Competing endogenous RNA network in newly diagnosed multiple myeloma by genetic microarray

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Multiple myeloma (MM) is a blood malignancy characterized by the clonal proliferation of plasma cells, which increases the number of monoclonal immunoglobulins in blood and urine and causes target organ damage. Long non-coding RNAs (lncRNAs) have been reported to have high genetic heterogeneity and to participate in malignancy formation, progression, and metastasis in MM.[1] Furthermore, lncRNAs and circular RNAs (circRNAs) act as microRNA (miRNA) sponges, by indirectly binding miRNAs and influencing the post-transcriptional regulation of target gene expression. This phenomenon is known as the competing endogenous (ceRNA) relationship, and it may be one of the most important lncRNA mechanisms in cancers. However, the only lncRNA maternally expressed gene 3 (*MEG3*) was reported to function as a ceRNA to regulate homeobox gene A11 mRNA expression by sponging miR-181a in MM.[2-3] Therefore, other ceRNA relationships, especially in newly diagnosed MM patients, remain to be investigated.

In recent years, genetic microarrays have become a credible technology to analyze nucleic acid sequences, and have been universally applied in precision medicine. Here, we adopted the Affymetrix microarray platform to detect RNAs from bone marrow cells of myeloma patients and to conduct comprehensive analyses of lncRNA, circRNA, miRNA, and mRNA expression of known sequences in the microarray database.

We recruited ten newly diagnosed, untreated MM patients from the Beijing Chaoyang Hospital between July 2018 and April 2019 and obtained bone marrow specimens from this test group; all MM patients met the 2014 International Myeloma Working Group (IMWG) updated criteria for the diagnosis of MM. Exclusion criteria

were: (1) recurrent MM patients, (2) refractory MM patients, (3) MM patients with critical disease, (4) MM patients preparing for autologous stem cell transplantation, and (5) patients with plasmacytoma. Bone marrow specimens were also obtained from ten healthy volunteers allocated as the control group. This research was approved by the ethics committee of Beijing Chao-yang Hospital (No. 2019-science-118). All participants provided their informed consent, and the study complied with the *Declaration of Helsinki* and its amendments.

Mononuclear cells were isolated from 8 mL of fresh bone marrow using lymphocyte separation medium (Ficoll PLUS; GE Healthcare, Amersham, UK) and red blood cell lysis buffer (Solarbio, Beijing, China) within 2 h of harvesting. All specimens were stored at −80°C after adding TRIzol RS reagent (Life Technologies, Carlsbad, CA, USA). Total RNA was purified using the miRNeasy Serum/Plasma kit (Qiagen, Hilden, Germany). The purity and integrity of total RNA were examined by agarose gel electrophoresis, recognizing 20 ng/μL as the minimum concentration required. The human Clariom™ D array (Affymetrix, Santa Clara, CA, USA) system was used to detect and analyze the expression of lncRNAs, circRNAs, miRNAs, and mRNAs by the intensity of hybridization signals. Furthermore, GeneChip Command Console Software (AGCC version 4.0; Affymetrix) was used to generate a ceRNA network by the target prediction database.

Statistical analysis used the *t*-test to compare the means of two samples with $P < 0.05$ indicating significance, and differentially expressed genes were identified when the threshold of fold-change (FC) was ≥2.0. Based on selected differentially expressed genes, Pearson correlation coefficients between matched molecules (eg, lncRNA and

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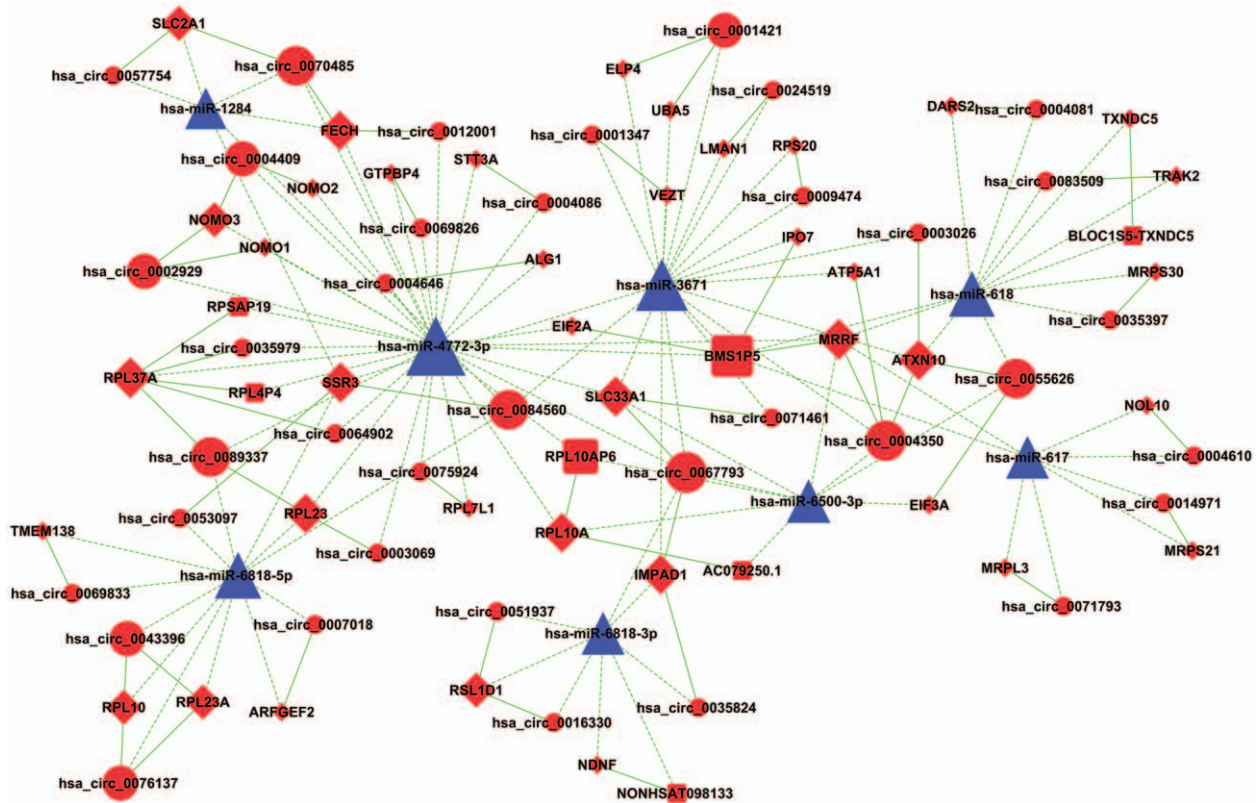E-Mail: 13910107759@163.com

Figure 1: The ceRNA network. Molecules with ceRNA relationships are shown in this network. Squares, triangles, rhombuses, and circles represent lncRNAs, miRNAs, mRNAs, and circRNAs, respectively. The solid line represents positive correlations, and the dotted line represents negative correlations. The size of the symbols signifies the degree value. Red indicates upregulation and blue indicates downregulation. ceRNA: Competing endogenous RNA; lncRNA: Long non-coding RNA; miRNA: MicroRNA; circRNA: Circular RNA; hsa: *Homo sapiens*; SLC2A1: Solute carrier family 2 member 1; ELP4: Elongator acetyltransferase complex subunit 4; UBA5: Ubiquitin-like modifier activating enzyme 5; FECH: Ferrochelatase; DARS2: Aspartyl-tRNA synthetase 2; NOMO1,2,3: NODAL modulator1,2,3; GTPBP4: GTP binding protein 4; STT3A: Subunit of the oligosaccharyltransferase complex; LMAN1: Lectin mannose-binding 1; VEZT: Vezatin adherens junctions transmembrane protein; TRAK2: Trafficking protein kinesin binding 2; ALG1: Hitobiosyldiphosphodolichol beta-mannosyltransferase; IPO7: Importin 7; BLOC1S5-TXNDC5: Biogenesis of lysosome-related organelles complex 1 subunit 5-hioredoxin domain containing 5; RPSAP19: Ribosomal protein SA pseudogene 19; ATP5A1: ATP synthase 5A1; MRPS30: Mitochondrial ribosomal protein S30; EIF2A: Eukaryotic translation initiation factor 2A; RPL2: Ribosomal protein L23; SSR3: Signal sequence receptor gamma; BMS1P5: BMS1 ribosome biogenesis factor pseudogene 5; MRRF: Mitochondrial ribosome recycling factor; ATXN10: Ataxin 10; SLC33A1: Solute carrier family 33 (acetyl-CoA transporter) member 1; NOL10: Nucleolar protein 10; TMEM138: Transmembrane protein 138; EIF3A: Eukaryotic translation initiation factor 3 subunit A; AC079250.1: ENSG00000230979; MRPL3: Mitochondrial ribosomal protein 3; MRPS21: Mitochondrial ribosomal protein S21; ARFGEF2: ADP-ribosylation factor guanine nucleotide-exchange factor 2; RSL1D1: Ribosomal L1 domain containing 1; NDNF: Neuron-derived neurotrophic factor; NONHSAT098133: Non-coding transcript identified by NONCODE.

miRNA) were calculated based on expression data; the cutoff was 0.99 and the threshold significance was set at $P < 0.05$. The quantity of miRNA response elements and combining scores allowed us to evaluate the competency of ceRNA molecules.

Using the Affymetrix human Clariom™ D chip we screened a total of 135,731 genes, from which we identified differentially expressed genes (FC >2, $P < 0.05$) as follows: 234 lncRNAs (1.7‰), 557 circRNAs (4.1‰), 122 miRNAs (0.9‰), and 709 mRNAs (5.2‰). Only a small number of RNAs were shown to be involved in the ceRNA network [Figure 1]: nine lncRNAs, 42 circRNAs, eight miRNAs, and 51 mRNAs. As shown in Figure 1, the degree value (DV) indicates the total quantity of a gene related to other genes.

This process identified molecules with the highest DVs: *hsa_miR-4772-3p* (DV:24), three upstream lncRNAs (*RPL4P4, RPSAP19,* and *BMS1P5*), and 13 circRNAs (*hsa_circ_0004646, hsa_circ_0069826, hsa_circ_0012001, hsa_circ_0070485, hsa_circ_0064902, hsa_circ_0089337,* *hsa_circ_0035979, hsa_circ_0067793, hsa_circ_0004409, hsa_circ_0002929, hsa_circ_0004086, hsa_circ_0075924,* and *hsa_circ_0003069*); all upstream molecules shared the downstream mRNA *RPL37A*. Occasionally, the downstream protein was ribosomal protein L37, which was first screened in a yeast promoter library.[4]

We conducted a preliminary analysis of correlations between lncRNAs, circRNAs, miRNAs, and mRNAs. GeneChip Command Console software found that the aberrantly expressed RNAs have a series of complicated ceRNA relationships. The ceRNA relationship was previously shown to be a compelling mechanism to explain MM pathogenesis, and activation or inhibition of the miRNA/mRNA axis was reported to control protein production.[2] Our results indicate that one of the most activated RNAs involved in the ceRNA network, *hsa_miR-4772-3p*, has a role in ribosome synthesis. Three LncRNAs and 13 circRNAs sponging *hsa_miR-4772-3p* forms the *miR-4772-3p/RPL37A* axis, while mRNAs *RPL10A, RPL23, RPL37A,* and *RPL7L1* code for structural proteins of the ribosome skeleton such as ribosomal

protein 37. This indicates that activities of the ceRNA network might increase protein production in patients with newly diagnosed MM, which is consistent with disease characteristics.

Our findings also indicate that *hsa_miR-618* and *hsa_miR-1284* may be involved in the MM mechanism as other centers of the ceRNA network. miR-618 was previously shown to play a role in tumor inhibition and to suppress metastasis in gastric cancer by negatively regulating the transcription of transforming growth factor-β2, which is a cytokine that promotes proliferation and suppresses apoptosis in endothelial cells.[5] Moreover, miR-1284 was reported to suppress the proliferation, migration, and invasion of breast cancer cells by targeting transcription factor zinc finger protein 2 to decrease tumor growth.[6] Thus, miRNAs can be proto-oncogenes or tumor suppressors in the mechanism of RNA action, which may cause protein expression disorders. We, therefore, speculate that ceRNA relationships cause an imbalance of gene expression, affecting the proliferation process of MM cells.

In summary, our study provides preliminary evidence of RNAs in a ceRNA relationship using genetic microarray analysis of a large number of aberrantly expressed RNAs from patients with newly diagnosed MM. This offers new insight into the pathogenesis of MM and identifies RNA molecules that could be used as novel targets for new countermeasures of MM.

Conflicts of interest

None.

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