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Expression profile of long noncoding RNAs and mRNAs in peripheral blood mononuclear cells from myasthenia gravis patients☆

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ABSTRACT

For the epigenetic characterization of myasthenia gravis (MG), we determined whether long noncoding RNAs (lncRNAs) and messenger RNAs (mRNAs) are expressed differentially in subjects with and without MG. Compared with healthy control subjects, the MG patients had 1561 upregulated lncRNAs, 1034 downregulated lncRNAs, 921 upregulated mRNAs, and 806 downregulated mRNAs (fold change > 2.0). Several GO terms including nucleic acid transcription factor activity, inflammatory response, regulation of leukocyte activation, lymphocyte proliferation and regulation of B cell proliferation were enriched in gene lists, suggesting a potential correlation with MG. Pathway analysis then demonstrated that cytokine-cytokine receptor interaction, intestinal immune network for IgA production, NOD-like receptor signaling pathway, NF-kappaB signaling pathway, cell adhesion molecules and TNF signaling pathway play important roles in MG. Co-expression network analysis indicated that 33 lncRNAs were predicted to have 31 cis-regulated target genes, and 65 lncRNAs appeared to regulate the patients' 45 trans target genes among differentially expressed lncRNAs. Our present study identified a subset of dysregulated lncRNAs and mRNAs in patients with MG, which may impact this disease process.

1. Introduction

Myasthenia gravis (MG) is a T and B cell-mediated autoimmune disease of the neuromuscular junctions (Lisak and Ragheb, 2012; Matsui et al., 2010; Thiruppathi et al., 2012). Muscle weakness and fatigue, as hallmarks of MG, resulting in the elicitation of an antibody-mediated autoimmune response against acetylcholine receptors (AChR) located at neuromuscular junctions. However, the epigenetic characteristics of this disease are not completely understood.

LncRNAs, which are >200 nucleotides in length, represent a new class of noncoding RNAs (Necsulea et al., 2014). They contribute to a variety of biological cascades and are reported to be involved in neurodegenerative diseases (Wan et al., 2016), diabetic mellitus (Jae and Dimmeler, 2015; Wessel et al., 2015), autoimmune disease (Duarte, 2015; Xu et al., 2016a; Xu et al., 2016b; Zhang et al., 2016), cancer (Leucci et al., 2016; Schmitt and Chang, 2013), and cardiovascular diseases (Viereck et al., 2016). Noncoding RNAs are emerging as a new regulatory layer that affects both development of the immune system and its function (Huang et al., 2015; Ranzani and Rossetti, 2015; Wang et al., 2014). Although thousands of lncRNAs have been identified in the mammalian genome by bioinformatics analyses of transcriptomic data, their functional characterization is still largely incomplete. Recent studies show widespread changes in the expression of lncRNAs during activation of the innate immune response, particularly noted in T cell development, differentiation, and activation. These lncRNAs control important aspects of immunity such as production of inflammatory mediators, altering differentiation, and management of cell migration by regulating protein–protein interactions or by base pairing with RNA and DNA (Cui et al., 2014; Heward and Lindsay, 2014; Roux and Lindsay, 2015). Although several lncRNAs have been implicated in diverse processes and diseases, few examples of their ability to regulate autoimmune diseases have been described.

In the present study, we performed an array of lncRNA and mRNA chip assays on peripheral blood mononuclear cells (PBMCs) from MG patients. Outstanding lncRNA functions were annotated based on co-expression genes and a gene ontology (GO) biological analysis process. The relationships among lncRNAs and mRNAs were revealed through cis and trans analyses. These results provide information for further studies of MG.

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Table 1
Baseline characteristics.

	Control ($n = 26$)	MG ($n = 48$)	*P* value
Gender, M/F	8/18	16/32	1.00
Age, years, median (range)	37 (24–53)	42 (27–62)	0.81
Age at onset, years, median (range)	–	37 (21–59)	–
Disease duration, median (range)	–	10.5 (0.8–17.5)	–
Anti-AchR + (%)	–	48/48 (100)	–
Anti-Musk + (%)	–	0 (0)	–
Thymoma (%)	–	0 (0)	–

MG, myasthenia gravis.

2. Methods

2.1. Study population and trial design

During an open enrollment, a total of 48 patients with type IV MG (Myasthenia Gravis Foundation of America, MGFA), who met the criteria of combined fluctuating muscle weakness with a positive neostigmine test, abnormal single-fiber EMG test, and positivity for AchR antibody, were recruited at Tianjin Medical University General Hospital from May 2013 to August 2015. These patients were within the peak timing of manifesting MG and before treatment with glucocorticoid or intravenous immune globulin. Exclusion criteria were the following: (Thiruppathi et al., 2012) co-presence of MG and other diseases of the immune system, (Matsui et al., 2010) presence of tumor(s) and systemic hematologic diseases, (Lisak and Ragheb, 2012) presence of recent infection, and (Necsulea et al., 2014) concomitant use of antineoplastic or immune-modulating therapies prior to blood sampling. We also recruited 20 age- and gender-matched healthy controls for the comparative study. The demographic and clinical features of all the patients and healthy controls are summarized in Table 1. The Tianjin Medical University General Hospital institutional review boards approved the trial protocol and supporting documentation. Informed consent was obtained at enrollment from all patients or legally acceptable surrogates.

2.2. Isolation of mononuclear cells from human peripheral blood (PBMCs)

Peripheral blood anticoagulated by ethylene diamine tetraacetic acid (EDTA) was obtained from all MG patients and healthy controls. Human PBMCs were isolated with Ficoll-Hypaque gradients.

2.3. RNA extraction and chip arrays

Total RNA was extracted from PBMCs using Trizol® reagent (Invitrogen, Grand Island, NY, USA). Approximately 200 ng of total RNA from each sample was used for the lncRNA microarray analyses. LncRNA expression was analyzed using OE_Biotech Human lncRNA chip software, containing 41,000 lncRNAs and 34,000 mRNAs. Those lncRNA and mRNA target sequences were merged from multiple databases: 23,898 from GENCODE/ENSEMBL; 14,353 from the Human LincRNA Catalog; 7760 from RefSeq; 5627 from USCS; 13,701 from ncRNA Expression Database (NRED); 21,488 from LNCipedia; 1038 from H-InvDB; 3019 from LncRNAs-a (Enhancer-like); 1053 from the Antisense ncRNA pipeline; 407 Hox ncRNAs; 962 UCRs; and 848 from the Chen Ruisheng lab (Institute of Biophysics, Chinese Academy of Sciences, Beijing, China). 4974 Agilent control probes. The lncRNA chip experiments were conducted at Capitalbio Corporation in Beijing, China. LncRNAs and mRNAs were analyzed using Cluster 3.0 software. The results were further analyzed using Tree View software. Green indicates low expression, and red indicates high expression in the output for these analyses.

2.4. Quantitative real-time PCR validation

Total RNA was extracted from PBMCs with Trizol® reagent (Invitrogen) following the manufacturer's instructions. RNA quantity and quality were assessed using a Nanodrop ND-100 Spectrophotometer (Nanodrop Technologies, Wilmington, USA) and a 2100 Bioanalyzer (Agilent RNA 6000 Nano Kit, Waldbronn, Germany), with a 260:280 ratio of ≥1.5 and an RNA integrity number of ≥7 for the majority of the samples. For the reverse transcriptase (RT) reaction, SYBR Green RT reagents (Bio-Rad, Indianapolis, USA) were used. The lncRNA PCR results were quantified using the $2^{\Delta\Delta ct}$ method against β-actin for normalization. The data represent the means of three experiments.

2.5. LncRNA co-expression analysis and gene function annotation

Volcano plot filtering was used to identify lncRNAs and mRNAs with statistically significant differences in expression. Hierarchical clustering was applied to present the diacritical lncRNA and mRNA expression patterns among the samples. LncRNA classification was carried out to explore the potential function of the differentially expressed lncRNAs. GO analysis and pathway analyses were also performed to describe more fully the roles of the differentially expressed mRNAs. Furthermore,

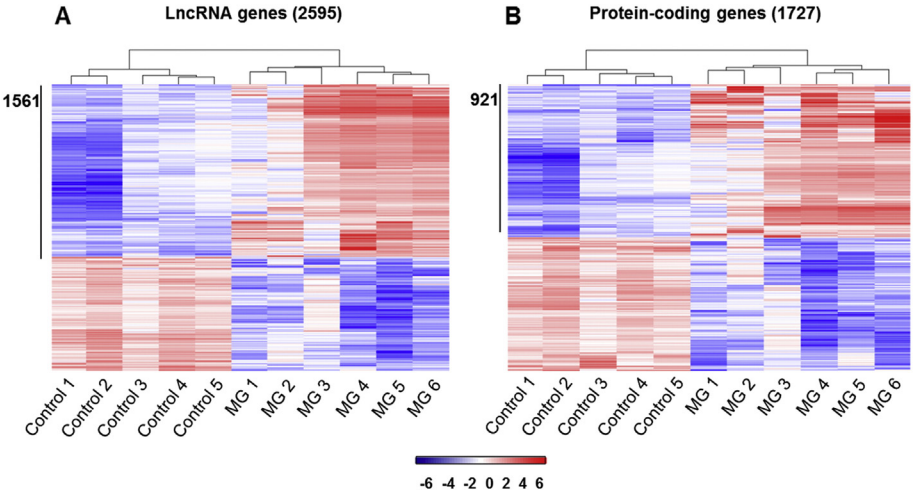

Fig. 1. LncRNA and mRNA profiles of microarray data. Hierarchical clustering shows a distinguishable (A) lncRNA and (B) mRNA expression profile between the two groups. Plots here represent analysis of RNA extracted from PBMCs obtained from 6 MG patients and 5 healthy control subjects.