ T cells from EAE mice were also investigated by Li et al \[54\]. There are 1112 differentially expressed LncRNAs and 519 mRNAs are observed in MOG35-55 specific CD4+ T cells from EAE mice. After bioinformatics analysis, they turned attention to LncRNA-lncRNA Dileu2, which might bind with Foxo1 and produce pre-miR-15a. Detailed analysis revealed that LncRNA Dileu2 is able to negatively regulate Foxo1 expression and reduces the expression level of Foxo3 through producing mature miR-15a. This bioinformatics analysis illuminates that significantly dysregulated LncRNAs might take part in the development of EAE and could be a promising biomarker of EAE. In addition, Liu et al reported the expression profiles of LncRNAs both in the brain of EAE mice and in IL-9-induce astrocytes \[55\]. A total of 3300 differentially expressed LncRNAs are found in the brain tissues of EAE mice and 3748 LncRNAs in IL-9-induce astrocytes. Among them, 5 co-regulated LncRNAs are identified both in the brain tissues and in activated astrocytes, including Gm14005, Gm12478, mouselincRNA1117, AK080435, and mouselincRNA0681. Furthermore, the biological func-
tions of differentially expressed mRNAs are relevant to metabolism, development and inflammation. Nevertheless, the underlying mechanism and biological functions of these IncRNAs involved in MS still require to be further clarified.

**HOTAIR**

IncRNAs have been shown to involve in the pharmacogenetics of a number of therapeutics. Sulfasalazine (SF) is an anti-inflammatory and immune-modulating agent, which is generally used in the treatment of inflammatory bowel disease (IBD) [68-71]. SF exerts pro-remyelinating property in EAE mice [68]. Recently, Duan et al. provided evidence that SF could promote repair of cuprizone-induced demyelination through regulating microglia reprogramming in mice [69]. Furthermore, mechanistic study suggested that SF mediates remyelination in microglia through inhibiting AKT2-NF-xB signal pathway by ceRNA regulation of HOTAIR and miR-136-5p. Concretely, in cuprizone-treated microglia, SF could down-regulate HOTAIR while up-regulates miR-136-5p, and then inactivates AKT2-NF-xB signal pathway. Oppositely, HOTAIR overexpression results in an increase of miR-136-5p and activation of AKT2-NF-xB in the cuprizone-treated microglia. Their findings point out the therapeutic potential of SF for MS and the important role of IncRNAs in the treatment of SF.

**1700040D17Rik**

IL23R-CHR, a truncated IL23R extracellular domain, is able to block the cellular signal between IL-23 with endogenous IL23R and Th17 differentiation in vitro [61]. In addition, in vivo study in EAE mice demonstrated that IL23R-CHR exhibits effectiveness for the treatment of EAE through suppressing the inflammation of CNS and the production of pro-inflammatory cytokine [67]. To understand the pharmacological action of IL23R-CHR, IncRNA-1700040D17Rik was identified by profiling IncRNAs between EAE and normal mice [60]. LNcRNA-1700040D17Rik, a mouse intergenic IncRNA nearby RORγ gene, was down-regulated in EAE and significantly increased after the treatment of IL23R-CHR. When overexpression of 1700040D17Rik, the expression level of RORγ was significantly reduced and the production of Th17 cells was decreased in vitro. These findings suggested that the biological function of 1700040D17Rik might be associated with the differentiation of Th17 cells by regulating the expression of RORγ. Taking together, 1700040D17Rik has the potential role in the pathogenesis of EAE and the effects of IL23R-CHR could be due to balancing the immune responses.

**GAS5**

Growth-arrest-specific transcript 5 (GAS5) is a functional IncRNA that has been shown to abnormally express in many types of tumors and to inhibit T-cell proliferation [65]. In a recent study by Sun et al., GAS5 was identified as an epigenetic regulator of microglial polarization by using microarray screening [64]. Functional studies revealed that GAS5 could suppress microglial M2 polarization in vitro. Interference with GAS5 in transplanted microglia attenuates the progression of EAE and promotes remyelination in a lyssolecithin-induced demyelination model. Moreover, GAS5 was found to be highly expressed in amoeboid-shaped microglia in MS patients. Further studies demonstrated that GAS5 inhibited M2 polarization through recruiting the polycomb repressive complex 2 (PRC2) to suppress transcription of TREF4 (a key factor controlling M2 macrophage polarization). These results indicated a role for GAS5 in microglial polarization and the pathogenesis of MS, and suggested that GAS5 may be a promising target for the treatment of neurological disorders associated with microglia polarization.

**The Clinical Relevance of IncRNAs**

Identifying the roles of IncRNAs in MS may be the key to clarify the mechanisms that contribute to the development of this disease. And it has been recognized that IncRNAs may serve as therapeutic targets and diagnostic markers in many diseases. Thus, IncRNAs may have significant clinical relevance for MS treatment.

**IncRNAs served as diagnostic markers**

As diagnostic biomarkers, IncRNAs are particularly advantageous. First, it has been clear that the expression patterns of IncRNAs are highly cell-type-, tissue- and developmental stage-specific [23]. These expression signatures make IncRNAs be good candidates for accurate disease diagnostics and classification. Second, it is easily to identify an IncRNA in various biological fluids, such as urine, saliva, plasma and blood [65]. Hence, IncRNAs are ideal candidates for efficient diagnostic assays of diseases, especially those diseases where disease site is largely inaccessible. Third, mRNAs play their roles by being translated into functional proteins, while IncRNAs mainly rely on themselves. Thus, the expression level of IncRNAs perhaps could become a valuable diagnostic biomarker of diseases. To date, individual IncRNA has been employed in the clinical practice and utilized as a biomarker for diagnosis. The first IncRNA, PCA3, was approved by FDA for the usage as a biomarker of prostate cancer in 2012 [66]. PCA3 is a specific-lncRNA significantly overexpressed in prostate cancer, which can be detected in urine of prostate cancer patients by noninvasive means [60]. Although the validation and application of the unique biomarkers in MS are still under development, there were a large number of IncRNAs being forward to propose as biomarkers for MS diagnosis. For example, it has been found the ANRIL genetic variants have a significant association with MS risk, thus it may be a useful biomarkers for MS diagnosis.

**IncRNAs served as therapeutic targets**

As therapeutic targets, IncRNAs also have certain advantages compared with current protein-based targets [68]. One of the main advantages is that nuclear IncRNAs are gene specific regulators, which could be used as targets to develop drugs that specifically target the chromatin at a particular locus. Moreover, IncRNAs can be utilized to modulate diseases that are not applicable to protein targets. For some diseases in which repressed or silenced genes are pathologically
involved, up-regulation and/or activation of the expression of proteins would be beneficial for the treatments. Given that most small molecule drugs are not effective to induce protein up-regulation and/or activation, most of these proteins are presently undruggable targets. However, when targeting regulatory lncRNAs to regulate the expression of endogenous genes is concerned, the therapeutic benefits can be easily achieved through oligonucleotide-based drugs. Furthermore, through targeting the regulatory lncRNAs, it is possible to remarkably improve the specificity that microRNAs and small molecules are usually less favorable. Additionally, the expression abundance of lncRNAs are generally low and they can exert the functions with a small number of copies. Consequently, lncRNA-targeted drugs could be administered at a lower dose than conventional oligonucleotide drugs.

Basically, there are following possible approaches to realize lncRNA-targeted therapy [69-72].

(i). Silence of lncRNAs. Similar to mRNAs, lncRNAs can be down-regulated at RNA level by targeting their specific sequences. Several technologies are available to apply in this strategy. Short interfering RNAs (siRNAs) can bind specific lncRNAs overexpressed based on complementarity and lead to argonauta-mediated degradation of lncRNAs. Indeed, the down-regulation of GAS5 by siRNAs could relieve the progression of EAE and promote remyelination in a lysol-ecithin-induced demyelination model, suggesting that GAS5 may be a promising target for the treatment of MS [64]. It was also found that silence TUG1 by shRNA attenuates EAE through inhibition of inflammation by sponging miR-9-5p via targeting NF-xB1/p50, suggesting that TUG1 is a potential therapeutic target for MS treatment [70]. An additional therapeutic strategy that target lncRNAs is to use antisense oligonucleotides (ASOs). ASOs are single stranded oligonucleotides that range from 8 to 50 nt in length, and can offer specific complementarity and RNase H-mediated degradation of the target sequence [74-75]. In comparison to siRNAs, ASOs are lesser toxic and can enter more easily into the nucleus due to their lower proinflammatory response and smaller size [71]. A potential therapeutic intervention for Angelman syndrome (AS) by reducing Ube3a-ATS, a nuclear-localized lncRNA related to AS, with ASOs was reported by Meng et al. In their study, ASO treatment achieved specific reduction of Ube3a-ATS and sustained unsilencing of paternal Ube3a in neurons in vitro and in vivo, and partial restoration of UBE3A protein in an AS mouse model, which ameliorates several cognitive deficits associated with the disease [76]. Another technology relied on RNase H-dependent knockdown can also be used to down-regulate lncRNAs by targeting the unique sequences, namely Locked Nucleic Acid (LNA) GapmeRs. These single stranded oligonucleotides contain a DNA stretch flanked by LNA nucleotides, and can bind target lncRNAs based on complementarity, resulting in RNase H-mediated degradation of lncRNAs. Since LncRNA BCAR4 plays a role in breast cancer metastasis by interacting with transcription factors SNIP1 and PNOTs, the therapeutic delivery of LNAs targeting BCAR4 could strongly suppress the metastasis of breast cancer in mouse models [77]. Furthermore, the degradation of lncRNAs can be achieved by using ribozymes that targets RNA and cleaves in a site-specific manner with protein-independent mechanism. Hammerhead ribozymes, characterized by a central loop flanked by two “arms”, are one of widely used ribozymes, which uses both ~20 nt long arms to bind their targets to cleave RNA at specific sites. Recently, Watson ZL et al delivered a hammerhead ribozyme latency-associated transcript (LAT) to HSV-1-infected neurons of latently infected rabbits with an adeno-associated virus (AAV) vector, showing that this treatment could reduce the reactivating ability of the virus to the majority of rabbits [78].

(ii). Transcriptional block of lncRNAs. Regulation of the expression of lncRNAs at genomic level through a site-specific integration of RNA destabilizing elements (RDEs) into the genomic locus of lncRNAs is a useful way to interfere the functions of lncRNAs. This method relies on the Zinc finger nucleases (ZFNs)-mediated integration of RDEs into the genome to silence gene expression. The potential RDEs that could be used for this gene-targeting approach include AU-rich elements, miRNA-binding sites, poly (A) signals, RNase P substrates, self-cleaving ribozymes and others. These RDEs possess different silencing mechanisms: AU-rich elements and miRNA-binding sites influence the stability of the whole transcript; poly (A) signals functioning as termination elements and destabilizing downstream sequences only silence downstream sequences; RNase P substrates and self-cleaving ribozymes can destabilize both upstream and/or downstream sequences, depending on the position and sequences used. Among these approaches, a successful example is to reduce the expression of lncRNA MALAT1 [79-80]. Beside it has been proved that lncRNA MALAT1 played an important role in pathogenesis of MS, based on anomalous splicing/backsplicing profiles of MS-relevant genes [81].

(iii). Functional disruption of lncRNAs. This approach is suitable for those lncRNAs that may not be possible to be silenced by RNAi technology due to their secondary structures. On the one hand, small molecule inhibitors can be used to change or mimic the secondary structures of lncRNAs to compete for the binding sites, and oligonucleotides can also be used to block correct folding of lncRNAs, leading to the structural disruption and functional impairment of lncRNAs. On the other hand, small molecules can also be used to block the binding of lncRNAs with proteins, DNAs, RNAs or other interacting complexes, leading to the functional damage of lncRNAs. Therefore, nucleotidic and protein aptamers may be a good example for this therapeutic strategy. Given that aptamers are a group of recognition units that can specifically bind to target molecules, aptamers can bind their targets by forming tertiary structures, whose binding in principle resembles that of antibodies, resulting in the inhibition of the functions of proteins or RNAs. Because aptamers can utilize both primary structure and secondary or tertiary structures of
target RNAs, they may provide a greater specificity than siRNAs, ASOs, LNA or ribozymes.\(^{[8]}\)

(iv). Utilization of highly expressed IncRNAs. In some cases, highly expressed IncRNAs in tumor cells can be used to avoid unintended toxicity to normal cells during antitumor treatment. Based on this idea, A. Mizrahi\(^{[1]}\) et al reported a potential therapy for human ovarian cancer ascites fluid (OCAF).\(^{[2]}\) The expression level of H19 is high in the OCAF according to their previous study on the expression profile of H19 in epithelial ovarian cancer. They constructed a therapeutic toxin vector carrying the "A" fragment of diphtheria toxin (DT-A) gene under the control of H19 regulatory sequences. In the presence of H19, H19 regulatory sequences were able to drive DTA expression to kill cells. As a result, this toxin vector could selectively kill tumor cell lines and inhibit tumor growth in vitro and in vivo.

(v). Specific delivery of IncRNAs. Compared with protein coding genes, the functions of IncRNAs could be mediated faster to reduce side effects after specific delivery of IncRNAs. Thus, IncRNAs can be directly delivered to function. For example, beneficial IncRNAs, such as tumor suppressor IncRNAs, can be delivered into high risk tissues to prevent the tumorigenesis.

Up to now, IncRNA-targeted therapeutics are still in the stage of pre-clinical phase, and there remains many challenges for further development.\(^{[3-4]}\) Similar to other gene therapies, off-target toxicity is the most serious obstacle. Due to the fact that IncRNA-targeted drugs have the potential to hybridize both on- or off-target, leading to unwanted and unanticipated responses, the off-target toxicity is difficult to control. Furthermore, achieving safe and efficient in vivo delivery is another problematic issue. The efficiency of delivery in some inaccessible sites, such as brain and heart, is especially difficult, which may result in poor treatment effects and even off-target toxicity. Moreover, the carriers of IncRNA-targeted drugs may induce immune activation, causing various unexpected adverse effects. Although there are some initial developments in the therapeutic application of IncRNAs, the field is still in its infancy, whose development needs to be promoted by further understanding of the involvement of IncRNAs in cell biology and the mechanisms of diseases.

**Conclusions**

With the advancements in RNA sequencing technologies, the speed on the discovery of various IncRNAs has become very rapid, and the number of novel IncRNAs are increasing on a daily basis. However, the corresponding functional analysis are still tedious and challenging. Similar situation is also true to those IncRNAs potentially involved in the pathogenesis of MS. Although increasing evidences have revealed that IncRNAs were important players in MS pathogenesis, their specific functions, in terms of how to regulate protein function, gene expression and the interaction with other RNAs are still poorly understood, which require extensive investigations. Nevertheless, it has been clear that IncRNAs could be served as valuable biomarkers or therapeutic targets. Given current limitations on further development of IncRNAs, such as various safety issues and precise and effective delivery, collective efforts should be focused on overcoming these obstacles. Consequently, making the full use of our understanding on the structures and functions of IncRNAs will help us develop IncRNAs associated drugs, which was looking forward to making up the demand of the clinical diagnosis and therapeutics of devastating diseases including MS.

**References**

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