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Research on Ping-Pong models for biomarkers selection and disease prediction

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Abstract. Biomarkers are the biochemical indexes that indicate the changes or possible changes of systems, organs and tissues, which have very extensive clinical application. Based on the high-throughput data, it is very important to study the biomarkers of complex diseases using the computer aided method.In this study, we proposed a novel approach to identify biomarkers of complex diseases. **Methods**: The biomarkers of complex diseases were identified referring to 'omics' data through constructing the lncRNA-mRNA interaction network based on Ping-Pong models.Then, a random walk algorithm was used to calculate the biomarkers of complex diseases and compare them with t-test results. **Results**:Using this method, lncRNAs CCAT1, MEG3, Snhg1, MALAT1, HOTAIR, UCA1, PVT1, CASC9, LOC100130476, TUG1, BC200, POU6F2-AS2, TP73-AS1and ZEB1-AS1 and mRNAs SPARC, CMTM7, SphK1, NANOG, LOXL2, HMGCS2, FZD7, PTOV1, CADM1, CTHRC1, MGMT and RECK were identified as biomarkers of esophageal cancer, which were related to the occurrence and development of esophageal cancer. Compared with the other identification method (t-test), four new lncRNA BC200, POU6F2-AS2, TP73-AS1 and ZEB1-AS1) and three new mRNAs (CADM1, SphK1and RECK)were identified. **Conclusions**: This method was verified to be more effective to predict biomarkers related to the complex disease.

Key words. Ping-Pong model, biomarker, complex diseases, lncRNA-mRNA interaction network.

1. Introduction

Complex diseases[1] are attributed to the joint action of multiple factors and the imbalance of many genes, proteins and the interaction of related molecules, which cause all kinds of disease symptoms. The early diagnosis of complex diseases is very difficult and recurrence rate and mortality rate of complex diseases are very high. Therefore, the study of complex diseases is of great realistic significance. High-throughput technique provides a powerful tool for the study of occurrence

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and development of complex diseases at the functional genomics level[2]. Based on the high-throughput data of gene expression array, biomarkers of complex diseases identified by data mining techniques could help us to understand the mechanism of diseases, predict the risk of diseases, furthermore, and aid the diagnosis and treatment of diseases.

Traditionally, the biomarker study of complex diseases is based on differentially expressed genes. Through the statistical methods such as t-test[3], the expression of some genes or proteins was detected to be obviously different in various samples or phenotypes (such as tumor and healthy tissues). Furthermore, the up-regulated and down-regulated genes in the different diseases or in the different stages of diseases were identified as biomarkers of cancers. In this method, normally, it has been assumed that all genes are independent of each other. However, it has been reported that only a few of differential gene expression sets to the same disease were alike from different laboratories. Furthermore, the results of one experiment were very difficult to be reproduced by another experiment. The reason of this situation might be due to the neglect of the interaction between various molecules in organisms. The results are not complete and cannot be repeated. Therefore, seeking complex diseases biomarkers is always the big challenge in the biomedical research.

In this study, we proposed a novel approach to identify biomarkers of complex diseases based on thePing-Pong model[4]. In this model, we performed the duel cluster analysis on gene expression data, and constructed the lncRNA-mRNA interaction network using Ping-Pong Algorithm. Biomarkers of complex diseases were identified by the Random Walk Algorithm, which compensated the defect of traditional methods. Compared with the other identification methods, some mRNAs and lncRNAs were identified effectively as biomarkers of esophageal cancer by this new approach. These mRNAs and lncRNAs were correlated to the disease significantly and had important directive significance in the henceforth mechanism study of disease occurrence and development.

2. Materials and Methods

2.1. Data sources and processing

2.1.1. Differentially expressed gene data Expression profile data of esophageal cancer (GSE53624) were adopted from GEO(Gene Expression Omnibus http://www.ncbi.nlm.nih.gov/geo/).In GSE53624 data, the tumor tissues and tumor adjacent tissues of 119 esophageal cancer patients were examined.

2.1.2. Re-annotation of genes and differential gene screening Gene re-annotation for the expression profile data of esophageal cancer (GSE53624) was performed and the corresponding expression profile data were obtained. The detailed procedure was described as followed. Firstly, the comparison was done between the probe sequences of platform and the standard sequences of lncRNA and mRNA provided by Genecode using BLAST method. The correspondence between probes and lncRNA / mRNA was extracted and compared. Then, probe sequences corresponding to

many lncRNAs and mRNAs, or lncRNA and mRNA at the same time, or less than three lncRNAs or mRNAs were excluded. Finally, the probe coordinate was deleted and the corresponding expression profile was obtained. The differential expression was recognized with multiple analysis method. Through setting the threshold value of multiple analysis method (FC>2 or FC<-2), the differentially expressed genes were identified from the expression profile data (lncRNA6252mRNA17434)

2.2. Methods

Firstly, the dual clustering was performed on the lncRNAs and mRNAs obtained through gene re-annotation using Ping-Pong Algorithm method. The lncRNA-mRNA interaction network was established. Following that, the interesting differential genes as seed sites were annotated to network. The weight was distributed to some sites using Random Walk Algorithm, and significances of candidate lncRNAs and mRNAs were evaluated through the network disturbance. Finally, the biomarkers of diseases were identified through the significance threshold. The flowchart of the method was shown in Figure 1.

2.2.1. Construction of mRNA-lncRNA interaction network using Ping-Pong Algorithm Ping-Pong Algorithm[4] can predict the interaction between two large scale datasets confidently. In this method, the seed sites were first assigned randomly in the dataset, and then dual clustering was performed between two datasets in the way similar to the running track of table tennis. The higher the Ping-Pong score between two data, the stronger the correlation. The flowcharts of Algorithm were described as followed.

$$n = 0\ ;\ g^{(0)} = random\,(N_G) \in [0,1]^{N_G}\ (initial\ random\ seed)$$

$$c = E_G^T \bullet \hat{g}^{(n)}; c_j^{(n+1)} = \begin{cases} c_j : if\ \left|c_j - \mu\,(c)\right| > t_c \sigma\,(c) \\ 0 : otherwise\ \ (j = 1, \ldots, N_C) \end{cases} \quad d = R_C \bullet \hat{c}^{(n)}; d_k^{(n+1)} = \begin{cases} d_k : if\ |d_k - \mu\,(\\ 0 : otherwise\ \ (k \end{cases}$$

$$\tilde{c} = R_D^T \bullet \tilde{d}^{(n)}; \tilde{c}_l^{(n+1)} = \begin{cases} \tilde{c}_l : if\ |\tilde{c}_l - \mu\,(\tilde{c})| > t_c \sigma\,(\tilde{c}) \\ 0 : otherwise\ \ (l = 1, \ldots, N_C) \end{cases}$$

$$g = E_C \bullet \tilde{c}^{(n)}; g_m^{(n+1)} = \begin{cases} g_m : if\ |g_m - \mu\,(g)| > t_G \sigma\,(g) \\ 0 : otherwise\ \ (m = 1, \ldots, N_G) \end{cases}$$

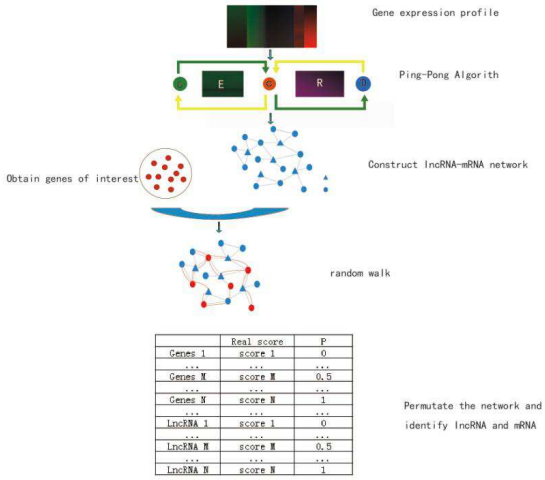
$$n = n + 1$$

$$g^* = g^{(n)}; \hat{c}^* = \hat{c}^{(n)}; \hat{d}^* = \hat{d}^{(n)}$$

Where; t_C was the conditional threshold; t_G was the gene threshold; t_D was the threshold of lncRNA;$\sigma(*)$ was the average value;$\tilde{x}$ was standard dealing;$E_G$and E_C were the gene expression profile and its conditioned matrix after standardization;$R_D$andR_C were the lncRNA expression profile and its conditioned matrix after standardization.

In this study, lncRNA and mRNA after gene re-annotation were used as the base for constructing the integral network and Ping-Pong score between each gene pair was calculated using Ping-Pong Algorithm. If the score between two gene pair was

more than 0.7, these two genes were recognized to be correlated significantly. The interaction network which integrated lncRNA and mRNA was constructed successfully once all these correlated gene pairs were connected (figure 2).



	Real score	P
Genes 1	score 1	0
...	...	...
Genes M	score M	0.5
...	...	...
Genes N	score N	1
...	...	...
LncRNA 1	score 1	0
...	...	...
LncRNA M	score M	0.5
...	...	...
LncRNA N	score N	1

Fig. 1. Flow diagram of the methodology

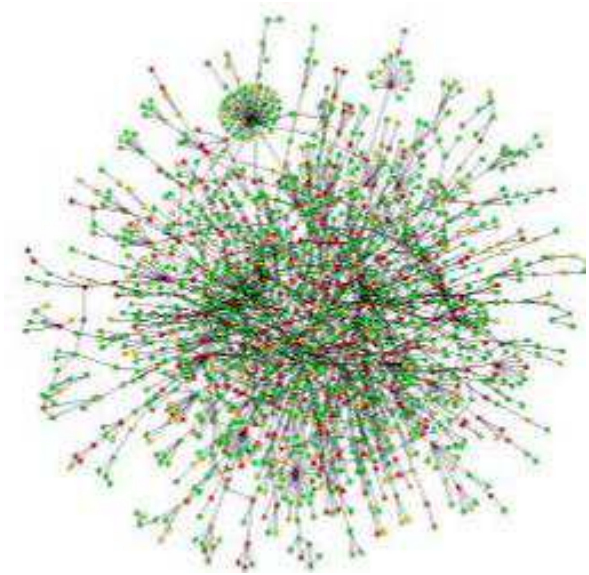


Fig. 2. Subgraph of mRNA-LncRNA interaction network constructed by Ping-Pong Algorithm

2.2.2. Optimization of the network by the RW Algorithm Random Walk (RW)[5] is a classical algorithm to optimize the network. In this algorithm, the interesting sites in the network were assigned weights as seed sites. The weights were distributed to the other neighborhood sites from the seed sites along with the net structure. Thereby, the site closely related to the seed site was apt to gain more weights. The formula of RW Algorithm could be defined as:

$$\mathrm{p}_{t+1} = (1-r)W\mathrm{p}_t + rp_0 \tag{1}$$

where, $r$represented the probability of weight distribution of some site to the neighborhood site, with the default value 0.7;$W$was the standardized adjacency matrix;$p_0$was the initial weight vector of the site; $p_t$was the new weight of the site after network was randomly walked for t times.

In this study, the sites whose degree ranking in a network graph was top one-third were assigned as seed sites. The initial weight of seed sites was the degree value after standardization. The weight of other sites in the network was set as zero. The iteration was terminated when

$$|\mathrm{p}_{t+1} - \mathrm{p}_t| \leq 10^{-10} \tag{2}$$

$p^{\infty}$was the final score of the site. The higher the score of the site, the closer the correlation to the disease. Furthermore, the gene was apt to be recognized as the biomarker of the disease.

2.2.3. Evaluation of the significance of disease biomarkers by the net disturbance To eliminate the bias of RW Algorithm that occurred during the network walking, we did random perturbation processing on data. In this method, disturbed seed sites with equal amount to seed sites were randomly selected and recognized from the lncRNA-mRNA interaction network. The standardized weights of seed sites were randomly distributed to these disturbed seed sites as their weights, then the Random Walk Algorithm was performed and the score of each site was calculated. After the disturbance was performed for 1000 times, frequency N of each site was added up and recorded when its random score was more than its real score. The significance of the candidate site was calculated following the formula

P=N/1000 (3)

If the P value was less than 0.01, this site was significant. This method could calibrate the influence of the results caused by the bias and identify biomarkers related to the diseases significantly. Finally, P value was used to evaluate the relationship between the biomarkers and diseases.

3. Results

We applied the identification methods of complex diseases biomarkers based on the Ping-Pong Algorithm to the expression profile data of esophageal cancer (GEOGSE53624). The interaction network was established with Ping-Pong Algorithm, Random Walk Algorithm was used to optimize the network and P value was ob-

tained after examined by net disturbance. In table 1, 12 mRNAs and 14 lncRNAs which were related to esophageal cancer were listed and their P value was less than 0.01.

To examine the validity of our method, the comparison with the traditional biomarker identification method (t test) was performed. With t test method, 15 mRNAs and 11 lncRNAs which were related to esophageal cancer were recognized. Through the comparison of these two methods, some new lncRNAs and mRNAs were identified with our method (table 2). The comparison results were showed in table 1. In this table, there were 18 RNAs and 15 lncRNAs which were related to the esophageal cancer and they were the union of the significant genes identified by our method and t test method.

To directly compare and analyze the results from these two methods, we depicted the bar chart (figure 3). The comparison result of mRNAs was shown in figure 3a and that of lncRNAs was in figure 3b. In the figure, 'red bar' represented the results from our method and 'blue one' was from t test. The length of the bar chart was calculated using the formula L=-lg(p), in which p meant the significance probability value for these two methods. In our study, the unified threshold of lncRNA and mRNA identified by our method was set as that p was less than 0.01 (that meant L was more than 2). Meanwhile, in the Y-axis of figure 3, mRNAs and lncRNAs marked in red color were only identified with our method, and those in blue color were only from t test. From table 2 and figure 3, we found that the newly identified mRNAs were CADM1, SphK1 and RECK, meantime, the newly identified lncRNAs were BC200, POU6F2-AS2, TP73-AS1 and ZEB1-AS1. The Invasion of peripheral tissues and metastasis of distal tissues occur in the very early stage of esophageal cancer, therefore, the effective inhibition of these two pathological process in cancers are key points for the treatment[6].

Table 1. mRNAs and LncRNAs identified by our method

mRNA	**P**	**lncRNA**	**P**
SPARC	0	MEG3	0
CMTM7	0	Snhg1	0
SphK1	0	CCAT1	0
NANOG	0.001	MALAT1	0
LOXL2	0.001	HOTAIR	0
HMGCS2	0.001	UCA1	0.001
FZD7	0.001	PVT1	0.001
PTOV1	0.002	CASC9	0.001
CADM1	0.002	LOC100130476	0.001
CTHRC1	0.004	TUG1	0.002
MGMT	0.008	BC200	0.004
RECK	0.008	POU6F2-AS2	0.004

Table 2. The comparison of mRNAs and LncRNAs identified by our method and t test

mRNA	**Rank 1**	**Rank 2**	**lncRNA**	**Rank 1**	**Rank 2**
SPARC	1	6	MEG3	1	1
CMTM7	2	10	Snhg1	2	11
SphK1	3	3	CCAT1	3	10
NANOG	4	7	MALAT1	4	8
LOXL2	5	1	HOTAIR	5	2
HMGCS2	6	2	UCA1	6	6
FZD7	7	13	PVT1	7	3
PTOV1	8	14	CASC9	8	9
CADM1	9		LOC100130476	9	5
CTHRC1	10	15	TUG1	10	7
MGMT	11	3	BC200	11	
RECK	12		POU6F2-AS2	12	
			TP73-AS1	13	
			ZEB1-AS1	14	

*Rank1: The order of the mRNAs and LncRNAs identified by our method sorted

by the significance. Rank2: The order of mRNAs and LncRNAs identified by the t test sorted by the significance.

CADM1 ranked No. 9. CADM1 is transmembrane glycoprotein which is composed of 442 amino acids. The structure of CADM1 is divided into three parts, extramembrane domain, transmembrane domain and cytoplasm domain. It is widely existed in epithelial tissues. In many tumors, the expression of CADM1 is abnormal. It has been reported that the expression of CADM1 was low in esophageal cancer and it was negatively related to the lymph node metastasis of the cancer. The inactivation of CADM1 was related to the occurrence and development of esophageal cancer. In the clinical practice, CADM1 was recognized as the biomarker to predict the prognosis of the esophagus squamous cell carcinoma patients at locally advanced stage[7]. RECK ranked No. 12 and it is a new antioncogene. It is widely presented in all kind of normal and non-transformed cell lines. In some tumor cell lines and fibroblast cells transformed by tumor genes, the expression of RECK is inhibited. It has been reported that RECK was involved in the occurrence and development of esophageal cancer, furthermore, it was related to the differentiation level, infiltration and lymph node metastasis of cancer cells. RECK could not only behave as a relatively reliable and important biomarker for the occurrence and development of esophageal cancer, but also work as a valuable reference index for judging the malignant level, infiltration ability and lymph node metastasis ability of esophageal cancer[8]. In lncRNAs, BC200 ranked No. 11. It has been reported that the expression of BC200 was significantly higher in tumor tissues than tumor adjacent tissues. The expression of BC22 was not related to the clinical pathological features such as age, stage and grade of cancer. However, it was closely related to the poor prognosis of esophageal cancer. The overall survival time of esophageal cancer patients with high expression level of BC200 was much shorter than those with low expression level of BC200, which meant that the high expression of BC200 reflected the poor prognosis of esophageal cancer patients and could be used as a new type of predictive biomarkers for esophageal cancer[9]. POU6F2-AS2 ranked No. 12. The squamous cell carcinoma of esophagus is the major subtype of esophageal cancer, which has been proved to be related to the unhealthy dietary habits, smoking and other behaviors. The progress of squamous cell carcinoma of esophagus is slow, however, it is normally at the advanced stage when it is diagnosed. Therefore, it is necessary to study the mechanism of the occurrence and development of squamous cell carcinoma of esophagus. It has been reported in some papers that POU6F2-AS2 is expressed in the squamous cell carcinoma of esophagus and involved in the DNA lose response, regulation of cell survival and other related functions. TP73-AS1 ranked No. 13. It has been reported by some literatures that TP73-AS1 was highly expressed in the tissues of esophageal cancer and closely related to the tumor size and TNM stages. The knock-out of TP73-AS1 could increase the sensitivity of chemotherapy on the esophageal cancer and it could be a potential target for the therapy of esophageal cancer. ZEB1-AS1 ranked No. 14. It has been indicated by some papers that comparing with the non-tumor tissue adjacent to tumor mass, ZEB1-AS1 was upregulated in the tumor tissue and was involved in the tumor grading, infiltration depth and lymph node metastasis. The overall survival rate of esophageal cancer

patients with high expression level of ZEB1-AS1 was low, therefore, ZEB1-AS1 could be recognized as the individual prognosis factor of esophageal cancer patients.

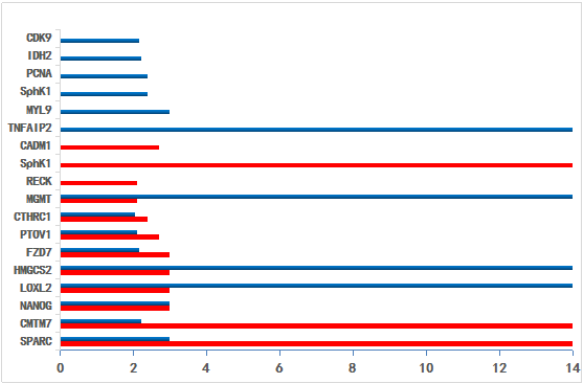


Fig. 3. The comparison of mRNAs and lncRNAs identified by our method and t test

The comparison of mRNAs identified by our method and t test. (b) The comparison of lncRNAs identified by our method and t test.

Besides those newly identified markers, the disease biomarkers obtained from traditional methods were also recognized by our method, such as CMTM7, NANOG, FZD7, PTOV1 and CTHRC1 for mRNA and CCAT1, MALAT1 and CASC9 for lncRNA. Comparing with t test, the ranking orders of these mRNAs rose from originally the 10^{th}, 7^{th}, 13^{th}, $14^{th}$and 15^{th} to the 2^{nd}, 4^{th}, 7^{th}, 8^{th} and 10^{th}; the ranking orders of these lncRNAs rose from originally the 10^{th}, 8^{th} and 9^{th} to the 3^{rd}, 4^{th} and 8^{th}. The No.2 mRNA was CMTM7, the expression level of which in esophageal cancer was lower than that in the normal tissue. The gene of CMTM7 locates in the 3^{rd} chromosome, with the full length of 1369bp and 4 introns and 5 exons. It has been reported that mRNA and protein expression levels of CMTM7 in the normal epithelial cell of esophagous were obviously lower than those in the cell line of esophageal cancer (CE81T), which was consistent with our result, i.e., CMTM7 acted as an antioncogene in the occurrence and development of esophageal cancer. The No.4 gene was NANOG, which is the transcription factor which is involved in the differentiation of embryo stem cells. It is the key gene to maintain the proliferation of stem cells and the biomarker of stem cells. It has been indicated by many studies that the expression level of NANOG protein was very low in the normal tissues and most of proteins were expressed in the cytoplasm of basal cells of esophageal mucosa. The reason for that may be that the basal cell has the features of stem cells and belongs to the hyperplastic layer. NANOG protein could control the proliferation of cells. The expression of NANOG protein in the tissue of esophageal cancer was very high, and its positive rate was positively correlated to the malignant degree of tissues. When the pathological grades and lymph node metastasis are increased, the differentiation degree of tumor drops down, the expression level of NANOG protein gets increased. The expression level of NANOG which belongs to stem cells specific protein is closely related to the occurrence, development and

metastasis of esophageal cancer. The No. 7 gene was FZD7, which is an important oncogene related to many malignant tumors. It has been discovered by many researchers that both protein expression profile and mRNA expression profile of FZD7 in the esophageal cancer cell lines were up-regulated. The knock-out of FZD7 protein could improve the sensitivity of chemotherapy on esophageal cancer cells and inhibit the drug tolerance of protein 1. FDZ7 could work as the therapeutic target for the treatment of esophageal cancer. The No. 8 gene was PTOV1, which is an overexpressed gene in prostate cancer. PTOV1 is involved in the regulation of cell cycle during the occurrence of tumors and has been recognized as the potential markers for studying the occurrence and progress of cancers. The relationship between PTOV1 and clinical pathological features of esophageal cancer was studied using survival analysis and multiple cox regression analysis methods. Results showed that the expression of PTOV1 in the esophageal cancer cell line was much more remarkable, and it was closely related to the gender and size of tumors. PTOV1 protein played a key role in the occurrence and development of esophageal cancer. The No. 10 gene was CTHRC1.The expression of CTHRC1 is elevated widely, which indicates that it has the features of oncogene. Results from some papers showed that CTHRC1 was expressed abnormally in esophageal cancer and the cancers with overexpressed CTHRC1 was apt to infiltration and metastasis, which made patients have much poorer prognosis. This hinted that CTHRC1 had an important effect on the occurrence and progress of esophageal cancer. Furthermore, it could become a potential target for the treatment of esophageal cancer. Among all lncRNAs, the No.3 was CCAT1. The acetylation of H3K27 can activate CCAT1. The knock-out of CCAT1 can inhibit the proliferation and metastasis of cancer obviously. CCAT1 can regulate the HOXB13 in the cytoplasm and promote the growth and migration of cells. The experiments indicated that CCAT1 played an important role in the formation of esophageal cancer and could become a target for the diagnosis and treatment of esophageal cancer. The No. 4 lncRNA was MALAT1. The overexpression of MALAT1 inhibited the activity of cells and down-regulation of Cks1. Therefore, when MALAT1was overexpressed, the up-regulated Csk1 promoted the proliferation of tumors, especially under the condition of radiotherapy. MALAT1 became an active regulation factor for esophageal cancer through regulating Csk1, therefore, MALAT1 could behave as a potential target for the treatment of esophageal cancer. The rank No. 8 lncRNA was CASC9. Some papers pointed out that CASC9 was up-regulated in the tissues of esophageal cancer. The knock-out of CASC9 could inhibit the migration and infiltration of cells significantly. The overexpression of CASC9 was related to the induced differentiation, which meant that CASC9 could work as a new marker for the poor prognosis of esophageal cancer and become a potential target for the intervention therapy of esophageal cancer.

4. Conclusions

The traditional methods to identify complex diseases biomarkers are to individually analyze the each lncRNA or mRNA and emphasize on considering the importance of one aspect, therefore, there are always some lncRNAs and mRNAs being

neglected, which are normally proved to be closely related to the mechanism of the formation of diseases. Therefore, in our study, we constructed the lncRNA-mRNA interaction network using Ping-Pong Algorithm followed by the Random Walk Algorithm. In the optimization process, the interaction between lncRNAs and mRNAs, and difference of genes when they executed their biological function were considered. In conclusion, the theory of constructing the interaction network based on the Ping-Pong Algorithm was much more effective and accurate on screening the biomarkers of disease. Moreover, it provided a new idea to recognize and analyze the biomarkers of diseases.

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