ORIGINAL ARTICLE

Expression analysis of mTOR-associated lncRNAs in multiple sclerosis

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Abstract

mTOR has been shown to be involved in the regulation of immune responses and differentiation of immune cells. This protein is a candidate molecule for unraveling the molecular mechanisms of autoimmune disorders such as multiple sclerosis (MS). We designed the current study to assess expression of *MTOR*, and four associated long non-coding RNAs (lncRNAs), namely *SNHG1*, *SNHG3*, *SHNG5* and *DANCR* in the peripheral blood of patients with MS compared with healthy controls. Analysis of real-time PCR results has shown down-regulation of *SNHG5* and *DANCR* in MS patients compared with controls. Sex of study participants had no significant effect on expression of either genes and the interaction of sex and disease on expression levels of all studied genes were insignificant. There was a significant negative correlation between expression levels of *MTOR* gene and disease duration. No other significant correlations were detected between genes expressions and clinical/demographic data. *SNHG5* and *DANCR* transcript levels had AUC values of 0.88 and 0.68 in separation of patients with MS from healthy controls, respectively. Taken together, our study suggests participation of two mTOR-related lncRNAs, i.e. *SNHG5* and *DANCR* in the pathophysiology of MS.

Keywords mTOR · SNHG1 · SNHG3 · SHNG5 · DANCR · lncRNA · Multiple sclerosis

Introduction

The PI3K/Akt/mTOR pathway has been shown to be involved in the regulation of T cell responses. TCR stimulation can activate mTORC1 and the degree of mTOR activation is strictly correlated with the extent of interaction between T cells and dendritic cells. Abnormal activity of PI3K/Akt/mTOR pathway has been found to increase risk of autoimmune conditions (Giacoppo et al. 2017; Liu et al. 2015). As a kinase, mTOR can regulate translation through phosphorylation of constituents of the protein synthesis system, such as p70 S6K and 4EBP-1, permitting involvement of eIF-4E in the construction of a complex that participates in the translational initiation (Hermida et al. 2017; Jhanwar-Uniyal et al. 2017). mTOR is also the fundamental constituent of mTORC1 and mTORC2 complexes and it is at the intersection of the PI3K/Akt pathway. mTOR signaling has the ability to induce immunoregulatory features in T cells. In

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Table 1 General data of enlisted cases

Parameters	Groups	Values
Sex (number)	Male	12
	Female	38
Age (Years, mean ± SD (range))	Male	37.5 ± 10.8
	Female	40.13 ± 9.52
Duration (Years, mean ± SD (range))	Male	4.5 ± 3.03
	Female	7.26 ± 6.18
Age of onset (Years, mean ± SD (range))	Male	32.83 ± 11.23
	Female	32.86 ± 9.32
EDSS	Male	3.41 ± 1.57
	Female	2.85 ± 1.26

fact, inhibition of mTOR has been demonstrated to increase expression of FoxP3 leading to expansion of Tregs populations (Chapman and Chi 2014; Sun et al. 2018).

Taken together, mTOR is a candidate molecule for unraveling the molecular mechanisms of autoimmune disorders such as multiple sclerosis (MS). The results of investigations in animal models of MS have also suggested targeting PI3K/mTOR pathway as an effective immunomodulatory strategy for prevention of relapses and attenuation of the disability course (Mammana et al. 2018).

mTOR has been shown to have interactions with a number of long non-coding RNAs, particularly *small nucleolar RNA host genes* (*SNHG*s) (Taherian-Esfahani et al. 2019). Moreover, the lncRNA *differentiation antagonizing non-protein coding RNA* (*DANCR*) has been reported to regulate expression of mTOR via binding to miR-496 (Lu et al. 2018).

In order to find the relevance of mTOR and its associated lncRNAs with the pathogenesis of MS, we evaluated expression of *MTOR*, *SNHG1*, *SNHG3*, *SHNG5* and *DANCR* in the peripheral blood of patients with MS compared with healthy controls.

Materials and methods

Patients and controls

The current project was conducted on blood specimen of 50 relapsing–remitting MS patients and 50 healthy controls. Persons enlisted in the control group had no history or sign of systemic disorders, particularly immune-related disorders. MS patients were assessed using the revised McDonald criteria (Polman et al. 2011). Patients were under treatment with IFN-β (CinnoVex, Cinagene Company, Iran). Informed consent forms were signed by all cases and controls. The study protocol was approved by the ethical committee of Shahid Beheshti University of Medical Sciences(IR.SBMU.MSP.REC.1399.163).

Expression studies

Three milliliters of venous blood were obtained from MS patients and controls in EDTA-containing tubes. These blood samples were subjected to RNA extraction using the Hybrid-R™ blood RNA extraction kit (GeneAll Biotechnology Co Ltd., South Koera). After confirmation of proper quality and concentration of RNA, cDNA was produced using High-Capacity cDNA Reverse Transcription kit (Applied Biosystems). Expressions of *MTOR*, *SNHG1*, *SNHG3*, *SHNG5* and *DANCR* were enumerated in the blood samples using SYBR® Premix Ex Taq™ (TaKaRa, Japan). PCR conditions and nucleotides sequences of primers were similar to our previous study (Taherian-Esfahani et al. 2019).

Statistical analysis

Analyses were performed using GraphPad Prism version 9.0 (GraphPad Software, La Jolla, CA, USA). Expressions of *MTOR* gene and four related lncRNA genes were calculated using the comparative –delta Ct method.

Table 2 General features of studied genes

Name/Gene ID	Accession number	Location	Description	Biological activity
SNHG1	NR_003098.2	11q12.3	small nucleolar RNA host gene 1	promoter of cell proliferation
SNHG3	NR_002909.2	1p35.3	small nucleolar RNA host gene 3	a novel lncRNA closely related to tumor development
SNHG5	NR_003038.2	6q14.3	small nucleolar RNA host gene 5	This RNA may regulate gene expression by acting as a sponge for microRNAs. This transcript may also stabilize mRNAs by blocking degradation by staufen double-stranded RNA binding protein 1
DANCR	NR_024031.2	4q12	differentiation antagonizing non-protein coding RNA	negative regulator of cell differentiation
MTOR	NM_001386500.1	1p36.22	mechanistic target of rapamycin kinase	mediate cellular responses to stresses such as DNA damage and nutrient deprivation

Fig. 1 Relative expression levels of *MTOR* gene and four related lncRNAs in Multiple Sclerosis (MS) patients (total) and healthy controls (total) as described by –delta Ct values (Ct Housekeeping gene- Ct Target gene). – delta Ct Data were plotted as box and whisker plots. Median [line], mean [cross], interquartile range [box], minimum and maximum values are shown. A non-parametric test (Mann–Whitney U test) was used to identify differentially expressed genes between two groups. (** P value < 0.001 and **** P value < 0.0001)
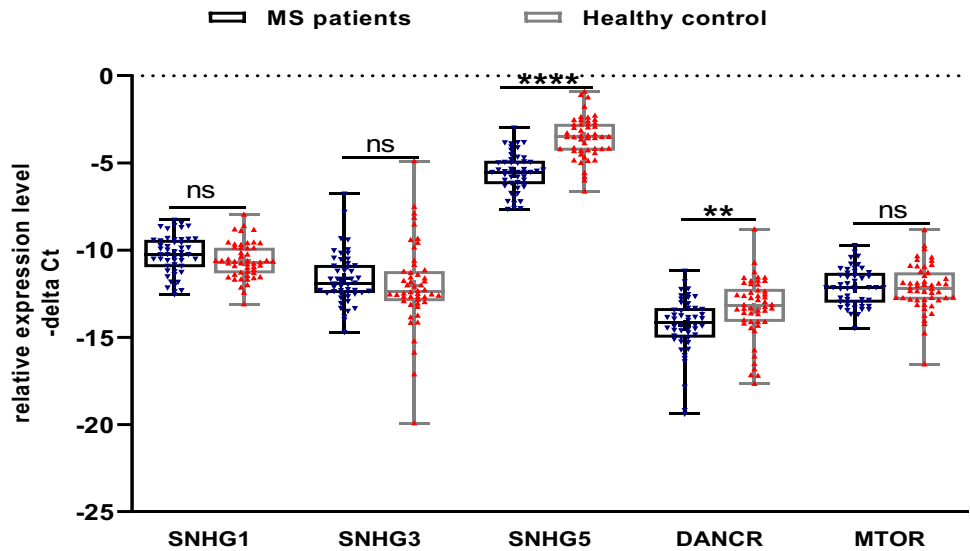
The normal/gaussian distribution of the values was accessed using the Shapiro–wilk test. A non-parametric test (Mann–Whitney U test) was used to identify differentially expressed genes between the patients and healthy controls. The two-way ANOVA and Tukey post hoc tests were used to analyze the effects of main factors (disease and gender) on expressions of genes. Since expression data was not normally distributed, correlations between gene expression levels were measured using Spearman's rank correlation coefficient. Spearman's rank correlation coefficient was also used for assessment of correlations between gene expression levels and clinical/demographic data such as age, disease duration, sex, age at onset and EDSS.

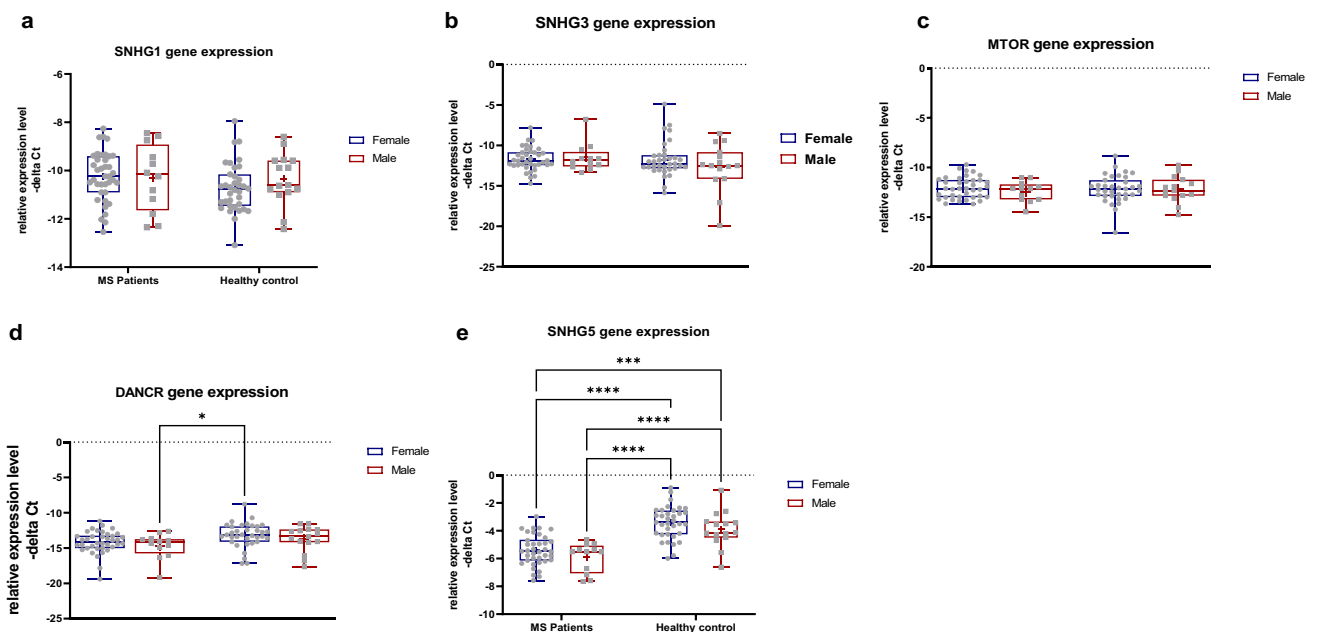The receiver operating characteristic (ROC) curves were depicted to appraise the diagnostic power of

Fig. 2 Relative expression levels of *MTOR* gene and four related lncRNAs in Multiple Sclerosis (MS) patients subgroups (male and female) versus control subgroups (male and female) as described by –delta Ct values (Ct Housekeeping gene- Ct Target gene). – delta Ct Data were plotted as box and whisker plots. The median [line], mean [cross], interquartile range [box], and minimum and maximum values are shown. The two-way ANOVA and Tukey post hoc tests were used to analyze the effects of main factors (disease and gender) on gene expression levels and their interaction in subgroups. (* P value < 0.05, *** P value < 0.001 and **** P value < 0.0001)

Table 3 Spearman's correlations between RNA expression levels among the MS patients ($N=50$) and healthy controls ($N=50$)

	SNHG1		SNHG3		SHNG5		DANCR		MTOR	
	Patients	Control	Patients	Control	Patients	Controls	Patients	Controls	Patients	Controls
SNHG1			0.46**	49**	0.43*	0.38*	0.5**	0.49**	0.39*	0.61**
SNHG3					0.3*	0.52**	0.44*	0.37*	0.57**	0.66**
SHNG5							0.39*	0.23	0.52**	0.57**
DANCR									0.46**	0.35*

* p-Value at a significance level of $p<0.05$.

** p-Value at a significance level of $p<0.001$.

expression levels of differentially expressed genes. Youden's J parameter was measured to find the optimum threshold. P value <0.05 was considered as significant.

Results

General data

Table 1 shows general data of MS patients. Enrolled controls were matched with cases in terms of sex ration and age.

Expression assays

Table 2 summarizes general features of studied genes.

Expression levels of SNHG5 and DANCR were significantly down-regulated in MS patients compared with controls (Fig. 1).

There was a significant effect of disease factor on expression levels of SNHG5 and DANCR lncRNAs. However, sex factor had no significant effect on expression of either genes and the interaction of sex and disease on expression levels of all studied genes were insignificant (Fig. 2).

There were significant correlations between expression levels of all gene pairs in both study subgroups (MS patients and controls), except for DANCR and SNHG5 genes whose expression levels were not correlated among controls (Table 3).

SNHG5 and DANCR transcript levels had AUC values of 0.88 and 0.68 in separation of patients with MS from healthy controls, respectively (Fig. 3).

Table 4 shows the details of statistical parameters related with ROC curves.

There was a significant negative correlation between expression levels of MTOR gene and disease duration. No other significant correlations were detected between genes expressions and clinical/demographic data (Table 5).

Discussion

In the present study, we have demonstrated down-regulation of two mTOR-associated lncRNAs, i.e. SNHG5 and DANCR in MS patients compared with controls. SNHG5 is an lncRNA which can activate several important signaling pathways, such as Wnt/β-catenin, p38/MAPK, mTOR and ROCK pathways (Han et al. 2020). Moreover, it has been shown to serve as a molecular sponge for several microRNAs, such as those being involved in the regulation of immune responses such as miR-205-5p, miR-154-5p and miR-26a-5p (Han et al. 2020). Notably, miR-205-5p has a possible role in the pathology of autoimmune disorders, since its silencing can alleviate the inflammatory responses in allergic rhinitis through influencing expression of B-cell lymphoma 6 (Zhang et al. 2021). Furthermore, miR-154-5p can mediate allergic inflammatory
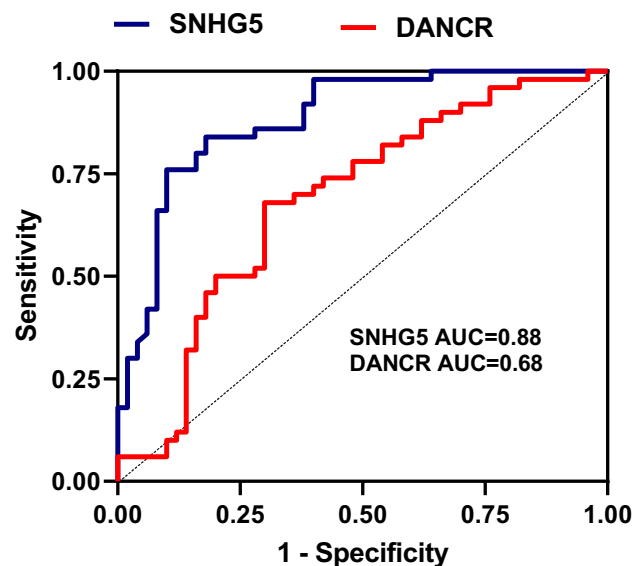

Fig. 3 ROC curves of SNHG5 and DANCR transcript levels in separation of patients with MS from healthy controls

Table 4 Details of statistical parameters of ROC curves

	SNHG5				DANCR			
	AUC ± SD	Sensitivity	Specificity	P Value	AUC ± SD	Sensitivity	Specificity	P Value
	0.88 ± 0.03	0.84	0.82	< 0.0001	0.68 ± 0.05	0.68	0.7	0.0013

responses through facilitating cellular interactions (Kim et al. 2021). miR-26a-5p as another target of *SNHG5* is involved in the TLR signaling through targeting CTGF (Li et al. 2020). Thus, *SNHG5* down-regulation can participate in the pathoetiology of MS via several routes including up-regulation of miRNAs that induce immune responses.

DANCR has been suggested to be an inflammation-modulating lncRNA which is involved in the surveillance of inflammatory cholinergic blockade (Meydan et al. 2020). Moreover, it can induce expressions of TNF-α and IL-6 in blood mononuclear cells (Tong et al. 2015). Thus, this lncRNA can also affect pathogenesis of MS through diverse routes.

Notably, expression profile of these lncRNAs has not been assessed in previous studies, thus no report has not been detected on abnormal expression of these lncRNAs. This is mostly due to the fact that the importance of lncRNAs in the pathophysiology of MS is just recently being elucidated.

Sex of study participants had no significant effect on expression of either genes and the interaction of sex and disease on expression levels of all studied genes were insignificant. This finding indicates the importance of *SNHG5* and *DANCR* in the pathogenesis of MS in both sexes. Further analyses revealed a significant negative correlation between expression levels of *MTOR* gene and disease duration. Since expression of this gene was nor different among MS patients and controls, we suggest assessment of its expression in larger cohorts of patients with different disease duration times to verify the mentioned correlation.

Table 5 The results of Pearson correlation between expression of *MTOR* and four related lncRNAs and, age, disease duration, sex, age at onset and EDSS

	Age	Sex	EDSS	Age at onset	Disease duration
SNHG1	-0.008	-0.008	-0.047	-0.087	0.162
SNHG3	-0.089	-0.008	0.144	-0.066	-0.108
SHNG5	-0.152	-0.151	-0.027	-0.066	-0.104
DANCR	-0.029	-0.112	0.176	-0.033	-0.121
MTOR	-0.140	-0.127	0.180	0.061	**-0.396**[**]
age		-0.136	0.057	**0.826**[**]	0.157
sex			0.151	-0.037	-0.112
EDSS				0.150	-0.221
Age at onset					**-0.356**[*]

SNHG5 and *DANCR* transcript levels had AUC values of 0.88 and 0.68 in separation of patients with MS from healthy controls, respectively. Thus, *SNHG5* can be suggested as an appropriate disease marker. Previous studies have reported different AUC values for lncRNAs in MS. For instance, Amiri et al. have reported AUC values of 0.861, 0.941 and 0.951 for *MIAT*, *H19* and *NRON*, respectively (Amiri et al. 2022). Moreover, another study has demonstrated that AUC values of HNF1A-AS1, LINC00305, and LNC-MKI67IP are 0.744, 0.926, and 0.703, respectively (Safa et al. 2021).

Taken together, our study suggests participation of two mTOR-related lncRNAs, i.e. *SNHG5* and *DANCR* in the pathophysiology of MS. These results should be confirmed in future functional assays. Moreover, our analysis was limited to peripheral blood samples. Since it remains unclear if the levels of circulating lncRNAs in peripheral blood coordinate with their levels in the central nervous system, further studies assessing the mentioned lncRNAs in CNS would be advantageous.

Authors' contributions SGF wrote the manuscript and revised it. MDO and MT supervised and designed the study. MG, MA, BMH and FE performed the experiment. SE analyzed the data. All authors read and approved the submitted version.

Data availability The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to Participant All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent forms were obtained from all study participants. The study protocol was approved by the ethical committee of Shahid Beheshti University of Medical Sciences. All methods were performed in accordance with the relevant guidelines and regulations.

Consent of publication Not applicable.

Competing Interest The authors declare they have no conflict of interest.

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