Regulatory network analysis of transcription factors, microRNAs, target genes and host genes in human multiple myeloma

Zhuoyan Huang[1,2], Zhiwen Xu[1,2 *], Kunhao Wang[1,2], Ning Wang[1,2] and Shang Wang[1,2]
[1]College of Computer Science and Technology
[2]Key Laboratory of Symbolic Computation and Knowledge Engineering of Ministry of Education,
Jilin University, Changchun, Jilin, P.R. China

Abstract: In recent years, molecular biologists have achieved great advance in micro RNA (miRNA) and gene investigation about the pathogenesis of multiple myeloma (MM). Existing research data of the transcription factors (TFs) and miRNAs is disperse and unorganized, which prevents researchers from investigating the mechanism and analyze regulatory pathways of MM systematically. In our research, regulatory interactions among miRNAs, TFs, host genes and target genes were imported to construct regulatory networks at three levels, including the abnormally expressed network and the related network as well as the global network. The abnormally expressed network was primary investigated cause it was an experimentally validated topological network, and it systematically explained the regulatory mechanism of MM. Its outstanding significance lies in that if we correct each abnormally expressed gene and miRNA to normal expression level by transcriptional control adjustment, thus the whole genetic expression network will return to normal state, and MM may not relapse. Additionally, analyses and comparisons to upstream as well as downstream of abnormally expressed miRNAs and genes in three networks highlighted some important regulators and key signaling pathways. For example, STAT3 and hsa-miR-125b, PIAS3 and hsa-miR-21 respectively formed self adaptation feedback regulations. The current research proposed a novel perspective to systematically explained the regulatory mechanism of MM and may contribute to further research and therapy of carcinomas.

Keywords: Multiple myeloma, micro RNA, transcription factor, abnormally expressed, regulatory network.

INTRODUCTION

As a malignant hematological cancer, multiple myeloma (MM) encroaches bone marrow. Now MM has been a common and serious hematological cancer which has caused substantial deaths. MiRNAs and genes have been proved be important roles during MM's development by numerous researches. Especially, abnormally expressed genes and miRNAs are identified as pivotal hubs among them. For example, MYC, an oncogenic transcription factor, reduces plasma cell protein in MM by controlling the expression of a large set of miRNAs, such as hsa-miR-18a, 19a, 92-1 (Chen *et al*., 2013). Some researchers show that target genes of 16 down regulated miRNAs, including hsa-152, 24, 425 and so on, are directly involved in the pathogenesis of MM (Rio-Machin *et al*., 2013). Besides, those MM-related miRNAs and genes which don't show abnormally expressed influence MM's physiological activity as well. IRF4 promotes cell proliferation by JNK pathway in MM (Zhang *et al*., 2011), and restoration of hsa-miR-34b leads to reduce cellular proliferation and enhanced apoptosis of MM's cells (Wong *et al*., 2011; Ozbas-Gerceker *et al*., 2013).

Transcription factor (TF) and micro RNA (miRNA) are outstanding regulators for gene expression (Hobert, 2008). A protein called TF regulates genetic information from DNA to messager RNA through binding certain DNA sequences (Latchman, 1997; Tang and Wang, 2015). To transmit genetic information, it blocks or promotes RNA polymerase's recruitment to certain genes with other proteins (Roeder, 1996). MiRNA is a tiny noncoding RNA, which usually silences genes or targets genes to regulate certain biological processes during cancer development, including proliferation, differentiation and apoptosis (Chen and Rajewsky, 2007).

In general, miRNAs target those miRNAs, which are commonly called target genes. Target genes are valuable materials for our research with respect to regulatory interactions between genes and miRNAs. Up to now, numerous algorithms (Betel *et al*., 2008) and experimentally validated databases (Papadopoulos *et al*., 2009; Hsu *et al*., 2011) have been worked out to support our research.

Host genes are those genes where miRNAs locate in. Rodriguez A. *et al*. have declared there were two transcriptional kinds of miRNAs, exonic miRNA and intronic miRNA (Rodriguez *et al*., 2004). Research has shown that intronic miRNAs cooperated with host genes frequently expressed in MM' cells, and both are involved in some regulatory topological pathways (Baskerville and Bartel, 2005; Ronchetti *et al*., 2008; Xie *et al*., 2014).

**Corresponding author:* e-mail: xuzw@jlu.edu.cn

Researchers have taken tremendous effort to find out regulatory mechanism of genes and miRNAs within MM's cells. They usually concentrate on the biological function of single gene or miRNA or study certain signaling pathway, but few investigations are spent on studying interactions among all these MM-relevant miRNAs and genes. In this study, we paid close attention to all the elements mentioned above, including miRNAs, TFs, host genes and target genes. Numerous experimentally validated gene regulatory interactions retrieved from databases and literature were introduced to create three regulatory networks: the abnormally expressed network, the related network and the global network. The miRNAs and genes as well as their interations in the abnormally expressed network were experimentally validated. In this paper we discussed abnormally expressed miRNAs and genes emphatically. The abnormally network implied some significant biological regulators and stuck out several signaling pathways within it. To further study the MM's transcriptional mechanism, some TFs predicted by P-match algorithm were supplemented into related network. Related network extended the abnormally network, showing more evidences with regard to MM's inner biological regulations. Global network included whole human cancers' regulatory interactions between miRNAs and genes so far. Finally, similarities and differences of interactions among abnormally expressed genes, abnormally expressed miRNAs from three networks were discussed to highlight certain important regulatory pathways in MM. In our view, there exist some key components (specific miRNAs and TFs) in MM's regulatory network, further, these elements should attract more attention and their normal expression may contribute to MM's treatment to a large extent.

MATERIALS AND METHODS

Material collection and data processing

We retrieved human miRNAs and their experimentally validated target genes from the Tarbase and miRTarBase. Tarbase and MiRTarBase collects experimentally validated target genes with corresponding miRNAs. MiRNAs and genes symbols in the dataset (the same below) were unified by National Center of Biotechnology Information. This dataset was named as U_1.

Experimentally validated dataset about human TFs with corresponding miRNAs were extracted from the TransmiR (Wang *et al*., 2010), which was a TF-miRNA regulation database. This dataset was named as U_2.

We collected miRNAs' host genes from the NCBI and miRBase (Kozomara and Griffiths-Jones, 2011). Official symbols were introduced to mark all the host genes. This dataset was named as U_3.

In this study, abnormally expressed genes contained genetic mutation genes, over expressed genes, single nucleotide polymorphisms (SNPs) and abnormally expressed proteins. These genes are select from NCBI SNP database and Cancer Genetics Web. Related genes came from four sources. Abnormally expressed genes were also the related genes. Besides, part of related genes were supplied by Gene Cards, which was a database that provided genetic and functional information of all known and predicted human genes (Safran *et al*., 2010). Part of related genes were extracted from associated literature where genes affected cancer grownth, migration, prevention and spread. Finally, to obtain an improved understanding of the transcriptional network, we acquired the predicted TFs by P-match algorithm (Chekmenev *et al*., 2005), and we only extracted those TFs that appeared in the transmiR and identified them as part of related genes. During retrieve process, first we downloaded 1000-nt (nucleotide) promoter region located in target genes of abnormally expressed miRNAs from UCSC (Fujita *et al*., 2011). Then P-match algorithm that combined weight matrix approaches and pattern matching was used, to get transcription factor binding sites (TFBSs) in above 1000-nt promoter region and eventually identified these TFBSs at target genes' promoter region. Matrix library is a dataset of existing TFBSs selected by TRANSFAC and it can be used to predict more TFBSs located in genes' promoter region. The dataset of abnormally expressed and related genes was named as U_4.

Abnormally expressed miRNAs were selected from mir2Disease (Jiang *et al*., 2009), which collected numerous abnormally expressed miRNAs with corresponding cancers. In the same way, abnormally expressed miRNAs were considered as part of related miRNAs. Other related miRNAs was selected from relevant literature. The dataset of abnormally expressed and related miRNAs was named as U_5.

Three networks construction

All the regulatory interactions among genes, miRNAs and TFs were included in U_1, U_2 and U_3. All the extracted regulatory interactions from U_1, U_2 and U_3 were input into a software named CytoScape (CytoScape, a bioinformatics software for visualizing genes' interactions network, http://www.cytoscape.org/), then we generated the global network. Abnormally expressed miRNAs and genes were separately retrieved from U_5 and U_4, and regulatory interactions among abnormally expressed miRNAs and genes were collected from global network. So we gained the abnormally expressed network. In the analogous way, we constructed the related network. What's more, global network was extremely huge and comprehensive and it contained all human cancers' regulatory interactions.

RESULTS

The abnormally expressed network of MM

Significant regulatory interactions among miRNAs, TFs, host genes and target genes are clearly shown in fig. 1 (Isolated elements are omitted in the network). There are 16 miRNAs, 7 TFs, 13 host genes and 9 target genes in the network. Regulatory interactions including miRNA targeting at target genes, miRNAs being regulated by TF and host gene hosting miRNAs are distinctly shown, for example, hsa-miR-23b's host gene is C9orf3, AKT regulates hsa-let-7e and hsa-miR-106b targets VEGFA. A host gene can host one or more miRNAs, such as DLEU2 hosts hsa-miR-15a and 16. A TF can regulate one or more miRNAs while a miRNA can also target one or more target genes. For instance, STAT3 regulates hsa-miR-125b and 21, hsa-miR-34a target BCL2, CCND1 and so on. Furthermore, specific crucial regulatory hubs exist in the network. For example, hsa-miR-29b targets SP1 and SP1 regulates hsa-miR-29b in turn, in other words, hsa-miR-29b and SP1 form a self-adaptation feedback regulation. Amodio, N. have pointed that SP1 is a negative regulator of hsa-miR-29b (Amodio *et al*., 2012). Previous studies have indicated that SP1 has carcinogenic potential while hsa-miR-29b has tumor suppressor potential. In such self-adaptation feedback pathway, gene and miRNA are regulated and controlled by each other. It is supposed that the other may express abnormally once either is abnormally expressed. What's more, hsa-miR-192 and TP53, hsa-miR-194 and TP53, and hsa-miR-215 and TP53 show mutual feedback regulations in fig. 1. Relevant research has pointed out hsa-miR-192, 194 and 215 are positive regulators of TP53, and they can enhance TP53's activating effect, further, they co-regulate MDM2's expression (Pichiorri *et al*., 2010). Meaningfully, relevant researchers try their best to correct all these abnormally expressed elements' abnormally expression level back to normally expression level, thus MM would be cured. The abnormally expressed network, especially key hubs and remarkable pathways in it, partially uncover the regulatory mechanism of MM, and contribute to medical therapy of MM.

Some features about host genes and miRNAs in the abnormally expressed network of MM can be identified in fig. 1. Host genes are involved in numerous interactive regulations. MIR17HG hosts hsa-miR-20a that targets RB1, VEGFA, BCL2 and so on. MIRLET7E hosts hsa-miR-7e that is regulated by AKT and MYC. MIR125B1 hosts hsa-miR-125b which forms a self-adaptation feedback regulation with STAT3. Mostly, miRNAs that share the same host gene cooperatively get involved in some regulatory activities, like together targeting specific target genes or being regulated by certain TFs. For instance, DLEU2 hosts hsa-miR-15a and 16, and hsa-miR-15a and 16 co-target BCL2, CCND1, TP53 and VEGFA. Hsa-miR-194 and 215 share the same host gene named IRAS2, and both are regulated by TP53. These characteristics will help to fig. out the biological roles of miRNAs and host genes during MM's development.

The related network of MM

Compared to the abnormally expressed network, the related network contains more TFs, miRNAs and genes, in fact, there are 20 TFs, 48 miRNAs, 39 target genes and 42 host genes. The related network contains 267 regulatory interactions among MM-related TFs, miRNAs and genes, which implies more regulation mechanism. The fig. of the related network is omitted in consideration of its complexity. Clearly, related network contains abnormally expressed network. In the related network, extra regulatory interactions and pathways are supplied. For instance, MIR137HG hosts hsa-miR-137; hsa-miR-21 and 19a are regulated by PTEN; VEGFA and PTEN are targeted by hsa-miR-93; hsa-miR-152 and MYC form a self adaptation feedback pathway. These additionally expanded regulatory interactions offer a wider understanding with respect to proliferation, differentiation and apoptosis of MM' cells.

The global network

The global network is an overall human body's genetic regulatory network. It comprises all the TFs, miRNAs, host genes and target genes along with their regulatory interactions. The global network also contains the abnormally expressed network of MM. The global network offers valuable and comprehensive materials for relevant researchers who major in a certain cancer.

Transcription network between abnormally expressed miRNAs and predicted TFs in MM

Ten abnormally expressed miRNAs as well as predicted TFs are investigated. Fig. 2 indicates 6 predicted TFs (RELA, REL, E2F1, NFKB1, ZEB1 and E2F3) and it brightly exhibits regulatory functions among abnormally expressed miRNAs along with predicted TFs. It can be concluded that a miRNA can target one or more TFs, a TF can also regulate one or more miRNAs, a TF could indirectly influences else TFs through certain miRNAs and a miRNA could indirectly affect else miRNAs through certain TFs as well. For instance, E2F1 regulates hsa-miR-15a, 16, 106b and 20a, hsa-miR-21 targets E2F1 and NFKB1, E2F3 controls E2F1 through hsa-miR-34a, and hsa-miR-34a are controlled by hsa-miR-125b through E2F3. Abnormally expressed miRNAs and predicted TFs may form self-adaptation feedback, such as hsa-miR-34a and E2F3 are controlled by each other. Specific predicted TFs have momentous regulations within other cancers, for instance, E2F1 is a tumour suppressive gene in human breast cancer (Worku *et al*., 2008). It is to be validated that these predicted TFs play a role in MM's development. These involved interactions contribute to studying the transcriptional network of MM.

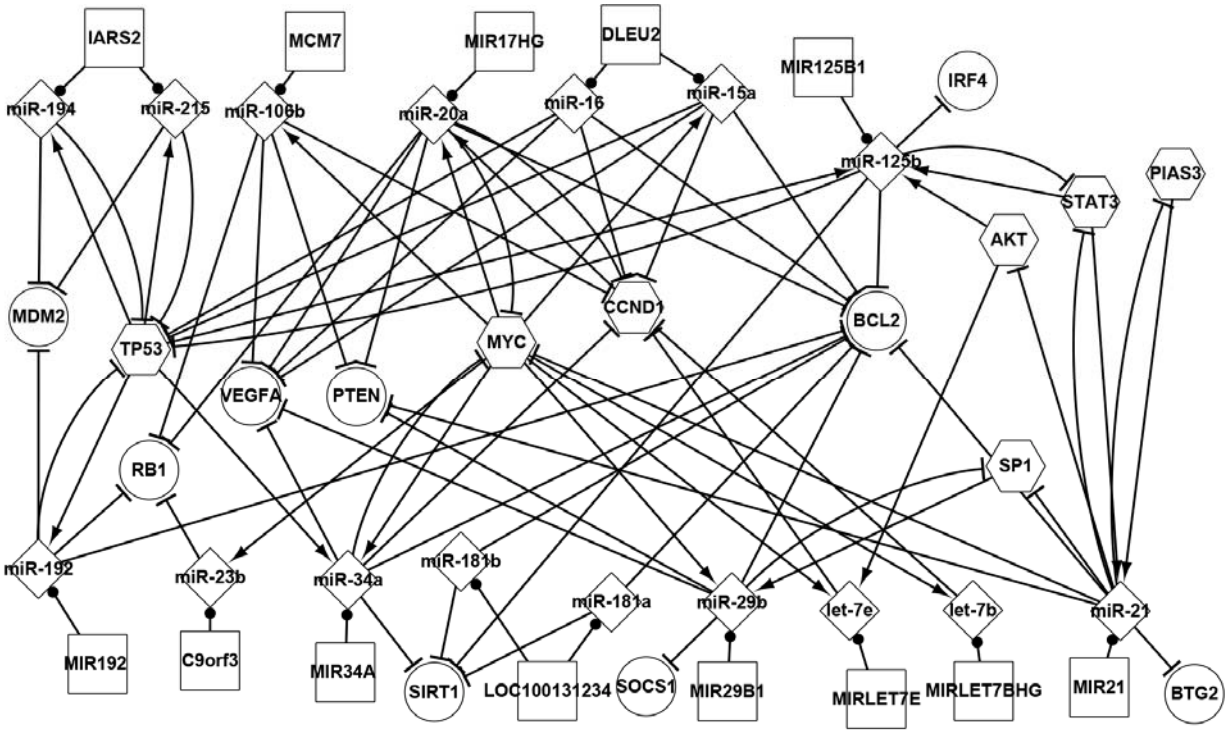

Fig. 1: The abnormally expressed network of multiple myeloma (MM)

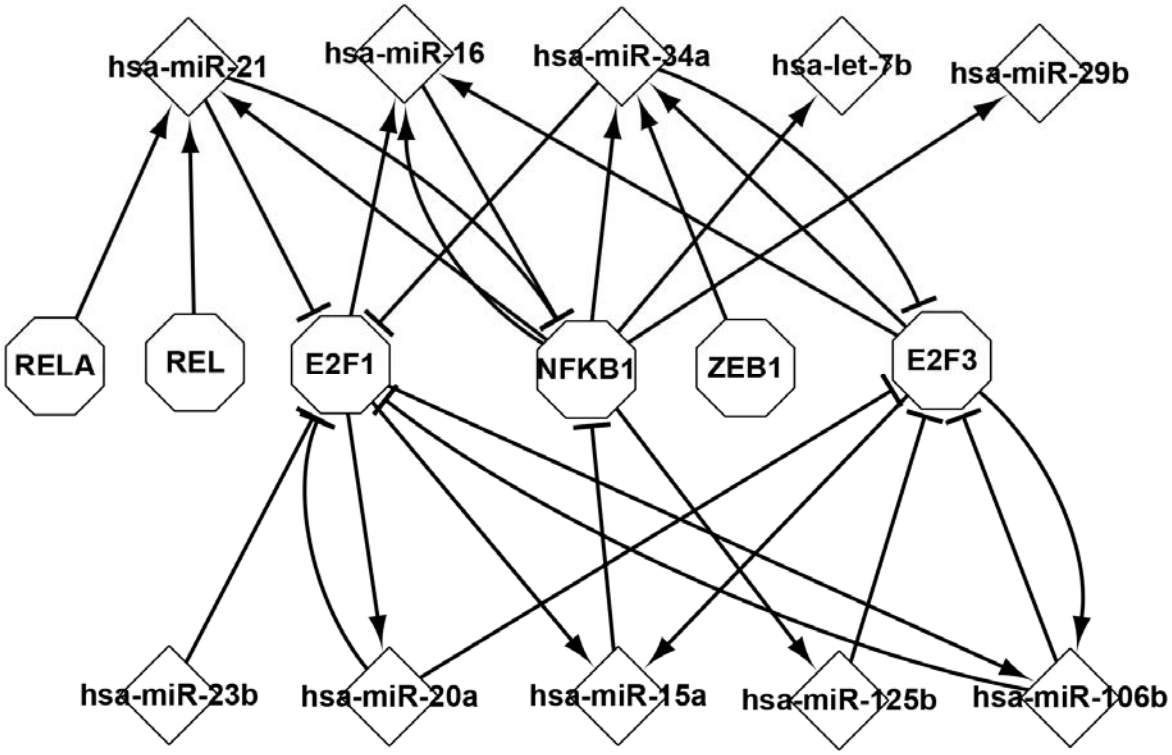

Fig. 2: Transcription network between abnormally expressed microRNAs (miRNAs) and predicted transcription factors (TFs) in multiple myeloma (MM)

Table 1: The upstream and downstream information of MYC in three regulatory networks of multiple myeloma (MM)

miRNAs that target MYC				miRNAs that are regulated by MYC		
Abnormally expressed network	Related network	Global network	Gene symbol	Abnormally expressed network	Related network	Global network
hsa-miR-20a	hsa-miR-152	hsa-let-7a	MYC	hsa-let-7b	hsa-let-7b	hsa-let-7
hsa-miR-21	hsa-miR-17	hsa-let-7c		hsa-let-7e	hsa-let-7e	hsa-let-7a
hsa-miR-34a	hsa-miR-20a	hsa-let-7g		hsa-miR-106b	hsa-miR-106b	hsa-let-7a-1
	hsa-miR-21	hsa-miR-145		hsa-miR-15a	hsa-miR-15a	hsa-let-7b
	hsa-miR-24	hsa-miR-17		hsa-miR-20a	hsa-miR-17	hsa-let-7c
	hsa-miR-34a	hsa-miR-20a		hsa-miR-23b	hsa-miR-17-3p	hsa-let-7d
	hsa-miR-405	hsa-miR-21		hsa-miR-29b	hsa-miR-17-5p	hsa-let-7e
		hsa-miR-24		hsa-miR-34a	hsa-miR-18a	hsa-let-7f
		hsa-miR-26a			hsa-miR-19a	hsa-let-7f-1
		hsa-miR-34a			hsa-miR-19b	hsa-let-7g
		hsa-miR-34b			hsa-miR-19b-1	hsa-let-7i
		hsa-miR-34c			hsa-miR-20	hsa-miR-106a
		hsa-miR-371a			hsa-miR-20a	hsa-miR-106b
		hsa-miR-373			hsa-miR-22	hsa-miR-141
		hsa-miR-378			hsa-miR-221	hsa-miR-15a
		hsa-miR-451a			hsa-miR-23b	hsa-miR-16-1
		hsa-miR-98			hsa-miR-29b	hsa-miR-17
					hsa-miR-34a	hsa-miR-18a
					hsa-miR-92-1	hsa-miR-195
					hsa-miR-92a	hsa-miR-19a
					hsa-miR-93	hsa-miR-19b
						hsa-miR-20a
						hsa-miR-20b
						hsa-miR-22
						hsa-miR-221
						hsa-miR-23a
						hsa-miR-23b
						hsa-miR-26a
						hsa-miR-26b
						hsa-miR-29a
						hsa-miR-29b
						hsa-miR-29c
						hsa-miR-34a
						hsa-miR-9
						hsa-miR-92a
						hsa-miR-93

Regulatory pathways of abnormally expressed genes among three networks

With the aim to clearly demonstrate the MM's regulatory network, the upstream as well as downstream information of the abnormally expressed genes, abnormally expressed miRNAs and predicted TFs was extracted.

Among three networks, the precursor and successor nodes of the abnormally expressed genes were selected out to investigate and compare the regulatory pathways. Results showed that 6 abnormally expressed genes (CCND1, MYC, PIAS3, SP1, STAT3 and TP53) with their adjacent miRNAs formed multiple self-adaptation feedback regulations.

Taking MYC for a typical instance, scientific research has validated that MYC is an outstanding regulator involved in cancer initiation and progression by regulating downstream genes and miRNAs (Guo *et al*., 2013). Table 1 indicates important interactions between MYC and its precursor and successor nodes. Two miRNAs (hsa-miR-34a and 20a) target MYC, then are regulated by MYC the other way round in the abnormally expressed network. Significantly, both feedback pathways are also contained in the other two networks. Above self-adaptation feedback regulations are meant to play key roles involved in the pathogenesis of MM. There are 7 miRNAs targeting MYC that regulates 21 miRNAs in the related network. The related network enlarges the underlying interactions between genes and miRNAs, which offers more regulatory mechanism than the abnormally expressed network. We can conclude hsa-miR-152 indirectly affects hsa-let-7b and 7e and hsa-miR-106b, 15a and so on through MYC' regulation. Compared to the front two networks, global network contains more precursor and successor nodes of MYC, which is of extraordinary

Table 2: The upstream and downstream information of hsa-miR-29b in three regulatory networks of multiple myeloma (MM)

Genes that regulate hsa-miR-29b				Genes that are targeted by hsa-miR-29b		
Abnormally expressed network	Related network	Global network	miRNA symbol	Abnormally expressed network	Related network	Global network
MYC	MYC	CEBPA	miR-29b	BCL2	BCL2	ADAM12
SP1	SP1	MYC		PTEN	CDK6	BACE1
		NFKB1		SOCS1	MCL1	BCL2
		TP53		SP1	PTEN	CDK6
		YY1		VEGFA	SOCS1	COL1A1
					SP1	COL3A1
					VEGFA	COL4A1
						DNAJB11
						DNMT1
						DNMT3A
						DNMT3B
						ESR1
						FGA
						FGB
						FGG
						GRN
						MCL1
						MMP2
						MMP24
						NCOA3
						NID1
						PTEN
						S100B
						SFPQ,
						SOCS1
						SP1
						TCL1A

importance to investigate signaling pathways involved in MM' cell activities.

Regulatory pathways of abnormally expressed miRNAs among three networks

With the same method as abnormally expressed genes, precursor and successor nodes of all the abnormally expressed miRNAs were selected. It turned out that 9 abnormally expressed miRNAs (hsa-miR-125b, 15a, 192, 194, 20a, 21, 215, 29b and 34a) as well as their corresponding genes formed significant self-adaptation feedback pathways.

Taking hsa-miR-29b for example, numerous regulatory pathways are involved in table 2. MYC and SP1 regulate hsa-miR-29b, and hsa-miR-29b targets SP1 in return among the abnormally expressed network, additionally, it targets another 4 genes. By means of hsa-miR-29b's adjustment, MYC and SP1 affect 5 genes (BCL2, PTEN, SOCS1, SP1 and VEGFA) in signaling pathways. In the related network, another NFKB1 regulates hsa-miR-29b, and another 2 genes (CDK6 and MCL1) are targeted by hsa-miR-29b. It is examined that abnormally expressed network is included by related network. Global network shows 5 genes have regulation to hsa-miR-29b and it targets 29 genes. The regulatory interactions focused on hsa-miR-29b in three network highlight hsa-miR-29b's regulation effect within various signaling pathways.

Regulatory pathways of predicted TFs among three networks

After analysis and comparison to precursor and successor nodes of predicted TFs, three TFs (E2F1, E2F3 and NFKB1) with their upstream and downstream elements formed 6 self-adaptation feedback pathways. These special feedbacks have direct effect in MM's complex regulatory pathways.

For example, regulatory pathways of E2F1 show several significant features. table 3 shows that hsa-miR-20a and 106b respectively construct self adaptation feedback pathways with E2F1. Five abnormally expressed miRNAs target E2F1, which regulates four miRNAs' expression among abnormally expressed network. Significantly,

Table 3: The upstream and downstream information of E2F1 in three regulatory networks of multiple myeloma (MM)

miRNAs that target E2F1				miRNAs that are regulated by E2F1		
Abnormally expressed network	Related network	Global network	TF symbol	Abnormally expressed network	Related network	Global network
hsa-miR-106b	hsa-miR-106b	hsa-let-7a	E2F1	hsa-miR-106b	hsa-miR-106a	hsa-let-7a
hsa-miR-20a	hsa-miR-20a	hsa-miR-106a		hsa-miR-15a	hsa-miR-106b	hsa-let-7i
hsa-miR-21	hsa-miR-21	hsa-miR-106b		hsa-miR-16	hsa-miR-15a	hsa-miR-106a
hsa-miR-23b	hsa-miR-23b	hsa-miR-126		hsa-miR-20a	hsa-miR-16	hsa-miR-106b
hsa-miR-34a	hsa-miR-34a	hsa-miR-149			hsa-miR-18a	hsa-miR-15a
	hsa-miR-93	hsa-miR-17			hsa-miR-19a	hsa-miR-15b
		hsa-miR-20			hsa-miR-20a	hsa-miR-16
		hsa-miR-203a			hsa-miR-25	hsa-miR-16-1
		hsa-miR-20a			hsa-miR-93	hsa-miR-16-2
		hsa-miR-21				hsa-miR-17
		hsa-miR-223				hsa-miR-18a
		hsa-miR-23b				hsa-miR-18b
		hsa-miR-330				hsa-miR-195
		hsa-miR-331				hsa-miR-19a
		hsa-miR-34a				hsa-miR-19b
		hsa-miR-93				hsa-miR-19b-2
		hsa-miR-98				hsa-miR-20a
						hsa-miR-20b
						hsa-miR-223
						hsa-miR-25
						hsa-miR-363
						hsa-miR-449a
						hsa-miR-449b
						hsa-miR-449c
						hsa-miR-92a
						hsa-miR-92a-2
						hsa-miR-93

miRNAs indirectly affect other miRNAs by E2F1's effect. Hsa-miR-106b has effect on hsa-miR-15a, 16 and 20a through E2F1's control. Compared to abnormally expressed network, related network shows more upstream and downstream information that more miRNAs target E2F1 and are regulated by E2F1. The related network also contains extra self-adaptation feedback regulations, such as hsa-miR-93 and E2F1 form feedbacks. The global network indicates abundant upstream and downstream information which offers a compositive unstanding of regulatory pathways of predicted TFs.

DISCUSSION

The current study mainly constructed and demonstrated three genetic regulatory network, abnormally expressed network and related network as well as global network. Results indicated complex regulatory interactions among TFs, miRNAs and genes, for instance, hsa-miR-192 targets BCL2, AKT regulates hsa-let-7e and MCM7 hosts hsa-miR-106b, which revealed latent regulatory mechanism associated with MM' development. The abnormally expressed network also showed certain remarkable signaling pathways. Hsa-miR-34a targets MYC, which regulates it conversely, which proves that self-adaptation feedback regulation plays a vital role in cancer progression. In addition, in multiple signaling pathways, upstream nodes of specific gene or miRNA always affect its downstream nodes, which give a further understanding of mechanism. Six abnormally expressed genes (CCND1, MYC, PIAS3, SP1, STAT3 and TP53) with their correlative miRNAs form self-adaptation feedbacks and nine abnormally expressed miRNAs (hsa-miR-125b, 15a, 192, 194, 20a, 21, 215, 29b and 34a) with their correlative genes are regulated from each other. Although specific pathways are not identified in MM's regulatory network, they can participate in biological regulation of other carcinomas. For instance, through the EGFR/AKT pathway, hsa-miR-133a targets the epidermal growth factor receptor and regulates breast cancer's proliferation eventually (Cui *et al*., 2013; Floriano-Sanchez *et al*., 2014). PTEN relieves YY1 to induce HIF-2's activity among renal carcinoma cells (Petrella and Brinckerhoff, 2009). Predicted TFs retrieved by P-match algorithm implied underlying interactions among abnormally expressed miRNAs and TFs, these interactions need further research efforts and experimental validation.

CONCLUSION

In conclusion, the present study, especially the abnormally expressed network, partially uncovered associated regulatory mechanism with regard to MM's

proliferation, differentiation and apoptosis. Our research points out the abnormally expressed network can be underlying fault graph of MM's canceration. And this network's significance lies in that if medical measure can be taken to correct each faulty regulation to normal regulation, thus each genes and miRNAs will return to its normal expression level, and canceration of MM may be prevented and not occur. Additionally, related network and global network offered more overall regulatory interactions and substantial materials for future investigation. The three genetic regulatory networks will contribute to further pathogenesis research and effective treatment of MM. In future research, low-expression, over expression and mutation of genes, and down regulation and up regulation of miRNAs should be put forward and discussed further. As a guess, we propose that there exist chief genes and miRNAs in carcinoma's whole regulatory network, which perform decisive regulation effects and control the network. These significant regulators are worthy to be laid great emphasis on.

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