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The Identification of Sub-pathways in Juvenile Idiopathic Arthritis by Integrating Expression Profiles between lncRNA-mRNA and Pathway Topologies

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ABSTRACT Juvenile Idiopathic Arthritis (JIA) is a systemic disorder with autoimmune chronic joint inflammation. The pathogenic mechanisms of JIA are still unclear. It has been reported that lncRNA can regulate the mRNA by competitively binding to miRNAs. Analysis of pathway underlying certain disease is a valuable strategy for exploring the functional roles of these transcripts. Therefore, identification of competitively regulated subpathways can not only contribute to understand the occurrence and development of diseases, but also further help to gain the functional roles of lncRNAs. In this work, an effective method was proposed to identify the subpathways that competitively regulated by lncRNAs in JIA, which integrated the lncRNA-mRNA expression profile and pathway topologies. Eventually, based on the expression profile of JIA, 38 subpathways involved in 31 complete pathways were confirmed. Some key lncRNAs associated with JIA may be detected by identification of lncRNA competitively regulated subpathways.

INTRODUCTION

Juvenile Idiopathic Arthritis (JIA) and Rheumatoid Arthritis (RA) are systemic disorders, characterized by autoimmune chronic joint inflammation, and have some similar clinical and pathological features, such as an intense pro-inflammatory response to joints and tissue destruction (Kobus et al. 2016; Wang et al. 2017). It has been reported that the occurrence of JIA is a complex genetic trait affected by multiple genes that associated with immunity and inflammation (Hinks et al. 2017). However, up till now, the pathogenic mechanisms of JIA are still unclear. Therefore, identifying the function of genes in JIA is useful to understand effectively the occurrence and development of JIA.

Long non-coding RNA (lncRNA) is a heterogeneous class of ncRNA which plays an important role in the occurrence and progression of diseases by regulating various biological functions (Pradeepa et al. 2017; Sun et al. 2017). A large number of reports demonstrated that lncRNAs can share identical miRNA binding sites with mRNAs to competitively regulate the expression levels of mRNAs, which is a significant universal layer of RNA regulation (Wang et al. 2016; Wang et al. 2015). Interestingly, a new regulatory circuitry identified by Lin et al. (2016) revealed that large intergenic non-coding RNAs (lincRNAs) can act as a competing endogenous RNA (ceRNA) to regulate the concentration and biological functions of miRNAs, since such ceRNAs can mediate the expression levels of miRNA molecules on their targets, and thereby causing a supplemental level of post-transcriptional regulation was imposed. Fu et al. (2017) found that lincRNA-RoR can act as a competing endogenous RNA (ceRNA) to regulate several tumor inhibitor miRNAs. The proliferative and invasive abilities of cells in pancreatic ductal adenocarcinoma can be inhibited by lincRNA-RoR. Additionally, it has been proposed by Sumazin et al. (2011) that the oncogenic pathways in glioblastoma can be regulated by the competing interaction networks between RNA-RNA. Since the mechanisms by which lncRNAs impact the activation of aberrant pathway associated with diseases are not fully understood, and the competitive interaction of RNA can affect significant functions of disease, thus the identification of lncRNA competitive regulatory pathways not only can insight into the potential mecha-

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nism and also contribute to investigate the functional roles of lncRNAs in disease.

Although it has been demonstrated that the competitive regulatory function of lncRNAs plays an important role in the occurrence and development of diseases. However, very few methods are provided to systematically predict aberrant pathways competitively regulated by lncRNAs in disease. Recently, several methods are proposed to explore the functional roles of lncRNAs in diseases (Wang et al. 2017). For example, Cabili et al. (2011) used chromatin-state maps to identify putative functions of numerous lincRNA. He et al. (2017) developed a lncRNA-protein coding gene co-expression network and applied it to predict the functions of lncRNAs. Although the methods that have been proposed are significant in identification of lncRNAs functions and regulation, the functional roles of lncRNAs that contribute to disease states cannot be investigated by these methods. Additionally, most of these methods can identify functions only for a single lncRNA. Since the occurrence and progression of the disease are hardly determined by a single factor alone, it is more informative for the evaluation of lncRNAs functions by identifying sets of risk lncRNAs. Some studies have reported that the different, but related pathways under different situations may be collectively affected by multiple risk-associated lncRNAs. Therefore, novel detection strategies and methods are required for analyzing the function of lncRNAs. Some studies have shown that abnormalities of lncRNAs in "subpathway regions" play a crucial role in etiology of disease (Xu et al. 2017; Zhang et al. 2017), and the destabilization of signaling pathways that contribute to human disease may be caused not only by dysfunctional nodes, but also by the interactions of dysfunctional molecular outside of those nodes (Sun and Zhang 2017).

Objectives

A possible inference is that dysregulation of lncRNAs may affect the development of disease by regulating subpathway regions. And founding subpathway regions related to dysregulated lncRNAs may contribute to explore the mechanisms by which lncRNAs act on disease states. In this work, an effective computational technique was proposed for evaluating the competitively regulated signaling subpathway of lncRNAs under certain conditions. In this process, the researchers integrated transcriptional expression, lncRNAs- mRNAs association network and pathway topologies to identify the subpathway of lncRNAs competitive regulatory and predict the function of lncRNAs in disease states.

MATERIAL AND METHODS

Juvenile Idiopathic Arthritis Datasets

In this work, datasets associated with JIA were obtained from ArrayExpress (https://www.ebi.ac.uk/arrayexpress/experiments/E-GEOD-26554/) that is recognized database of microarray gene expression data. The expression data of JIA that were analyzed in this paper from a genome-wide association study (GWAS), which was constructed by 814 Caucasian JIA cases and 3058 Caucasian controls. In this study, expression sets of 91 samples were selected for further analysis, including 23 healthy samples (control group) and 68 JIA samples (disease group). Consequently, after pretreatment, expression profile data of 20514 genes were detected by mapping between the genes and the probes.

Obtaining the Interaction Data between lncRNA-mRNA

Firstly, the interaction data of lncRNA-miRNA were extracted from starBase v2.0 whereas the TarBase, mirTarBase, mir2Disease and miRecords (V4.0) tools were used to collect the experimentally validated mRNA-miRNA interactions. Because miRNA was shared by lncRNA and mRNA, thereby the researchers could construct a competing triplet related to lncRNA (lncRNA-miRNA-mRNA relationships), and then obtained candidate competitively regulated relationship between lncRNA-mRNA. To ensure the reliability of the data, in the screening process of lncRNA-mRNA competitively regulated relationship, the following two criteria should be satisfied; (i) The hypergeometric test was used to evaluate the enrichment significance of the shared miRNAs that were interacted with lncRNA and mRNA, and PDR correction was performed for the obtained p values, eventually, screened out lncRNA-mRNA interactions that have p values less than 0.05 after adjustment; (ii) Ranking of Jaccard coefficient of lncRNA-

mRNA intersections at top twenty percent was selected. Based on lncRNA and mRNA shared miRNAs, the hypergeometric test formula used in this paper was as follows:

$$P = 1 - \sum_{k=0}^{m} \frac{\binom{n}{k}\binom{N-K}{M-K}}{\binom{N}{M}} \quad (1)$$

Where N represents all of miRNAs interacting with lncRNA or mRNA, M represents the number of miRNAs interacting with the given mRNAs, n represents the number of miRNAs interacting with the given mRNA, m represents the total number of miRNAs interacting with both the given lncRNA and mRNA. Ultimately, the candidate lncRNA-mRNA competitively regulated networks were acquired based on the above two criteria, which contains 7693 lncRNA-mRNA interactions among 835 lncRNAs and 1749 mRNAs.

Furthermore, integrating genes in JIA expression profile data, and taking the intersection with the mRNAs and lncRNAs in the above lncRNA-mRNA interactions. Eventually, the expression profile included 1650 mRNAs and 165 lncRNAs was obtained.

Constructing Co-expression Relationship Pairs

An association network of lncRNA-mRNA was constructed by introducing correlations between lncRNAs and mRNAs. Based on the matching expression profile of lncRNAs and mRNAs that have been detected and satisfied the above criteria, the Pearson correlation coefficient (PCC) of each gene pair was computed for evaluating the candidate lncRNA-mRNA interactions. Moreover, Fisher's asymptotic test was used to confirm the statistical significance of each PCC value that has been calculated, and then the lncRNA-mRNA-interaction (LncGenePairs) relationships with p less than 0.05 were screened out. Consequently, based on the above screening criteria, 277 LncGenePairs contains 72 lncRNAs and 191 mRNAs were obtained.

Identifying lncRNAs Competitive Regulatory Subpathway

Screening the Candidate Condition Specific lncRNA Competitively Regulated Signal Pathways

In this study, KEGG was chosen as the database of the lncRNA-accessed pathway to form new networks that retained the original pathway topologies. Based on Fisher's Z transformation, the pathways of gene enrichment in mRNAs expression profile were identified. Then the pathways with p value less than 0.01 after correction were selected, and were called candidate differential pathways.

Embedding lncRNAs Pathways

Based on the obtained LncGenePairs results, these lncRNAs were inset into candidate differential pathways as pathway nodes by connecting to mRNAs that were regulated by lncRNAs. Consequently, the condition specific lncRNA competitively regulated signal pathways (LRSP) were acquired using this method, and in this obtained pathways, we can also obtain the lncRNA nodes and the competitively regulated edges between lncRNA-mRNA.

Identifying lncRNA Competitively Regulated Subpathway

lncRNAs participated in the competitive regulation and mRNAs that have regulatory relationship with lncRNAs were considered as signature nodes. Combining these nodes with topology of LRSP would contribute to effectively locate the subregions regulated by lncRNAs. That is, signatures nodes were mapped into the LRSP, and "lenient distance" similarity was used by combining with network topology structure, then seeking out subpathways involved in competing regulation in lncRNAs. In a word, the shortest route between each two signature nodes was evaluated, and the signature pairs would be merged into one nodes if the number of their molecules was less than n. Furthermore, the number of the nodes in molecule sets within pathway was calculated for evaluating the candidate subpathways, and if the number of nodes was more than s, the path would be considered as candidate subpathways competing regulated by lncRNAs. In this detection method, the n and s were regarded as the parameters that modulate the intensity of regulation signals and the magnitude of candidate subpathways, respectively. It is worth mentioning that in this work, the default values for n and s were 1 and 8, respectively.

Evaluating lncRNA Competing Regulated Subpathways

Wallenius approximation method is a statistical model of the deviation sampling with the form of non-central hypergeometric distribution. It was used to evaluate the significance of candidate subpathways for confirming whether the candidate lncRNAs-related subpathways were competitively regulated. Additionally, the p value in each candidate subpathway was computed, and p less than 0.01 should be the standard criteria that screen the candidate subpathways. In this method, the following parameters were needed: (i) the number of mRNAs (x) that can be collected to the subpathways in LncGenePairs; (ii) the total number of background mRNAs (n); (iii) the number of background mRNAs participated in the given subpathways (m1); (iv) the number of background mRNAs that can be annotated into this subpathways (m2); (v) the weight of this subpathway (W), which indicated the intensity of the competitively regulated by lncRNAs participated in the subpathways. The computing formula of subpathway weight was as follows:

$$W = 1 + \beta\left(-\log_2\left(\frac{G_L}{P_G}\right)\right) \quad (2)$$

Where PG is the number of mRNAs involved in the given subpathway, GL is the number of mRNAs involved in the competitively regulated by lncRNAs in the given subpathway, ^{2} is a parameter as control (in present paper ^{2}=1). Specific use of Wallenius approximation method can refer to R package (BiasedUrn) (Epstein et al. 2012).

Identifying Hub-lncRNA

Based on the results of subpathway evaluation, researchers can obtain the lncRNA-related pathway to construct a network on the pathway-lncRNA-mRNA. And then, according to this network, lncRNAs that degree value greater than the average of the lncRNAs were calculated and were called hub-lncRNA.

RESULTS

Obtaining the lncRNA Competitively Regulated Subpathways

Subpathways competitively regulated by lncRNA were evaluated by integrating the JIA dataset. Firstly, expression profiles of candidate lncRNAs and mRNAs associated with JIA were obtained by selecting the intersection of mRNAs associated with JIA from ArrayExpress database and mRNAs from the candidate lncRNA-mRNA interactions for further studying the candidate co-expression relationship pairs between lncRNA-mRNA. Then the mRNAs regulated by lncRNAs in the candidate LncGenePairs were embedded into the candidate differential pathways acquired from the KEGG database, and thus obtaining the lncRNA competitively regulated signaling pathways. The results in Table S1 contained 43 candidate signaling pathways of competitively regulated by lncRNAs among 96 matched mRNAs and 53 matched lncRNAs**.** Finally, the candidate pathways with the number of signature nodes more than or equal to 8 as the subpathways of competitively regulated by lncRNAs were identified, and screened out the potential subpathways that have p values less than 0.01 with Wallenius approximation methods. The screening results in Table 1 showed 38 important lncRNA competitively regulatory subpathways containing 31 complete pathways with FDR<0.01. Most of these pathways in Table 1 were demonstrated to be associated with cancers, and related to the occurrence, progression and metastasis of numerous tumor. Additionally, the key subregions identified by lncRNAs competitive regulation relationships would be more effective (Xu et al. 2017). Therefore, pathways that were biologically meaningful and were competitively regulated by lncRNAs were confirmed by subpathway detection methods.

Evaluating the Statistical Significance of Identified Subpathways

The top 3 subpathways with FDR<0.01 that were competitively regulated by lncRNAs in Table 1 were selected for further analysis. The first subpathway with significant difference is the path: 04066_1, which was a subregion of HIF-1 signaling pathway. The interaction networks for lncRNA-mRNA were plotted and showed in Figure 1. The blue nodes represent the mRNAs, yellow nodes represent the lncRNAs. Studies have reported that the suppression of hypoxia-inducible factor-1 (HIF-1) signaling pathway to the therapy of malignancies has significant clinical applications (Semenza 2012), and the activation of HIF-1 signaling path-

Table 1: The identification of subpathways in JIA datasets

Pathway_id	*Pathway_name*	*FDR*
04066_1	HIF-1 signaling pathway	0.00E+00
04110_2	Cell cycle	0.00E+00
04115_1	p53 signaling pathway	0.00E+00
04151_2	PI3K-Akt signaling pathway	0.00E+00
04510_1	Focal adhesion	0.00E+00
05200_2	Pathways in cancer	0.00E+00
05215_1	Prostate cancer	0.00E+00
05161_1	Hepatitis B	3.06E-14
04012_1	ErbB signaling pathway	3.28E-13
04722_2	Neurotrophin signaling pathway	3.28E-13
05214_1	Glioma	3.28E-13
04912_1	GnRH signaling pathway	3.71E-13
05218_2	Melanoma	7.57E-13
04150_1	mTOR signaling pathway	2.07E-12
05220_2	Chronic myeloid leukemia	2.07E-12
04540_2	Gap junction	2.26E-10
05212_1	Pancreatic cancer	2.26E-10
04010_1	MAPK signaling pathway	6.38E-10
04064_1	NF-kappa B signaling pathway	6.38E-10
04151_1	PI3K-Akt signaling pathway	6.38E-10
04330_1	Notch signaling pathway	6.38E-10
05221_1	Acute myeloid leukemia	1.61E-09
05169_4	Epstein-Barr virus infection	3.88E-09
05203_1	Viral carcinogenesis	3.88E-09
04144_1	Endocytosis	1.59E-08
05200_1	Pathways in cancer	1.59E-08
05214_2	Glioma	1.59E-08
05218_1	Melanoma	1.59E-08
05219_4	Bladder cancer	1.59E-08
04520_2	Adherens junction	1.07E-07
04210_3	Apoptosis	4.04E-07
05166_7	HTLV-I infection	4.04E-07
05202_3	Transcriptional misregulation in cancer	4.04E-07
04722_1	Neurotrophin signaling pathway	1.43E-06
05161_3	Hepatitis B	1.43E-06
05162_6	Measles	1.43E-06
05166_4	HTLV-I infection	1.43E-06
04370_1	VEGF signaling pathway	3.47E-06

way would enhance the angiogenesis, migration and invasion of tumors, thus causing higher mortality and worse treatment (Brocato et al. 2014; Lu et al. 2016). When further exploration on this subpathway was performed, it can be found that there are 9 lncRNAs and 13 mRNAs involved in this competitive regulatory subpathway (as seen as Fig. 1). In this subpathway, HIF-1α as a subunit of HIF-1 transcription factor functions as induction expression, and plasmacytoma variant translocation 1 (PVT1) as a newly founded lncRNA functions as oncogenic molecule in numerous tumors, such as kidney cancer, colorectal cancer and ovarian cancer (Hudson et al. 2002). Vascular endothelial growth factor (VEGFA) interacted with lncRNA PVT1 plays an important role in vascular formation and maintenance (Dimke et al. 2013; Wang et al. 2017). It has been demonstrated that the activation of angiogenesis, induction of hypoxia may modulate other characteristics that play an important role in the occurrence and progression of RA, and further, the expression levels of HIF-1± and VEGFA in fibroblast-like synoviocytes derived from rheumatoid arthritis (RA) and osteoarthritis (OA) under hypoxia were up-regulated, suggesting that HIF-1 signaling pathway is closely associated with the occurrence and progression of RA (Muz et al. 2009; Peng et al. 2013). It has been reported that lncRNA TUG1 functions as a tumor suppressor by regulating the expression in different tumors, and participates in multiple cellular process of tumors, such as cell proliferation, apoptosis and invasion. Zhang et al. (2013) has investigated the effect of TUG1 on the osteosarcoma, and found that the down-regulations of TUG1 would suppress the cell proliferation and enhance apoptosis. Insulin-like growth factor 1 receptor (IGF1R) regulated by lncRNA TUG1 is essential for cell survival. In RA, the expression of IGF1R was affected, and thereby activating the IGF1R signaling for regulating the expansion of the inflamed synovia (Andersson et al. 2016). The above results show that lncRNAs PVT1 and TUG1 in the subregion of HIF-1 signaling pathways are related to the RA, thus the subpathway named path: 04066_1 may play a key in JIA.

The second important subpathway is a subregion within cell cycle (path: 04115_1), and the interaction network in Figure 2 included 13 lncRNAs (yellow nodes) and 15 mRNAs (blue nodes) between JIA-related to lncRNA-mRNA. As it is known, JIA is one of RA, and RA is a chronic rheumatic illness, accompanied by progressive articular cartilage and bone destruction, which is affected by the immune regeneration of tissue proliferation. Cell cycle checkpoints can improve the cell relative viability and regulate the cell proliferation (Kastan et al. 1992). As one of competitively regulated by lncRNA LINC00116, WEE1, is a regulator protein in G2/M checkpoint of cell cycle, and may function as a good target for cancer treatment. It has been reported that the expression of WEE1 kinase in rheumatoid synovium was increased and thus inhibited the expression of mitosis promoting factor (Nakamura et al. 1999). Another competitive regulator of lncRNA LINC00116 in this sub-

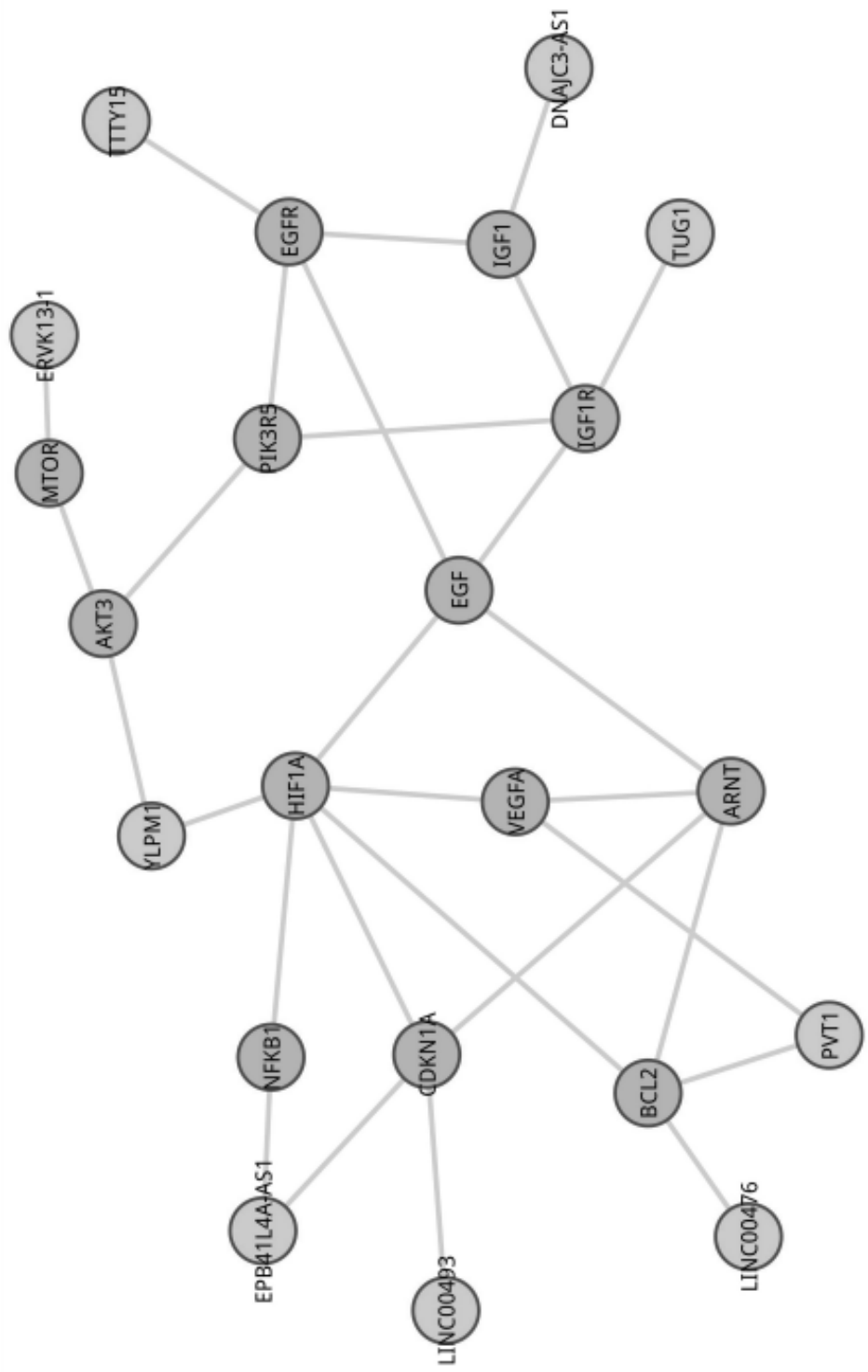

Fig. 1. The identification of subpathway of HIF-1 signaling pathway (path: 04066_1, FDR= 0). Blue and yellow node labels represent mRNAs and lncRNAs, respectively
Source: Author

Table S1: Identification of subpathways containing 53 competitively regulated lncRNAs and 96 matched mRNAs in Juvenile idiopathic arthritis

Path-name	*Matched lncRNA*	*Matched gene*
5323, 5166, 4012, 5221, 5200, 5222, 5218, 4064, 5219, 4520, 5211, 5216, 4910, 5215, 4115, 4150, 4912, 4370, 5169, 4330, 5210, 4320, 5120, 4920, 4510, 5161, 5212, 4110, 4210, 5162, 5213, 4144, 5220, 5203, 4066, 4540, 4722, 4010, 5014, 5202, 5223, 4151, 5214	FOXN3-AS1, LINC00482, CASP8AP2, EPB41L4A-AS1, ALMS1-IT1, PCBP1-AS1, C14orf169, PVT1, VPS11, DNAJC27-AS1, NEXN-AS1, MAGI1-IT1, LINC00242, PRKAG2-AS1, LINC00521, DAPK1-IT1, LINC00319, LINC00240, MCF2L-AS1, YLPM1, EMG1, WASIR2, SLC38A3, C11orf95, TUG1, PITPNA-AS1, ERVK13-1, RPS10P7, RUSC1-AS1, DLEU2, DNAJC3-AS1, LINC00173, MAFG-AS1, TTTY15, CROCCP2, LBX2-AS1, LINC00472, LINC00312, BOLA3-AS1, FAM201A, MIR4500HG, UBXN8, MIAT, MAGI2-AS3, DGKK, LINC00116, DCP1A, LINC00476, CWC15, LINC00493, ZNF503-AS2, LINC00094, UHRF1	LMO2, MAP3K12, ITGA3, ERBB4, MTOR, CCND2, ZAP70, PRKAR1B, TAF15, CSF1, LAMB3, KIDINS220, NOTCH1, CDC6, PPARG, BAX, RAB5A, ATF4, TP53INP1, ATP6V1F, VPS36, TNFAIP3, AKT3, IRS4, ACACA, CCL20, MCL1, NFKB2, BCL2, TERT, GLI1, IGF1, TJP1, HIF1A, LMO7, CTBP1, COL5A3, HRAS, TP53, FZD3, WNT5A, DUSP5, ASPSCR1, TUBB4B, AP2A1, POLR1C, PRKAA1, DUSP2, IL6, ACSL4, ITPR1, ARHGDIG, GYS1, TGFBR2, VEGFA, SERPINB5, MDM2, PARVB, TUBB3, NRAS, DFFA, FGF7, LPAR1, EGFR, NFKB1, ITGB4, IGF1R, HMGA2, HIST3H3, WEE1, TAB3, NCOR2, SP1, SRC, ICAM1, CDC42, MYC, IL18, CDKN1A, LDLR, TSG101, PPP1R12A, NTRK3, CCND1, NOTCH3, CCNT2, CAT, ABL1, NOTCH2, CDC14B, RAB9B, CCNE2, DDIT4, IL11, MLLT1, HLA-G

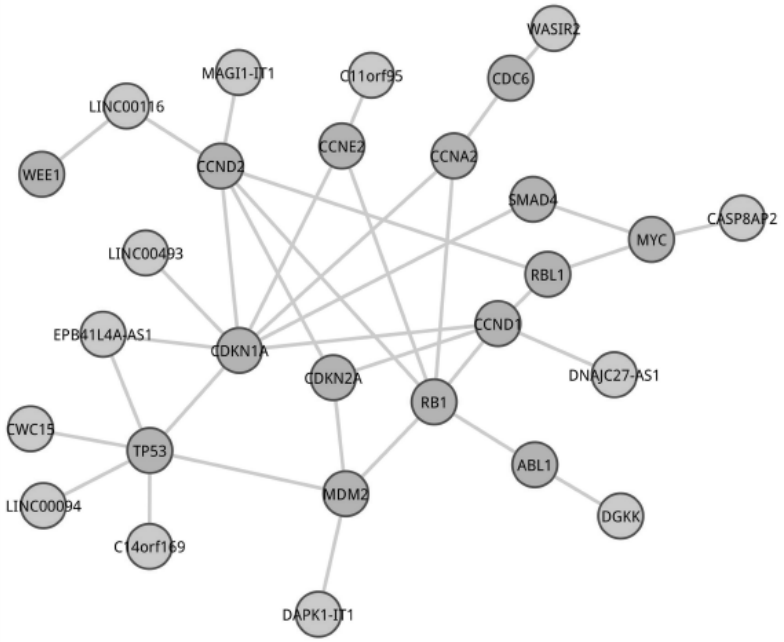

Fig. 2. The identification of subpathway of cell cycle (path: 04110_2, FDR= 0). Blue and yellow node labels represent mRNAs and lncRNAs, respectively
Source: Author

pathway is cyclin D2 (CCND2), which is a member of highly conserved cyclin family, with a remarkable periodicity in protein abundance through the cell cycle characteristics. CCND2 is a regulator of cyclin-dependent kinases (CDKs) that modulate the synovial cell cycling in rheu-

matoid arthritis, and changes the expression and degradation patterns of various cyclins (Evron et al. 2001; Sekine et al. 2008). Based on the above analysis, it can be found that CCND2 and WEE1 genes regulated by lncRNA LINC00116 play a key role in cell cycle,

Finally, the third subpathway named p53 signaling pathways (path: 04115-1) was analyzed. In this subpathway, 23 lncRNA-mRNA interaction networks showed in Figure 3 contained 13 lncRNAs (yellow nodes) and 10 mRNAs (blue nodes). It is well-known that p53-mediated signaling pathway plays a key role in inducing cell cycle arrest, promoting DNA repair for maintaining cellular normal life activities (Hien et al. 2016; Wu et al. 2015). Additionally, p53 as a key protein of this pathway, is a well-known tumor suppressor protein and can be encoded by TP53 gene. It has been reported that for patients with RA, the dysregulation or functional abnormality of p53 protein would affect the synovial lining hyperplasia, and p53 could function as repairing the DNA that was destroyed by the immune and inflammatory reactions related to RA (Lee et al. 2000; Taranto et al. 2005). Murine double minute-2 (MDM2) as the major negative regulator of p53 protein, has diverse biological functions, such as promoting tissue inflammation, enhancing cell proliferation (Thomasova et al. 2012; Yuan et al. 2011). Taranto et al. (2005) has indicated that the abundant expression of MDM2 in RA was the result that the p53 downregulation affected by hypoapoptotic phenotype of lining tissue. Another study showed that the expression of MDM2 was positively correlated with RA disease, and the anti-inflammatory effect was presented by the inhibition of MDM2 (Zhang et al. 2016). Some studies showed that TP53 is susceptible in several autoimmune diseases, such as RA, indicating that TP53 gene is crucial to maintain immune homeostasis (Butt et al. 2006). Furthermore, because of the formation of TP53 mutations, an unfavorable prognostic effect occurred in B and T cells associated with RA (Hoshida et al. 2005). Furthermore, genes IGFBP-3 and IGF-1 regulated by lncRNA DNAJC3-AS1 were reported to play an important role in dynamic exercise, and gene IGF-1 decreased

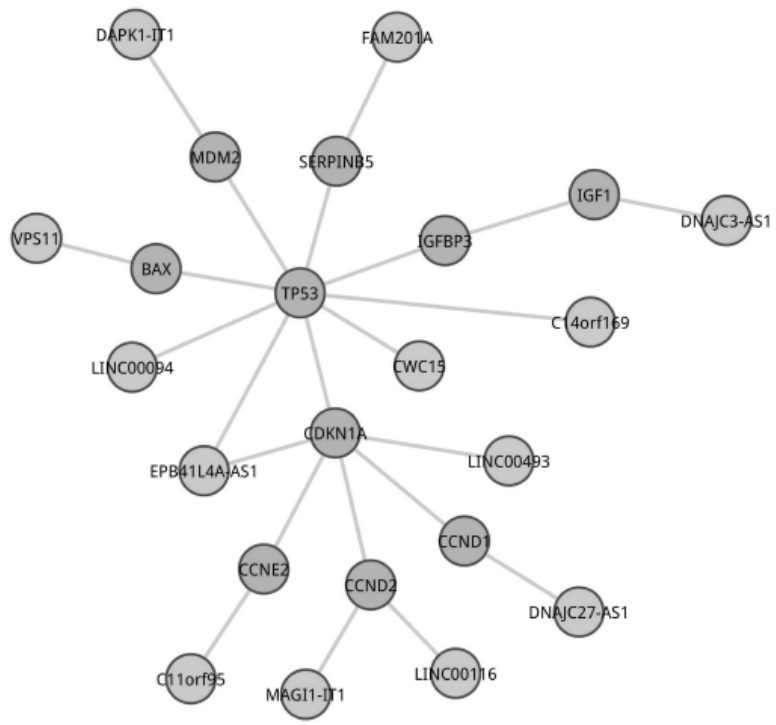

Fig. 3. The identification of subpathway of p53 signaling pathway (path: 04115_1, FDR= 0). Blue and yellow node labels represent mRNAs and lncRNAs, respectively

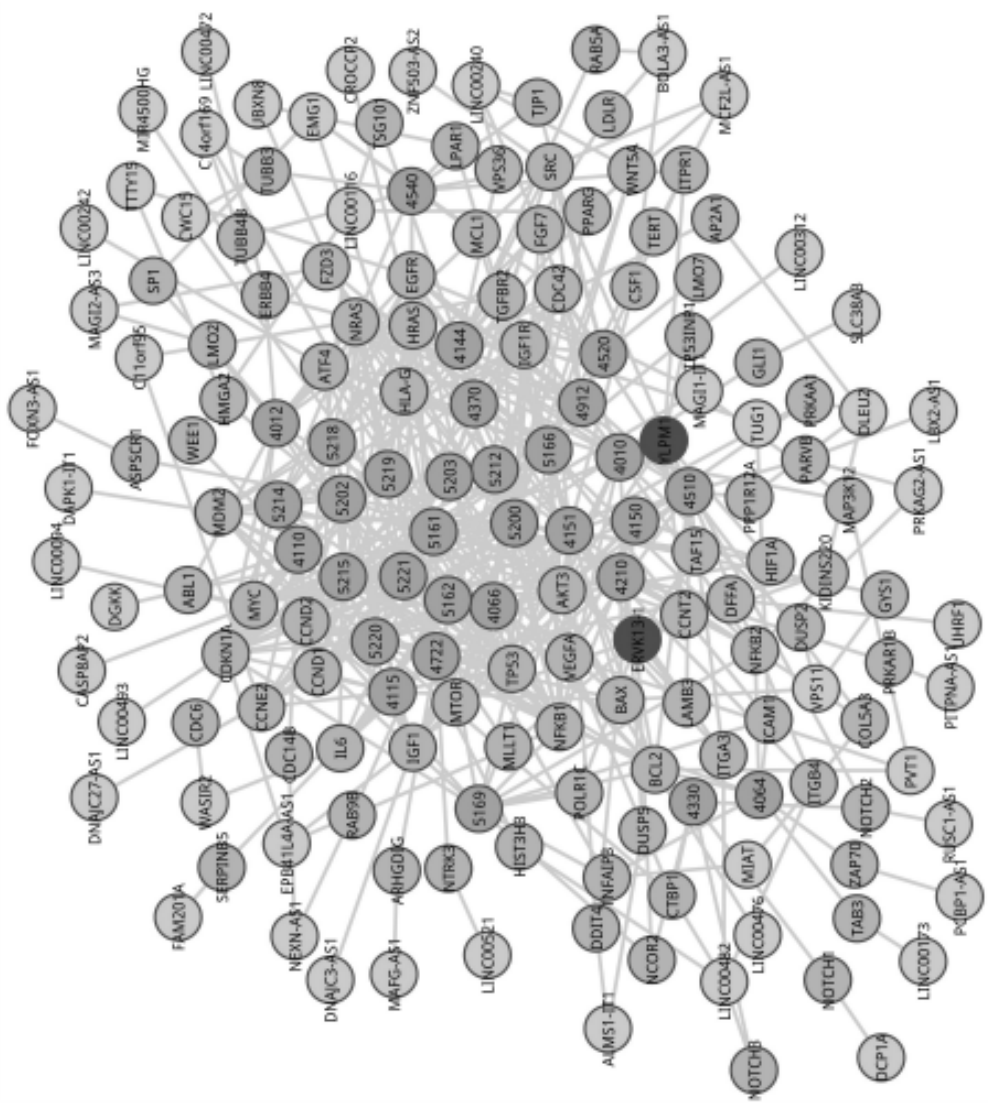

Fig. 4. Network of JIA-associated lncRNA competitively regulated signal subpathways. Blue and yellow node labels represent mRNAs and lncRNAs, respectively. Green and red node labels represent subp
Source: Author

the expression of IGFBP-3 in RA patients (Kim et al. 2015). Additionally, the expression of lncRNA DNAJC3-AS1 was altered in arthritis (Frankbertoncelj et al. 2016). All these results indicate that lncRNA DAPK1-IT1 that can regulate TP53 and MDM2 genes is important for the regulation of functions in RA, and further show the effect of p53 signaling pathway on disease with immune and inflammatory defects.

Evaluating the Hub_lncRNAs

The interaction network between lncRNA-mRNAs included the correlation subpathways that was plotted and shown in Figure 4. lncRNA was marked with yellow, mRNA was marked with blue, subpathway was marked with green, and hub_lncRNA was marked with red. It can been seen from Figure 4 that two hub_lncRNAs, ERVK13-1 and YLPM1, with degree value more than the average can be detected. Gene mTOR interacted with lncRNA ERVK13-1 has been reported to direct the cellular response to inflammatory stimuli, and play a multifaceted regulatory role in RA. Furthermore, the protein AKT3 interacted with lncRNA YLPM1 is a member of the AKT family, and it is well known that AKT is key protein in many signaling pathways, and plays an important role in cell survival and apoptosis. It is reported that AKT1 in chondrocytes regulates the cartilage calcification by suppressing pyrophosphate, and AKT2 as the target of miR-650 suppresses the proliferation, migration and invasion of rheumatoid arthritis cells (Fukai et al. 2010; Xu et al. 2017). Therefore, AKT3 may play a key role in RA. Based on the above results, it can be indicated that the subpathway with biological significance not only can be identified with the researchers' method, but also some key lncRNAs under disease condition are highlighted.

In summary, the above results showed that the confirmation of subpathway with this identification method is effective and reliable. Therefore, researchers can identify the functional roles of lncRNAs and find novel lncRNAs related to disease through the identification of subpathway.

DISCUSSION

Recently, lncRNA as a crucial molecule that modulates the physiological and pathological processes of various diseases, has been widely concerned. Additionally, increasing studies have indicated that lncRNA may be significant in regulating gene expression (Todeschini et al. 2017). For example, it can be a competitor of mRNAs for miRNA binding, thus competitively regulating the expression of mRNA and mediating normal biological functions (Liang et al. 2015; Tay et al. 2014). Based on this regulatory mechanism, biological problems and organism complexity of diseases may be easier to understand. Although disturbances of competitively regulated functions by lncRNAs may cause the onset of diseases, it provides opportunities for new therapies by better understanding this regulation. However, up till now, few approaches are specifically developed to identify the competitive regulatory functions of lncRNAs, and detect their functional roles in human disease. In present work, a novel method was proposed for identifying lncRNAs competitive regulatory signaling subpathways, and detecting the regulation function of lncRNAs in certain disease.

In this study, through integrating the expression profile of lncRNA-mRNA and the pathway topologies, the new method was specially developed to identify the lncRNAs competitive regulatory subpathways and detect the functions of lncRNAs. Several crucial aspects are considered in this approach. Firstly, lncRNA that functions as regulating various biological processes are considered in the pathway analysis as a new regulatory layer. Further, mRNAs competitively regulated by lncRNAs and pathway topologies are embedded in different candidate pathways to identify the potential subpathways and thereby better reflect the transmission of disease signals. It has been reported that lncRNAs play an important role in autoimmune and immune-related disorders etiology by participating in some signaling pathways, such as JIA (Ren et al. 2017). Increasing studies show that lncRNAs play an important role in epigenetic regulation, cell cycle regulation and cell differentiation regulation, and particularly can competitively regulate biological pathways in human diseases (Chen et al. 2018; Wang et al. 2017). Therefore, based on the pathway topologies, it is necessary to comprehensively analyze the interactions of lncRNA-mRNA that are competitively regulated. Finally, the etiology of diseases was confirmed from more subtle aspects by analyzing the subpathways rather than the completely pathways. Additionally, analysis from the subpathways may be more conducive to recall

pathogenesis, find more pathways with biological significance and identify the functional roles of lncRNAs. The method used in this paper can effectively find lncRNA competitive regulatory signaling subpathways and detect the functional roles of lncRNAs in certain condition.

To ensure the reliability of data, databases, starBase, TarBase, mirTarBase, mir2Disease and miRecords (V4.0) were used to collect the interactions of lncRNA-miRNA and miRNA-mRNA. Furthermore, hypergeometric test was used to evaluate the enrichment of miRNAs that both interacted with lncRNAs and mRNAs. Wallenius approximation method was used to evaluate the statistical significance of lncRNA competitive regulatory signaling subpathways. Eventually, 38 JIA-related subpathways involved in 31 complete pathways were identified. Three different subpathways with FDR<0.01 were selected to elaborate the effectiveness of researchers' methods, that is, path: 4066_1, path: 4110_2 and path: 4115_1. As the subregion of HIF-1 signaling pathway, the functions of genes VEGFA and Bcl-2 in the path: 4066_1 on RA that share some clinical and pathological features with JIA have been reported (Paradowskagorycka et al. 2016; Rodríguez et al. 2015) suggesting that the lncRNA PTV1 interacted with mRNAs VEGFA and Bcl-2 may be related to the RA since RA and JIA are autoimmune chronic arthritis, with complex genetic traits and autoantibodies, and have associations with human leukocyte antigen genetic markers. Thereby, the researchers can predict that the lncRNA PVT1 may be associated with JIA. The second is the path: 4110_2, which is the subregion of cell cycle. Similarly, genes WEE1 and CCND2 that competitively regulated by lncRNA LINC00116 were reported to participate in the biological processes in RA (Lee et al. 2015). Finally, the researchers analyzed the third subpathways (path: 4115_1), which is the subregion of p53 signaling pathway. The results indicated that the genes MDM2 and TP53 regulated by lncRNA DAPK1-IT1, and genes IGFBP-3 as well as IGF-1 regulated by lncRNA DNAJC3-AS1 may participate in the biological function of arthritis, thereby showing that the functional roles of lncRNAs DAPK1-IT1 and DNAJC3-AS1 in arthritis.

CONCLUSION

In summary, the method proposed in this work is effective for identifying the subpathways with biological significance, and the lncRNAs that play an important in certain disease can be highlighted by the identification of subpathways. Therefore, the identification of subpathways can not only contribute to understand the molecular mechanism underlying certain disease, but also is helpful to explore the functional roles of lncRNAs in pathological states.

RECOMMENDATIONS

No experiment was performed to verify the results obtained from this paper. Thus, in the next study, more experiments should be conducted.

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