Transcriptome Analysis of lncRNA-mRNA Interactions in the Non-Small Cell Lung Cancer

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Research article

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Abstract

As a serious malignancy with high incidence and fatality, non-small cell lung cancer (NSCLC) has affected millions of people each year worldwide. However, the 5-year survival rate of NSCLC is still not satisfactory. Therefore, it is particularly imperative and necessary to explore the underlying molecular mechanisms of NSCLC to contribute to the potential therapeutic targets and strategies. Six clinical samples including three normal tissues and three cancer tissues obtained from patients diagnosed with NSCLC were sequenced through Illumina Hiseq2500 sequencer. Differentially expressed lncRNAs and mRNAs analysis were implemented using the edgeR, and the function of differentially expressed lncRNAs and mRNAs were analyzed with GO and KEGG enrichment analysis. Then, top 10 dysregulated lncRNAs and mRNAs were screened out. Finally, the co-expression, biological function and pathway networks of dysregulated lncRNA with the predicted target genes were constructed. Differentially expressed lncRNAs and mRNAs could distinguish cancerous tissues from normal tissues. The functions and KEGG pathway of differentially expressed lncRNAs and mRNAs mainly were enriched in immune response, metabolism, phagosome, cytokine receptor interaction and IL-17 signaling pathway. The co-expression network revealed that top ten dysregulated lncRNAs were interacted with IGH gene cluster. Furthermore, the biological function and pathway network also indicated that dysregulated lncRNAs were involved in immune process. The results demonstrated that the biologic function of dysregulated lncRNAs that may be involved in immune process. Our data provides a foundation of the biologic significances of lncRNAs to contribute to the therapeutic targets and strategies for NSCLC to promote the survival for patients.

1 Introduction

Lung cancer is one of most serious and common disease with high incidence and mortality, which has affected millions of people each year worldwide (Bray, et al., 2018). Lung cancer comprises non-small cell lung cancer (NSCLC) and small-cell lung cancer (SCLC) (Davidson, et al., 2013). Among them, NSCLC accounts for more than 85% of all lung cancers (Pastorino, 2010). Similarly, NSCLC mainly contains adenocarcinoma and squamous cell carcinoma two subtypes according to its pathological appearance (Pastorino, 2010, Sève, et al., 2010). Although substantial efforts, such as radiotherapy and chemotherapy have targeted on NSCLC, the 5-year survival rate of NSCLC is just about 15% due to the absence of apparent symptoms at the early stages (Peters, Siegel, 2019, Travis, 2011). Therefore, it is particularly necessary to explore the underlying molecular mechanisms of NSCLC to contribute to the potential therapeutic targets and strategies.

Currently, more and more researches have demonstrated that non-coding RNAs (ncRNAs) play a vital role in several biological processes (Slack and Chinnaiyan, 2019). As one of most important ncRNA, the long non-coding RNA (lncRNA) containing > 200 nucleotides is known to involve in cancer (Reis and Verjovski-Almeida, 2012, Spizzo, et al., 2012). The dysregulated expression of lncRNAs has been proved to promote a variety of tumors development and progression. In NSCLC, over-expression of lncRNA-HIT promotes cancer cells migration and invasion by association with ZEB1 (Jia, et al., 2016); upregulated lncRNA SNHG1 contributes to the cancer progression by inhibition of miR-101-3p and activation of WNT/β-

catenin signaling pathway (Liu); lncRNA MALAT1 contributes to the cancer development through regulating miR-206 and miR-124 (Deng, 2018, Hu) and lncRNA UCA1 evokes nonT790M acquired resistance to EGFR-TKIs via activation of AKT/mTOR pathway (Cheng, et al., 2015). Additionally, lncRNAs were also reported to be a potential biomarker in NSCLC widely (Damjan, Osielska and Jagodziński, 2018). Although the role of different kinds of lncRNAs and their underlying mechanisms in NSCLC have been partially reported, it is also imperative and obligatory to understand the mechanisms of the interactions between lncRNAs and protein-coding genes mainly mRNAs in NSCLC.

Thus, in this present study, we studied the expression and interactions of lncRNA and mRNA in the cancer tissues of patients with NSCLC. We wish the results could contribute to the therapeutic targets and strategies for NSCLC to promote the survival for patients.

2 Materials And Methods

2.1 Clinical samples

All the experiments protocol complied with ethical standards as determined by the ethical committee of Sichuan Cancer Hospital (Chengdu, China), and written informed consent was permit from every participant. The clinical samples including three normal tissues and three cancer tissues were obtained from patients diagnosed with NSCLC at Sichuan Cancer Hospital (Chengdu, China) from January 2018 to June 2018. The normal tissues were taken at a distance of at least 5 cm from the tumor and there were no obvious tumor cells. All patients were diagnosed histologically. All the clinical specimens were stored at -80 °C for further use. The detailed information of patients is summarized in Table 1.

2.2 Total RNA extraction

Total RNA of all six samples was extracted using Animal Total RNA isolation Kit (FOREGENE, RE-03014) according to the manufacturer's instruction. The quality of the obtained RNA was detected with a Nanodrop 2000 spectrophotometer (Thermo Scientific, Worcester, MA, USA), and the absorbance ratio of 260/280 nm was limited to 1.8-2.0.

2.3 LncRNA library construction and Illumina sequencing

Total RNA was depleted ribosomal RNA using Ribo-Zero™ Magnetic Kit (Epicentre) in accordance with the manufacturer’s instructions. After purification, divalent cations were used to fragment RNA into small fragments at high temperature. Subsequently, the RNA fragments were reverse-transcribed to create the final cDNA library using the RNA-Seq sample preparation kit (Illumina, San Diego, USA) according to the manufacturer’s instructions. The single-end sequencing (50 nt) were executed in accordance with the vendor’s recommended protocol for the Illumina Hiseq2500 sequencer at the LC Biotech (Hangzhou, China). Differentially expressed lncRNAs and mRNAs analysis was implemented using the edgeR (Robinson, et al., 2010). The screening criteria for the differentially expressed genes changed more than twofold, and the differences were considered to be statistically significant at $P<0.05$.

2.4 Functional annotation of differentially expressed lncRNAs and mRNAs

Functional annotation were executed through Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis using GO seq R package and KOBAS software, respectively (Mao, et al., Young, et al., 2010) to decide the biological significance of lncRNA targeting differentially expressed mRNA.

2.5 Construction of lncRNA-mRNA networks

The functional links between dysregulated lncRNAs and the predicted mRNAs were identified, and lncRNAs contained the cis-prediction and trans-prediction (Stadler, 2014). The dysregulated lncRNAs were been selected as the hub with the respective differential mRNAs (Correlation≥0.99 or Correlation<-0.99, and P-value<0.05). The interaction networks of lncRNAs and mRNAs were constructed with the Cytoscape Software (version 3.1.0).

2.6 Statistical analysis

All statistical analyses of data were analysed by one-way analysis of variance and Duncan's test using the SPSS 19.0 package (SPSS Inc. Chicago, IL, USA), and the differences were considered statistically non-significant and significant when $p > 0.05$ and $p < 0.05$, respectively.

3 Results

3.1 Differentially expressed mRNAs and lncRNAs

Six clinical samples including three normal tissues and three cancer tissues were obtained from patients diagnosed with NSCLC and used for sequencing analysis. The differentially expressed mRNAs and lncRNAs in different groups were calculated. Compared with normal tissues, 1327 mRNAs in case 1, 3493 mRNAs in case 2 and 1315 mRNAs in case 3 were upregulated and 1349 mRNAs in case 1, 4280 mRNAs in case 2 and 1273 mRNAs in case 3 were downregulated in NSCLC tissues. Additionally, there were 2709 upregulated-mRNAs and 1846 downregulated- mRNAs in three total NSCLC tissues compared with three total normal tissues (Fig. 1A). Consistently, compared with normal tissues, 544 lncRNAs in case 1, 707 lncRNAs in case 2 and 438 lncRNAs in case 3 were upregulated and 660 lncRNAs in case 1, 2062 lncRNAs in case 2 and 480 lncRNAs in case 3 were downregulated in NSCLC tissues. And 449 upregulated-mRNAs and 281 downregulated- mRNAs present in three total NSCLC tissues compared with three total normal tissues (Fig. 1B). Briefly, these results suggested that these genes could distinguish cancerous tissues from normal tissues.

3.2 GO and KEGG enrichment analysis

Functional annotation were executed through GO and KEGG enrichment analysis of the differentially expressed mRNAs and lncRNAs above, thereby contributing to the biological significance of the lncRNA and mRNA. The results revealed that the majority of the top 20 GO function according to P value were

involved immune process, such as immune system process, regulation of immune response, adaptive immune response, antigen binding and complement activation, classical pathway and so on (Fig. 2A). Moreover, according to the KEGG pathway analysis, the top 20 KEGG pathway according to P value were related to metabolism, such as mineral absorption, nitrogen metabolism, alanine, aspartate and glutamate metabolism and arginine biosynthesis and so on, phagosome, cytokine-cytokine receptor interaction, chemokine signaling pathway and IL-17 signaling pathway and so on(Fig. 2B). Overall, the data indicated that the process of immune response, metabolism, phagosome, cytokine receptor interaction and IL-17 signaling pathway mainly functioned in NSCLC.

3.3 The dysregulated expression of lncRNAs and mRNAs

To explore the biologic function of lncRNAs and mRNAs, we firstly screened out the top 10 dysregulated lncRNAs and mRNA (Fig. 3A and B). Among 10 dysregulated lncRNAs, eight lncRNAs (HOXC-AS2, IGHV3-66, MIR8071-2, IGHV1-69, AL590666, AL365181, MIR8071-1 and FAM30A) were upregulated, and two lncRNAs (ADAMTS9-AS2 and AC092053) were downregulated in NSCLC (Table 2). However, all the 10 dysregulated mRNAs (EEF1A2, PPP2R2C, MUC5AC, AKR1B10/15, ONECUT1, B3GNT3, GNG4, NPY, DKK1 and IGF2BP1) were upregulated in NSCLC (Table 2). Furthermore, four deregulated lncRNAs still remained novel, and the HOXC-AS2, AL590666, AL365181, FAM30A, ADAMTS9-AS2 and AC092053 have been known to be involved in NSCLC (Table 2).

3.4 The construction of lncRNA-mRNA co-expression networks

To further detect the biologic function of dysregulated lncRNAs, we then constructed the co-expression network of dysregulated lncRNAs. Using the dysregulated lncRNAs as the center of the network, we can clearly see possible regulated target genes (Fig. 4). The results showed that the entire network was composed of 7 independent parts, the largest part had 28 nodes, and the smallest is only one-to-one regulation. Importantly, among these ten dysregulated lncRNAs, half of them (FAM30A, IGHV3-66, IGHV1-69, MIR8071-2 and MIR8071-1) interacted with IGH gene cluster, indicating that dysregulated lncRNAs may be association with immune process (Fig. 4).

3.5 The construction of lncRNA-mRNA biological function and pathway network

To verify the biologic function of dysregulated lncRNAs, the lncRNA-mRNA biological function and pathway network was constructed. Besides the basic biological functions, such as plasma membrane, external side of plasma membrane, extracellular region and membrane, the dysregulated lncRNAs were mainly involved in immune process, such as immune response, immune system process, immunoglobulin receptor bingding, adaptive immune response, Fc-gamma receptor signaling pathway involved in phagocytosis, antigen binding, immunoglobulin complex, circulating and positive regulation of B cell activation. Additionally, complement process, such as regulation of complement activation and complement activation, classical pathway, phagocytosis process such as phagocytosis recognition and phagocytosis engulfment were also correlation with the dysregulated lncRNAs (Fig. 5). Shortly, the data suggested that the dysregulated lncRNAs were indeed related with immune process.

4 Discussion

Non-small cell lung cancer is one of most common malignancy worldwide, and the majority of cases of NSCLC are diagnosed at an advanced stage clinically owing to the absence of apparent symptoms at the early stages. Therefore, it is more significant to elucidate the underlying molecular functions and potential enrichment pathways of NSCLC. In this present study, we investigated the function and pathway of differently expression of lncRNAs and mRNAs as well as the co-expression, biological function and pathway network of dysregulated lncRNA with the predicted target genes in the cancer tissues of patients with NSCLC. This study revealed that differently expression of lncRNAs and mRNAs could distinguish cancerous tissues from normal tissues, and both the differently expression of lncRNAs and mRNAs and the dysregulated lncRNAs were involved in immune process.

Over the past decade, with the advanced development of whole-genome and transcriptome sequencing technologies and the ENCODE project, it is generally acknowledged that the vast majority of genomic DNA is represented by processed transcripts that lack protein-coding capabilities (2012). Among the non-coding genes, lncRNAs are non-negligible. Previous studies have demonstrated that lncRNAs are association with metabolism, cell cycle regulation, migration and survival (Gupta, et al., 2010). More importantly, the dysregulated lncRNAs always contributed to the occurrence and development of multiple human cancers through cell metastasis, proliferation, invasion and apoptosis (Malouf, et al., 2015, Thai, et al., 2013, Vance, et al., 2014). In our study, the differently expression of lncRNAs and mRNAs also present in the cancerous tissues from patients of NSCLC. Moreover, we found that both the numbers of the differently expression of lncRNAs and mRNAs were higher in case 2 than these in case 1 and case 3. We guessed this phenomenon may be due to the different histology subtype in line with the results reported previously (Li, et al., 2020). Although adenocarcinoma and squamous cell carcinoma two subtypes are very similar in clinical manifestations, they are significantly different in pathogenesis, treatment and prognosis (Zhan, et al., 2015). Hence, the differently expression of lncRNAs and mRNAs in case 2 belonging to squamous cell carcinoma may be different from the two others in case 1 and case 3. Additionally, 80% of the top ten dysregulated lncRNAs are upregulated, indicating that increased lncRNAs may play a more vital role than the decreased lncRNAs. Furthermore, six of the top ten dysregulated lncRNAs have been identified. Therein, the downregulated ADAMTS9-AS2 has been reported recently (Acha-Sagredo, et al., 2020). Encouragingly, there are still four novel lncRNAs in the top ten dysregulated lncRNAs. Due to significant roles of lncRNA in the cancer biomarkers (Damjan), further study can be focus on these novel lncRNAs.

As one of most fundamental biological functions of lncRNAs, the regulation of neighboring coding genes has been revealed in past researches (Ponjavic, et al., 2009). In our study, we constructed the networks of co-expression and biological function and pathway of the dysregulated lncRNAs and the target genes. The results both suggested that the dysregulated lncRNAs have been related in the immune processes. Besides the various cellular responses, such as cell proliferation, differentiation, and apoptosis (Kretz, et al., 2013, Kretz, et al., 2012, Wang, et al., 2014), more and more studies have demonstrated that lncRNAs are crucial in the regulation of the immune system (Chen, et al., 2017, Hur, et al., 2019). More importantly,

it has found that immune-related lncRNAs are association with cancers. For example, lncRNA LINK-A decreased cancer cell antigen presentation (Hu, et al., 2019). LncRNA Lnczc3h7a promotes a TRIM25-mediated RIG-I antiviral innate immune response (Lin, et al., 2019). NKILA lncRNA promotes tumor immune evasion by sensitizing T cells to activation-induced cell death (Huang, et al., 2018). In this present study, the networks of the dysregulated lncRNAs indicated that lncRNAs may play the roles of in cancer immunology. Thus, as the immunotherapy has emerged as a hopeful cancer treatment strategy (Kaufmann, 2019), further experimental study need to apply to contribute to the therapeutic strategies for NSCLC.

In conclusion, the results from our study indicated the dysregulated lncRNAs in the cancerous tissues from patients in NSCLC were involved in immune process. Our finding can lay a foundation of the therapeutic targets and strategies for NSCLC.

Declarations

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Consent for publication

All authors agree to publish.

Availability of data and material

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

Competing interests

The authors declare that they have no competing interests.

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Not applicable

Authors' contributions

BL, JL and YSW proposed the hypothesis, analyzed the results, and wrote the manuscript. BL, JL and YSW designed and executed the majority of the experiments. BL, JL, JML, GYL and YSW assisted in the execution of a part of the experiments. All authors read and approved the final version of the manuscript.

Ethics approval

All the experiments protocol complied with ethical standards as determined by the ethical committee of Sichuan Cancer Hospital (Chengdu, China)

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Tables

Table 1
Clinicopathological features analysis of NSCLC patients

Characteristic	Case_1	Case_2	Case_3
Age (years)	54	67	64
Gender	Male	Male	Female
Smoking history	Yes	Yes	No
Histology subtype	ADC	SSC	ADC
EGFR mutation	Mutated	Wild type	Mutated
Tumor size (cm)	≥ 5	≥ 5	≤ 5
Tumor capsular	Complete	Complete	Incomplete
TNM stage	T2b	T2b	T2a

Table 2
List of the dysregulated expression of the top 10 lncRNAs and mRNAs and the possible mechanisms of their involvement.

lncRNA	Statue		mRNA		Pathway
HOXC-AS2	known	up	EEF1A2	up	RNA transport
IGHV3-66	novel	up	PPP2R2C	up	PI3K-Akt signaling pathway/ AMPK signaling pathway/ Hippo signaling pathway/
MIR8071-2	novel	up	MUC5AC	up	IL-17 signaling pathway
IGHV1-69	novel	up	AKR1B10/15	up	Pentose and glucuronate interconversions
AL590666	known	up	ONECUT1	up	Signaling pathways regulating pluripotency of stem cells
AL365181	known	up	B3GNT3	up	Glycosphingolipid biosynthesis - lacto and neolacto series
MIR8071-1	novel	up	GNG4	up	PI3K-Akt signaling pathway/ Ras signaling pathway/ Chemokine signaling pathway
FAM30A	known	up	NPY	up	cAMP signaling pathway/ Adipocytokine signaling pathway
ADAMTS9-AS2	known	down	DKK1	up	Wnt signaling pathway
AC092053	known	down	IGF2BP1	up	MicroRNAs in cancer

Figures

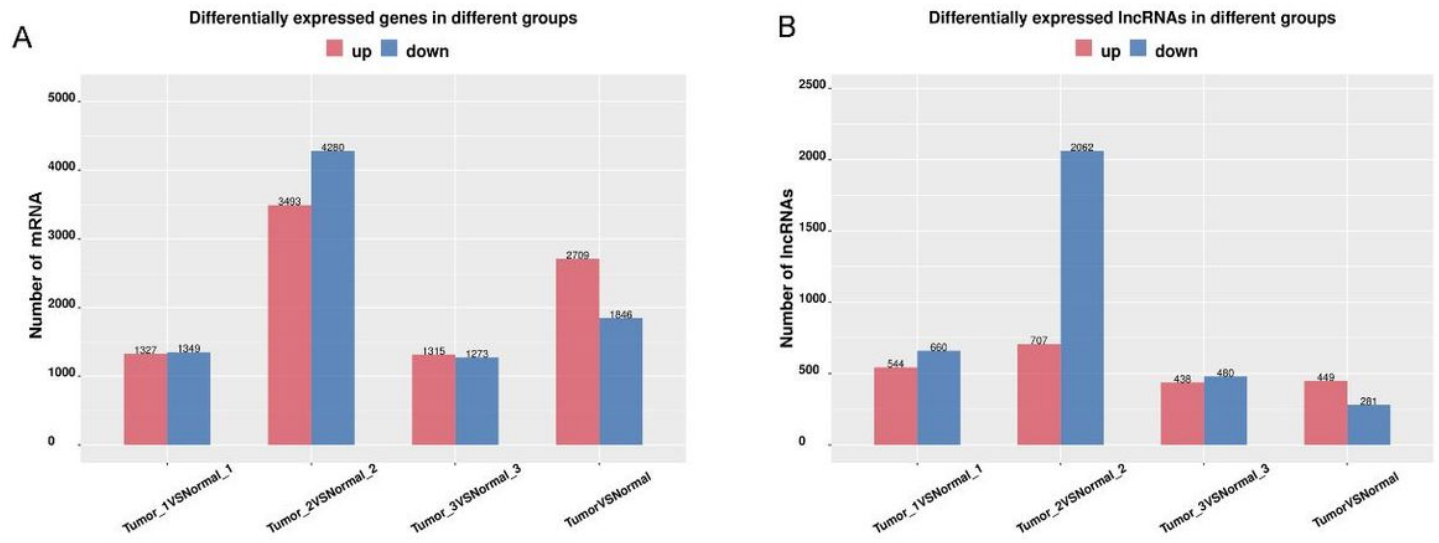

Figure 1

Differentially expressed mRNAs and lncRNAs. (A) Numbers of upregulated-mRNA and downregulated-mRNA. (B) Numbers of upregulated-lncRNA and downregulated-lncRNA.

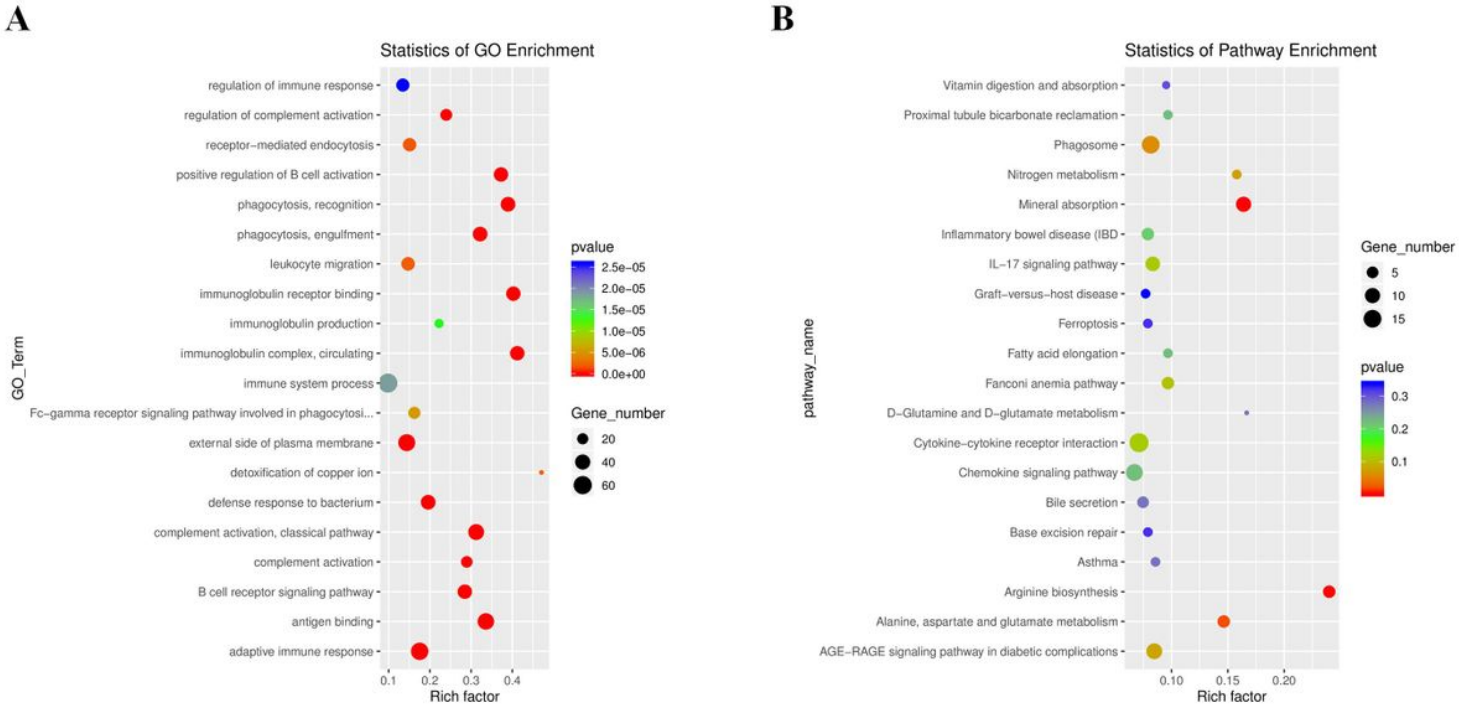

Figure 2

The GO and KEGG enrichment analysis. (A) Top 20 GO function of differentially expressed mRNAs and lncRNAs according to P value. (B) Top 20 KEGG pathway of differentially expressed mRNAs and lncRNAs according to P value.

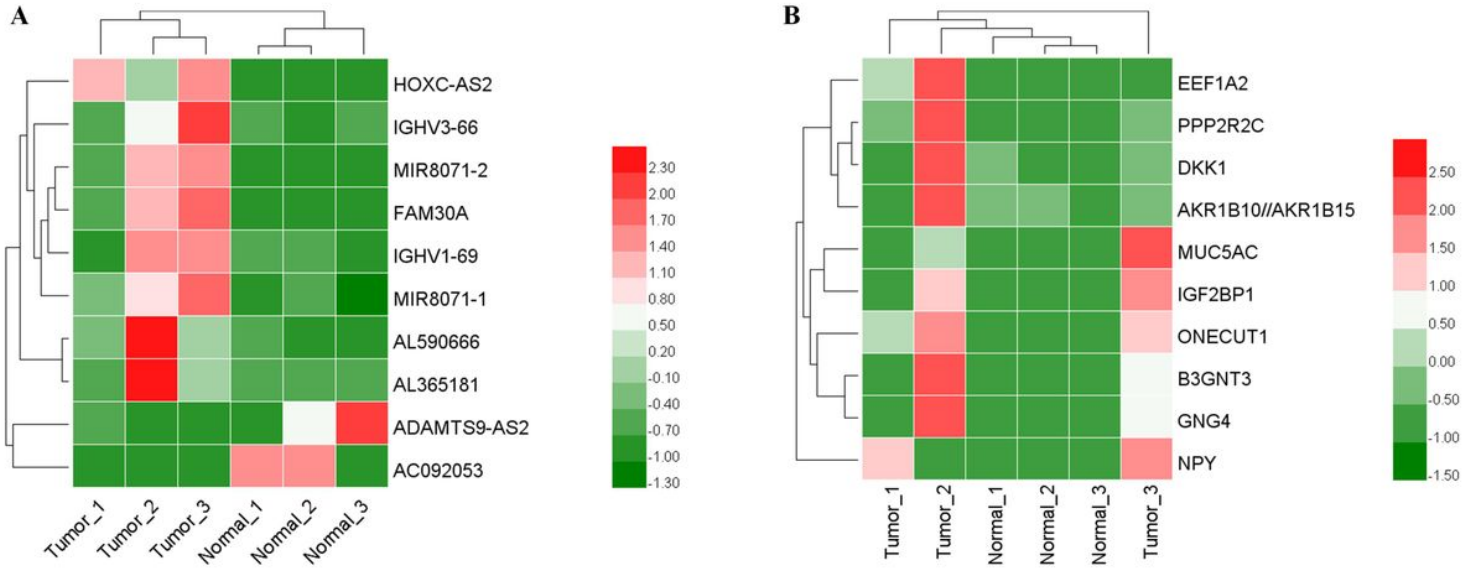

Figure 3

The dysregulated expression of the top 10 lncRNAs and mRNAs with a heat map. (A) Heat map of the dysregulated expression of the top 10 lncRNAs. (B) Heat map of the dysregulated expression of the top 10 mRNAs.

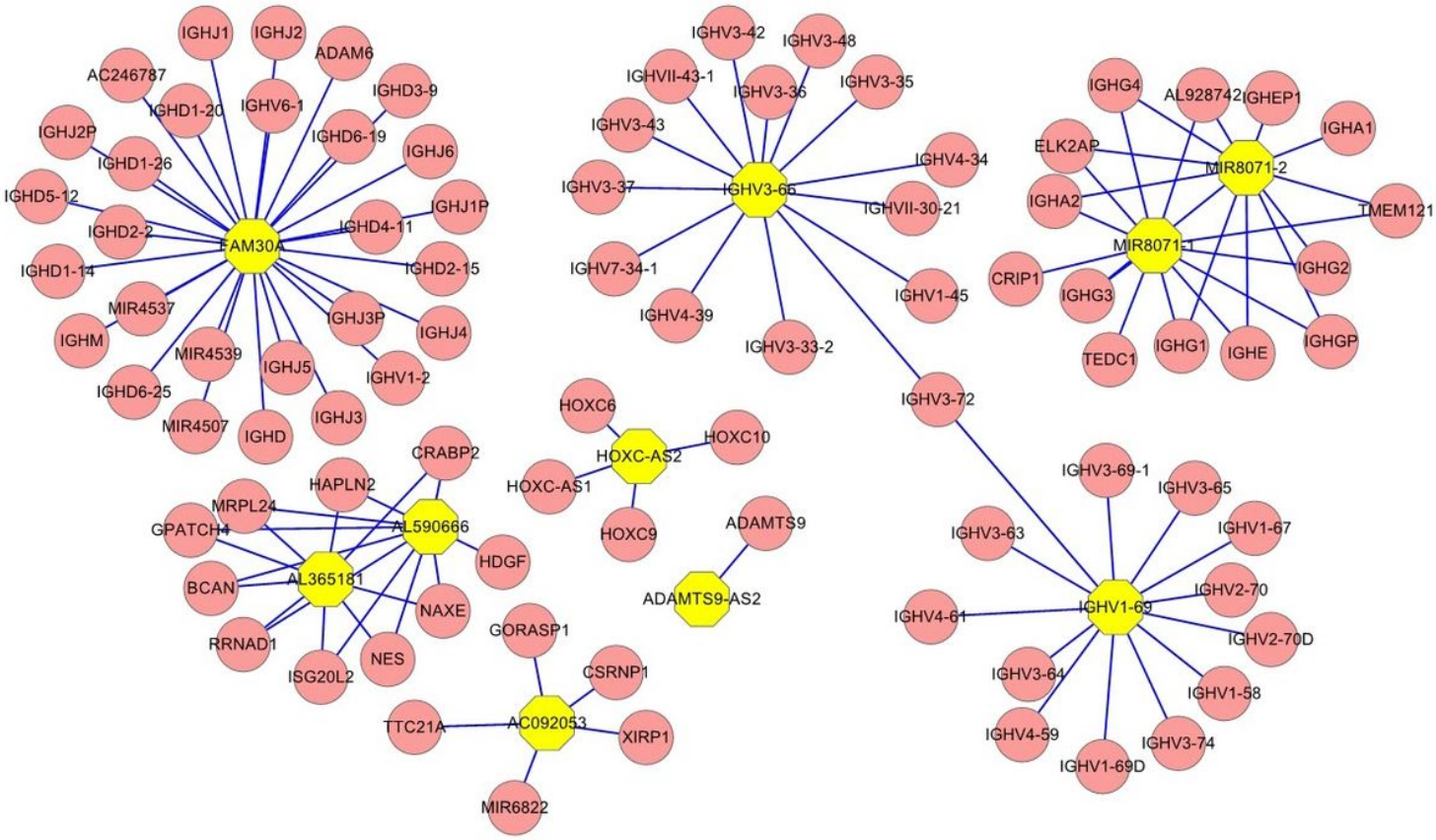

Figure 4

The predicted target genes of the dysregulated 10 lncRNAs. In the networks, dysregulated lncRNAs were as the center marked with yellow and the predicted target genes were marked with red.

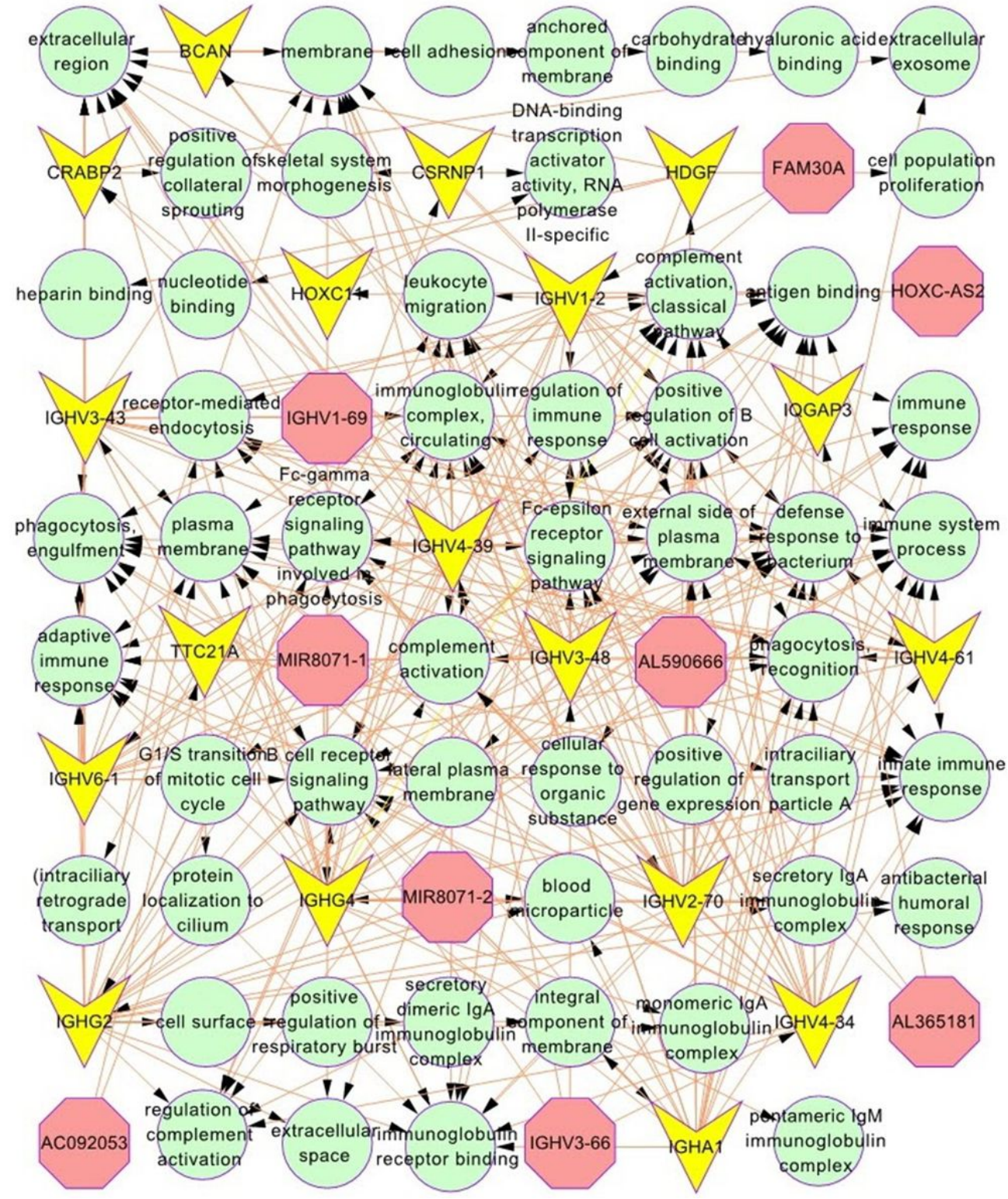

Figure 5

The lncRNA-mRNA biological function and pathway network. The dysregulated KEGG pathways of lncRNAs and the predicted target genes were constructed. In the network, dysregulated lncRNAs were as the center marked with red and the predicted target genes were marked with yellow.