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Significance of LncRNA KCNQ1OT1 expression in diagnosis and prognosis judgment of myelodysplastic syndrome

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Abstract. – OBJECTIVE: To study the expression level of the long non-coding ribonucleic acid (lncRNA) KCNQ1 overlapping transcript 1 (KCNQ1OT1) in the patients with myelodysplastic syndrome (MDS), as well as the correlation between the expression level of lncRNA KCNQ1OT1 and the clinical diagnosis and prognosis of MDS.

PATIENTS AND METHODS: A total of 60 MDS patients were selected as the MDS group and 20 healthy people as the control group. The expression levels of lncRNA KCNQ1OT1 in the serum of the two groups of participants were compared. The associations of the expression level of lncRNA KCNQ1OT1 with the clinicopathological parameters of the MDS patients were analyzed. MDS was divided into various subtypes in accordance with the World Health Organization (WHO) classification. The expression level of lncRNA KCNQ1OT1 in all the subtypes was detected, and its correlation with the prognosis was judged.

RESULTS: There was a statistical difference in the expression level of lncRNA KCNQ1OT1 between the control group and the MDS group ($p<0.001$). The MDS patients with the low expression of lncRNA KCNQ1OT1 had remarkably longer survival and progression-free survival (PFS) in comparison with those with the high expression of lncRNA KCNQ1OT1 ($p<0.001$). The survival status and chemosensitivity of the MDS patients were closely related to the prognosis ($p<0.001$).

CONCLUSIONS: LncRNA KCNQ1OT1 presents high expressions in the MDS patients, indicating that it has a correlation with the prognosis of the MDS patients, and thus providing a new direction for the future treatment of the MDS patients.

Key Words:
Myelodysplastic syndrome, LncRNA KCNQ1OT1, Clinical significance, Prognosis.

Introduction

Myelodysplastic syndrome (MDS) can develop into acute leukemia[1]. The proportion of the MDS patients accompanied with the gene mutation can reach over 80%. According to a great number of experiments, long non-coding ribonucleic acid (lncRNA) KCNQ1 overlapping transcript 1 (KCNQ1OT1) has been verified to be involved in the genesis, progression, and clinical prognosis of many hematologic malignancies[2].

LncRNAs refer to the RNAs, which cannot be translated into the proteins. They are correlated with multiple biological processes of various cells, e.g., the activities of the neurons, the generation process of the red blood cells, and the immunoreaction of the organisms[3,4]. Firstly, as signaling modules of diversified biological events, lncRNAs indicate their abnormal expressions[5]. Secondly, lncRNAs can affect the protein expressions[6] and take part in the intracellular signal transmission[7,8]. Many studies[9,10] have verified that lncRNAs will become important biomarkers for the diagnosis, prognosis, and targeted therapy of tumors in the future. However, the action mechanism of lncRNAs in MDS patients has not been proved yet. The experiment aims to investigate the expression of lncRNA KCNQ1OT1 in MDS patients and healthy people and judge whether it is associated with the prognosis of the MDS patients or not.

Patients and Methods

Data of Cases

A total of 60 patients who were diagnosed with MDS in the Affiliated Yixing Hospital of Jiangsu University from March 2015 to March 2019 were collected as the MDS group, and 20 healthy people were selected as the control group (Table I). Venous blood was taken from every study subject, 3 mL of ethylenediaminetetraacetic acid (EDTA) anticoagulant was added, and then, venous blood was centrifuged and preserved in a refrigerator at -80°C.

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Table I. General information in MDS group and control group.

Characteristic	Control (n=20)	MDS (n=60)	χ^2	p
Gender			1.111	0.292
Female	10	38		
Male	10	22		
Age/year			0.817	0.366
≤50	8	31		
>50	12	29		
Complication			0.112	0.945
Diabetes	7	13		
Heart disease	8	12		
Hypertension	10	16		

The follow-up visits in various forms were conducted for the 60 MDS patients, with the time of definite pathological diagnosis of the MDS patients as the starting time of the follow-up visits in this experiment. Up to the last follow-up visit, the death rate of the MDS patients was as high as 46.67% (28 cases), without loss of any medical record. This study was approved by the Ethics Committee of The Affiliated Yixing Hospital of Jiangsu University. Signed written informed consents were obtained from all participants before the study.

Detection of Expression Level of LncRNA KCNQ1OT1 in Healthy People and MDS Patients Via Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) Assay

TRIzol (Invitrogen, Carlsbad, CA, USA) reagent method was adopted to extract the total RNAs in the serums of the healthy people and MDS patients in this experiment. The total RNAs were reversely transcribed into the complementary deoxyribonucleic acids (cDNAs) at 37°C for 15 min and at 98°C for 5 min. Then, the PCR amplification was conducted with the transcribed cDNAs (0.4 μmol/L) as the templates, as well as the forward and reverse primers designed corresponding to the target genes[11]. For lncRNA KCNQ1OT1, forward primer sequence: 5'-CCCAGAAATC-CACACCTCGG-3' and reverse primer sequence: 5'-TCCTCAGTGAGCAGAT-GGAGA-3'. For the internal reference, glyceraldehyde 3-phosphate dehydrogenase (GAPDH), forward primer sequence: 5'-TACATGGGCCGAGGCAAGA-TAA-3' and reverse primer sequence: 5'-ATAGC-CCAGGGAAGT-GAAGGTGTC-3'. The PCR was performed on a quantitative Real Time-PCR (qRT-PCR) apparatus.

MDS Classification

According to the World Health Organization (WHO) classification of MDS in 2008, the proportions of the blast cells in the peripheral blood and bone marrow and the presence of Auer rods, the MDS among the 60 patients could be divided into several subtypes, including refractory cytopenia with unilineage dysplasia (RCUD), refractory anemia with ring sideroblast (RARS), refractory cytopenia with multilineage dysplasia (RCMD), refractory cytopenia with excess of blasts-1 (RAEB-1) and RAEB-2. The expression level of lncRNA KCNQ1OT1 in patients with various MDS subtypes was comparatively analyzed.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 13.0 (SPSS, Chicago, IL, USA) software was utilized for the analysis in this experiment. Differences between two groups were analyzed by using the Student's t-test. Comparison between multiple groups was done using One-way ANOVA test followed by the post-hoc test (Least Significant Difference). The survival *via* Kaplan-Meier method. $p<0.05$ indicated statistical significance.

Results

Expression of LncRNA KCNQ1OT1 in Control Group and MDS Group

The expression level of lncRNA KCNQ1OT1 was detected using the qRT-PCR assay (Figure 1). The expression level of lncRNA KCNQ1OT1 in the 60 MDS patients was higher than that in the control group, and the difference between the two groups was statistically significant ($p<0.001$). The comparison between the lncRNA KCNQ1OT1 positive expression and the pathological diagno-

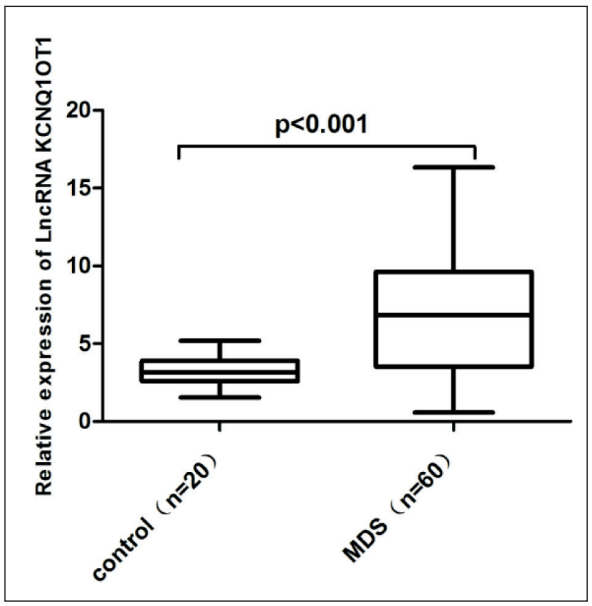

Figure 1. Expression of lncRNA KCNQ1OT1 in MDS patients detected via qRT-PCR assay (p<0.001, control group *vs.* MDS group).

sis results of the patients is shown in Table I. The false-positive rate was 54.5%, the false-negative rate was 5.45%, the true positive rate, namely sensitivity, was as high as 94.5%, and the true negative rate, namely specificity, was 45.45%.

Associations of Clinicopathological Parameters of MDS With Expression of LncRNA KCNQ1OT1

The average expression level of lncRNA KCNQ1OT1 in the 60 MDS patients was 6.89, according to which the patients could be divided into high-lncRNA KCNQ1OT1 group (n=29) and low-lncRNA KCNQ1OT1 group (n=31). The results are listed in Table II. Gender and age of the MDS patients had no evident relationships with the expression of lncRNA KCNQ1OT1, meaning that no statistical difference existed, but the survival status and chemosensitivity had correlations with the expression of lncRNA KCNQ1OT1, in other words, there were statistical differences (p<0.01).

Table II. Comparison between lncRNA KCNQ1OT1 positive expression and pathological results.

LncRNA KCNQ1OT1	Pathological result		Total
	Negative	Positive	
Negative	5	3	8
Positive	6	52	58
Total	11	55	66

Correlation Between Expression of LncRNA KCNQ1OT1 and Overall Survival (OS) of MDS Patients

To probe into the relationship between the survival of the MDS patients and the expression of lncRNA KCNQ1OT1, the data analysis was carried out using the Kaplan-Meier method in this experiment (Table III). The median progression-free survival (PFS) of the MDS patients with the high expression of lncRNA KCNQ1OT1 was evidently shorter than that of those with the low expression of lncRNA KCNQ1OT1 (17 months *vs.* 35 months, p<0.001) (Figure 2). The results indicated that the median OS (28 months) of the MDS patients with the high expression of lncRNA KCNQ1OT1 was

Table III. Associations of clinicopathological parameters of MDS with the expression of lncRNA KCNQ1OT1 (p<0.05 indicates statistical significance).

Clinical characteristic	LncRNA KCNQ1OT1 expression		χ^2	p
n	Low 31	High 29		
Age/year			0.601	0.438
≤50	14	16		
>50	17	13		
Gender			0.067	0.796
Female	16	14		
Male	15	15		
Survival status			19.221	<0.0001
Survival	25	7		
Death	6	22		
Chemosensitivity			8.109	0.004

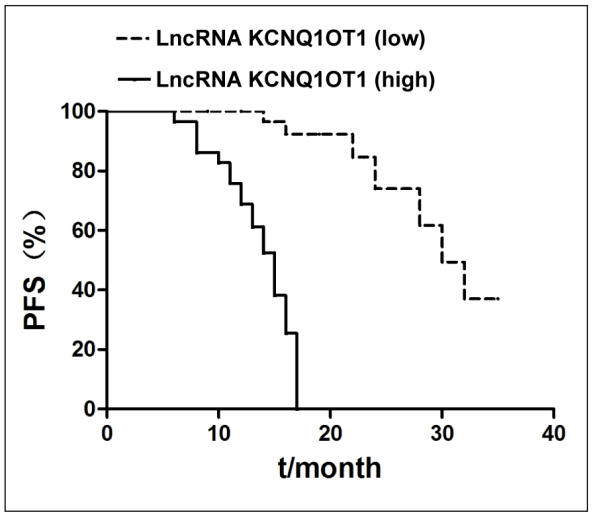

Figure 2. PFS curves of MDS patients [the median PFS (17 months) at the high expression *vs.* that (35 months) at the low expression, $p<0.001$].

statistically different from that (42 months) of those with the low expression of lncRNA KCNQ1OT1 (χ^2 =19.95, $p<0.001$) (Figure 3).

Expression of LncRNA KCNQ1OT1 in Different MDS Subtypes

The WHO classification results of the MDS patients are shown in Table IV. The relative expression of lncRNA KCNQ1OT1 in the MDS group to that in the healthy control group was calculated through the double Delta method. The expression level of lncRNA KCNQ1OT1 in various MDS subtypes is seen in Figure 4. The

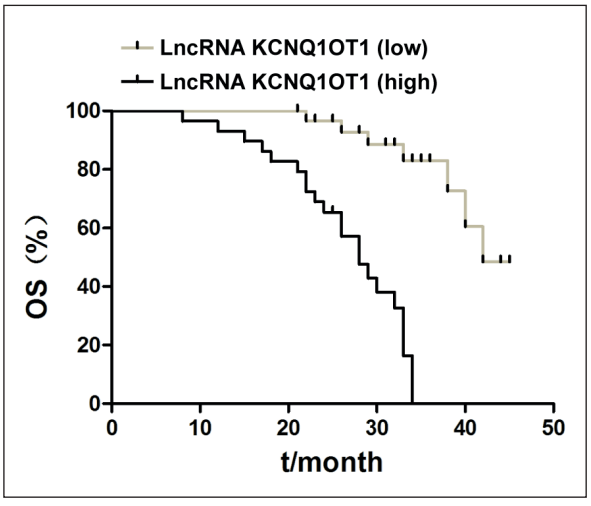

Figure 3. OS curves of MDS patients [the median OS (28 months) at the high expression *vs.* that (42 months) at the low expression, $p<0.001$].

Table IV. Data of 60 MDS patients.

WHO classification	n	%
RCUD	9	15
RCMD	21	35
RARS	5	8.33
RAEB-1	12	20
RAEB-2	13	21.67

relative expression level of lncRNA KCNQ1OT1 in RUCD, RCMD, RARS, RAEB-1, and RAEB-2 was (4.471±0.867), (4.108±0.601), (5.64±0.53), (7.1±0.813) and (11.4±1.32), respectively. The expression level of lncRNA KCNQ1OT1 in all MDS subtypes was significantly higher than that in the control group ($p<0.001$).

Relationship Between LncRNA KCNQ1OT1 and MDS Prognosis

The prognosis of the MDS patients was divided into good prognosis (RAEB-1 and RAEB-2) and poor prognosis (RUCD, RCMD, and RARS). The relative expression level of lncRNA KCNQ1OT1 in the two groups was (4.533±0.411) and (9.011±0.883), respectively, and the difference was of statistical significance (Figure 5).

Discussion

As a kind of clonal disease, MDS is very likely to be transformed into acute leukemia[12-14]. High-risk MDS can evolve into acute leukemia years later or even within a shorter time. As a

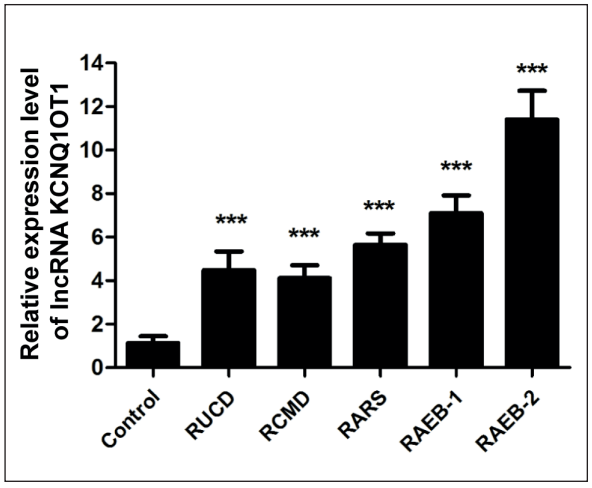

Figure 4. Relative expression level of lncRNA KCNQ1OT1 in all MDS subtypes (***$p<0.001$ *vs.* the control group).

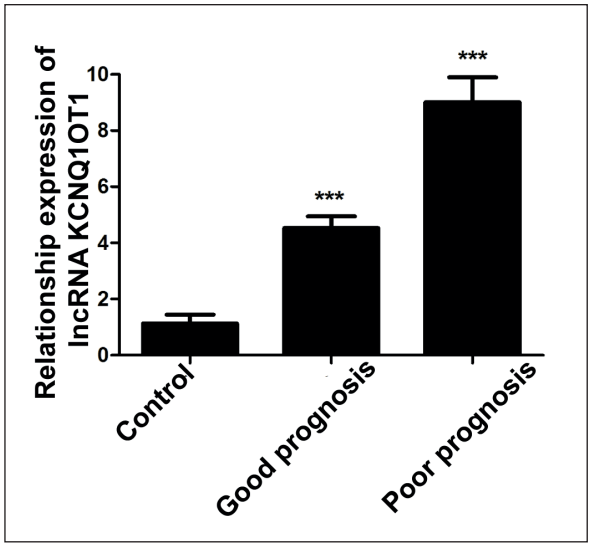

Figure 5. Relationship between relative expression level of lncRNA KCNQ1OT1 and MDS prognosis (***p<0.001 *vs.* the control group).

general rule, MDS patients are clinically treated with growth factors, blood transfusion, etc. High-risk MDS probably needs chemotherapy or transplantation therapy[15]. Even though the advent of Lenalidomide has been of great significance for the treatment of MDS, a minority of patients may still suffer from the continuous progression of the disease within 2 years[16]. Bone marrow transplantation has a good effect, but it is inapplicable to most MDS patients yet. Therefore, seeking a new MDS treatment program is extremely significant to the early diagnosis and targeted therapy of MDS.

As a hot issue in the modern tumor research, the reverse treatment of some genes is not only applicable to early diagnosis and prognostic evaluation of the tumors but also has become a new-targeted therapy for tumors. Nowadays, a great number of studies[17,18] have verified that lncRNAs are of vital importance in the genesis, development, and prognosis of the hematologic malignancies[19]. Before and after the treatment of the diseases, the detection of the level of some abnormally expressed lncRNAs has become an important method for the diagnosis and prognostic stratification of tumors in clinical practice[20]. Developing the targeted drugs for some abnormally expressed lncRNAs will certainly become a part of the clinical precision treatment of tumors. With the development of the modern detection technologies in recent years, research regarding lncRNAs has achieved rapid development. However, due to the conservativeness and specificity of the detection results of lncRNAs, more functions of lncRNAs have not been clarified yet. The current studies on lncRNAs in the hematologic system-related tumors are still in the initial phase. The relationship between hematologic malignancies and some lncRNAs has not been affirmed. The literature has verified that the precursor of miRNAs is lncRNAs, which can promote or inhibit the genesis and development of tumors. The mechanism is that lncRNAs code miRNAs or serve as endogenous competitive RNAs of miRNAs to influence the expression of its downstream genes[21]. Besides, the regulation of the epigenetics and the modification of the chromatins are also correlated with the expression of lncRNAs, so as to exert the promoting or inhibitory effect on the transcription[22,23]. The expression level of lncRNA KCNQ1OT1 in the control group and MDS group was detected in this experiment using the RT-PCR method, and whether the clinical prognosis of the MDS patients had a correlation with it was analyzed. The results manifested that the expression level of lncRNA KCNQ1OT1 was low in the control group, while it was high in the MDS group, showing a statistical difference. The sensitivity of the MDS patients to lncRNA KCNQ1OT1 reached as high as 94.5, which could act as a reference index for the diagnosis of MDS. The expression of lncRNA KCNQ1OT1 was related to age and gender of the patients, but it was associated with the survival status and chemosensitivity of the patients. Among the MDS subtypes, the expression level of lncRNA KCNQ1OT1 in RCUD, RCMD, and RARS was significantly lower than that in RAEB-1 and RAEB-2. The severer the progression of MDS is, the higher the expression level of lncRNA KCNQ1OT1 will be, verifying that the prognosis of MDS has a bearing on the expression level of lncRNA KCNQ1OT1.

Conclusions

To sum up, lncRNA KCNQ1OT1 not only participates in the genesis and development of MDS but also can serve as a potential therapeutic target and a prognostic factor for the MDS patients, but its concrete action mechanism still needs in-depth research.

Conflict of Interests

The authors declare that they have no conflict of interest.

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