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Focusing on long non-coding RNA dysregulation in newly diagnosed multiple myeloma

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ABSTRACT

Aims: Multiple myeloma (MM) is an incurable hematological cancer with a higher rate of relapse. Alterations in the function of long non-coding RNAs (lncRNAs) promote the progression and metastasis of cancer. We carry out this study to explore the expression profile of differently expressed lncRNAs in newly diagnosed MM.

Main methods: The Bone marrows we analyzed were obtained from five MM and five IDA patients (serving as controls). Arraystar Human LncRNA Array V4.0 was used to profile expression of lncRNAs and mRNAs. Gene ontology (GO) and pathway analysis were utilized to understand the biological roles of differently expressed genes, while Database for Annotation, Visualization and Integrated Discovery (DAVID) was used for constructing the lncRNA-mRNA co-expression network. Quantitative polymerase chain reaction (qRT-PCR) was performed to confirm the expressions of dysregulated lncRNAs.

Key findings: Bioinformatic analysis of the lncRNA expression identified > 3000 dysregulated lncRNAs (difference ≥ 2-fold) in MM samples. GO and pathway analysis revealed that ECM-receptor and cell cycle pathway-related genes were significantly associated with MM. Four dysregulated lncRNAs were confirmed by qRT-PCR. Among them, the expression of ST3GAL6-AS1, LAMA5-AS1and RP11-175D17.3wereassociated with stage and risk status of MM. On the basis of GEO public database analysis, LAMA5-AS1 was related with an overall survival rate of MM patients.

Significance: These results reveal the feasible functions of lncRNAs in pathogenesis of MM. Further studies are required to explore whether these lncRNAs could serve as candidate therapeutic targets and new molecular biomarkers for MM.

1. Introduction

Multiple myeloma (MM), the second most common hematological cancer, is characterized by the accumulation of malignant plasma in the bone marrow and the detectable secretion of monoclonal immunoglobulin in the serum or urine [1,2]. Significant improvement in outcomes has been observed for myeloma patients in the past few decades, mainly as a result of the use of novel drugs such as proteasome inhibitors [3] and immunomodulatory drugs, together with autologous stem cell transplantation and advancing supportive care [4,5]. However, further development and identification of prognostic markers or novel targets for MM treatment which could improve the survival rate of MM are still in the mist.

Long non-coding RNA (lncRNA) is emerging as an important regulator of involved in gene regulation at the transcriptional, posttranscriptional and epigenetic levels [6]. Human genomic data have shown that about 62–75% of the human genome is transcribed into RNA, and only 1% codes protein expressions, which indicated that a large number of the genome are compounded as regulators [7,8]. The dysregulation of lncRNA has been observed to play important roles in the development and progression of a variety of diseases [9] and tumors [10–14], such as RGMB-AS1 in lung adenocarcinoma [15], HOTAIR in breast cancer [16].

Previous studies showed that the upregulation of lncRNA MALAT1 was proved to play a role in pathogenesis of MM [17,18], while the downregulation of KIAA0459 was supposed to take part in the pathogenesis or progression of myeloma [19]. But the comprehensive lncRNA expression profiles of MM are hardly studied.

In the present study, we revealed the differential expression of lncRNAs in MM to gain a better understanding of pathogenesis and

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Table 1
Basic information of the microarray analysis samples.

Patient	Gender	Age	Diagnosis	D-S stage	Risk status	Chromosome	Group
1	Male	56	MM (IgG)	II A	Low	Normal	Experimental
2	Female	64	MM (IgG)	III A	Intermediate	Normal	Experimental
3	Male	68	MM (IgG)	III A	Intermediate	Normal	Experimental
4	Female	71	MM (IgG)	III A	Intermediate	Normal	Experimental
5	Male	53	MM (IgG)	III A	high	Normal	Experimental
6	Female	25	IDA	–	–	Normal	Control
7	Female	21	IDA	–	–	Normal	Control
8	Female	23	IDA	–	–	Normal	Control
9	Female	20	IDA	–	–	Normal	Control
10	Female	28	IDA	–	–	Normal	Control

D-S: Durie-Salmon; MM, multiple myeloma; IDA, iron deficiency anemia.

identify possible biomarkers and novel therapeutic targets of MM.

2. Material and methods

2.1. Patients and samples

Approval for this study was obtained from the Ethics Committee of the Second Affiliated Hospital of Xi'an Jiaotong University (Approval number: 2015186). When the written informed consent was obtained from all patients, bone marrow specimens were obtained from newly diagnosed MM patients at the Department of Hematology, Second Affiliated Hospital of Xian Jiaotong University, between 2015 and 2016. The diagnosis, stage and risk status of MM were made in accordance with the National Comprehensive Cancer Network (NCCN) (2015 version 3 &2017 version 3). To weaken the variation on samples as much as possible, bone marrow samples of iron deficiency anemia (IDA) patients were used as controls after informed consent, for the deficiency of bone marrow samples from normal donors in clinic. The general clinical and laboratory features of the five MM patients and five IDA patients are summarized in Table 1.

2.2. Profiling of lncRNA expression by Arraystar Human LncRNA Array V4.0

Arraystar Human LncRNA Microarray V4.0, performed by KangChen Biotech (Shanghai, China), is designed for global profiling of human lncRNAs and protein-coding transcripts, which could detect about 40,173 lncRNAs and 20,730 coding transcripts. Simply, mRNA was purified from total RNA using mRNA isolation kit (mRNA-ONLY™ Eukaryotic mRNA Isolation Kit, Epicentre). Then, each sample was amplified and transcribed into fluorescent cRNA as probes to hybridize to the Human LncRNA Array V4.0. RNA quantity and quality were monitored by NanoDrop ND-1000. Agilent Feature Extraction software (version 11.0.1.1) was used to analyze acquired array images. Differentially expressed lncRNAs and mRNAs were performed with statistical significance (P-value < 0.05) and a cut-off point of 2-fold between two groups.

2.3. Gene ontology and Kyoto encyclopedia of genes and genomes pathway analysis

Gene Ontology (GO) Enrichment analysis of differently expressed genes was performed using functional annotation tool Database for Annotation, Visualization and Integrated Discovery (DAVID) (http://david.abcc.ncifcrf.gov/summary.jsp), and the significant GO terms were identified as a *P*-value < 0.05. Pathway analysis of differentially expressed genes was performed based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (http://www.genome.jp/kegg).

2.4. Co-expression analysis of lncRNA and mRNA

The screening criteria of dysregulated lncRNA are as follows: i) fold change was > 2.0-fold; ii) *P*-value was < 0.05; iii) average raw intensities of replicate samples of MM group and the control group was > 100; iv) lncRNAs who located in sex chromosomes were removed.

String (http://www.string-db.org/) is online software to reveal protein-protein interaction network, which was used to track the core mRNAs in the pathogenesis of MM in this study. The dysregulated mRNA whose fold change was > 4.0-fold was included in String analysis.

The lncRNA-mRNA co-expression analysis was performed by Weighted Correlation Network Analysis (WGCNA, https://labs.genetics.ucla.edu/horvath/CoexpressionNetwork/), and the network was then constructed by Cytoscape software (the Cytoscape Consortium, San Diego, CA, USA). K-core scoring was used to identify core transcripts of co-expression networks. The higher k-core score means a more central location of a transcript within a network.

The procedures of searching for the possible co-expression coding gene of lncRNA ST3GAL6-AS1 included the following: i) scan the position of lncRNA in UCSC database (http://genome.ucsc.edu/) to seek for the possible related coding gene according to Flank 10 K theory [23]; ii) confirm the co-expression relationship between two genes in pan-cancer by CHIPbase v2.0 (http://rna.sysu.edu.cn/chipbase/); iii) validate the expression level of possible target coding gene by qRT-PCR.

2.5. RNA isolation and real-time quantitative reverse transcription-polymerase chain reactions (qRT-PCR)

Total RNA was extracted from bone marrow mononuclear cells (BMSCs) samples of MM and IDA using TRIzol reagent (Invitrogen, Germany), and stored at −80 °C until use. RNA purity and concentration were determined by NanoDrop ND-1000.

RNA samples were reversely transcribed into cDNA using a Primescript RT master mix with Oligo dT primers and random primers in accordance with manufacturer's protocols (Takara). Then, the qRT-PCR was performed by SYBR Premix Ex Taq™ II (TliRNaseH Plus) (Takara) and StepOne Software v2.1, according to manufacturer's instructions. Primers of detected lncRNA and β-actin were designed and synthesized by Invitrogen (Shanghai, China). And the primer sequences were as shown in Table 2. ΔCt value was used to reflect the expression level of lncRNAs.

2.6. GEO datasets of MM

Gene microarray expression data and the related clinical information of the patients with MM used in this study were obtained from publicly available Gene Expression Omnibus (GEO) database, GSE24080 (Affymetrix HG-U133_Plus_2.0 array) (www.ncbi.nlm.nih.