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The Prediction of miRNAs Target Genes Associated with Rheumatoid Arthritis by Using Novel Prediction Algorithm Called Targetscore

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ABSTRACT miRNAs as a biomarker of the immune system, have become an important role in disease biology. MRNAs as the downstream part of miRNAs, could be regulated by miRNAs. It is useful to understand the biological functions of miRNAs by identifying the target genes. In present work, the gene expression profiles of RA and normal groups were extracted from Gene Expression Omnibus (GEO). Furthermore, a novel probabilistic scoring algorithm called targetScore was developed to improve the prediction of miRNAs targets after differentially expressed genes (DEGs) screening. Targets as the transformed fold-changes have been weighted by the Bayesian posteriors. Eventually, targets with larger taregtScore value were selected and the interaction between miRNAs and mRNAs was shown. Optimal targets were detected by integrating the different miRNAs associated with RA, noting that the same target in different miRNAs has the similar tagretScore value, suggesting targetScore achieved significantly higher accuracy.

INTRODUCTION

Rheumatoid Arthritis (RA) is a common chronic, inflammatory synovitis-based autoimmune disease occurred on the joint tissue, accompanying with severe joint deformity and loss of function characteristics, and eventually may cause severe lifelong disability (Cheung and Mcinnes 2017; Vandormael et al. 2017). It is worth mentioning that the cell death and tissue destruction would not occur in the early stages of RA, but the activation and proliferation of interstitial tissue between the lesion locations are appeared. Moreover, some studies have demonstrated that early treatment of RA disease contributes to reduce joint damage and improve clinical outcome, and then prevents the irreversible destruction and disability of joints (Carpenter et al. 2017; Levitsky et al. 2017). Therefore, early diagnosis, treatment intervention or therapy can significantly prevent the development of severe RA disease. However, the pathogenesis of RA disease is still unknown till date. Studies have shown that the therapeutic outcome of RA disease could be improved by introducing appropriate predictive biomarkers (Gavrila et al. 2017). MicroRNAs (miRNAs) as a potential biomarker, have been demonstrated with expression levels altered in RA patient (Su et al. 2017). Whereas, the change of gene expression may be associated with a number of diseases. Therefore, since the specificity of target genes in certain disease, identifying the relevant targeted mRNAs of certain disease is helpful to understand the biological function of miRNAs and RA pathogenesis (Hong et al. 2017; Hruskova et al. 2016).

miRNAs are short single-stranded non-coding RNAs, with approximately 22 nucleotides long, that are involved in negatively regulating the expression of mRNA at the post-transcriptional level (Oliveto et al. 2017). The regulation of miRNAs is functioning predominantly by inhibiting the translation, degradation and direct cleavage of target mRNAs. Thereby, the expres-

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sion levels of target genes depend on the degree and character of complementarity between mRNAs and specific miRNAs (De and Sassonecorsi 2014; Seo et al. 2017). Additionally, one message can be regulated by multiple miRNAs, manifesting the cooperative translational control among miRNAs. Inversely, each miRNA could possess several target mRNAs, indicative of target multiplicity. The multiplicity of target mRNAs and cooperative signal integration on target mRNAs have become the key characteristic of the translation control of miRNAs. However, it is complicated on the relationship between miRNAs and target genes, and additionally, although numerous miRNAs have been found, the number of related target genes that have been identified is not much. Therefore, the prediction of miRNAs is urgently needed to understand the biological function of miRNAs in some specific diseases.

Objectives

Up till now, several computational programs have been used to predict the target genes of miRNAs. Unfortunately, it is still a challenge to accurately predict the target genes using these analysis tools. The amount of false positive or false negative is increased due to the limitation of each prediction algorithm, leading to the prediction results of miRNAs targets being inaccurate. In this work, a probabilistic scoring method without solely depending on evolutionary conservation, targetScore, was used to improve the target prediction. The new prediction algorithm is specifically developed for miRNA-overexpression experiments to identify the target genes of a specific miRNA under a particular cell-qualification. Moreover, the entire gene set of differential gene expression is handled by using the new approach, so the overall possibility of simulation is closer. Thereby, the miRNA (hsa-miR-223, hsa-miR-146a, hsa-miR-150, hsa-miR-16) target genes associated with RA disease in this paper are predicted using the targetScore algorithm.

MATERIAL AND METHODS

Collection of Samples Associated with RA Disease

In this work, Gene Expression Omnibus (GEO), that is a high throughput chip expression database repository, was used to collect the expression profiling of miRNAs and RNA-seq data from experimental samples associated with RA disease. The experiments contain some kinds of tissue-specific expression profiling analysis in the RA biopsy samples and developmental stages by adopting different platforms. The platforms comprise of the studies based on bead, and microarray platforms designed by different laboratories. A series of quality control checks of data from the GEO database were carried out to make sure the data sets with most undetected spots were eliminated. In this paper, the gene chip and sequencing-related datasets of RA disease were obtained by entering a specific accession numbers into the GEO database. Then samples associated with RA were obtained, and relevant data were converted into the corresponding expressionSet. Genes in the expressionSet were preprocessed by using the researchers' platform corresponding to the preprocessing software to obtain the relative expression level. This value was used for the calculation of subsequent genetic differences. It is worth mentioning that the expression level cannot contain the missing values. Additionally, small change in expression value, the maximum value and minimum value would be removed in the pretreatment, so there are no such data in the gene expression profile.

The Screening of Differentially Expressed Genes

The differentially expressed (DE) genes were obtained and analyzed by the limma software package. Limma contains rich functionality, can be used to handle complex experimental designs and to conquer the problem of small sample sizes. Furthermore, the DE and differential splicing analysis of RNA-seq data can be conducted by the limma package, and the expression profile of DE genes can be analyzed by co-regulated genome and higher-order expression features which provide an intensive likelihood on biological translation of gene expression differences. For RNA-seq data, it usually demands a specialized software developed on the bases of the negative binomial or similar distribution (Robinson et al. 2010). However, high precision analysis of RNA-seq read counts can be performed by using the limma package to transform counts of the log-scale and evaluate the mean-variance relationship empirically (Ritchie et al. 2015). Furthermore, the genes can be verified by t-test and

F-test on the expression matrix of genes, and linearly fits the data using the limFit function. It is worth mentioning that the empirical eBayes procedures in limma software package were used to compute the consensus pooled variance of each gene, besides, the statistical tests and associated p-values were carried out by the eBayes command. Accordingly, use of empirical eBayes procedures enhanced the accuracy and statistical function of data in a more flexible way. Ultimately, the results of correlation analysis, including the fold change (FC) value (logFC absolute value is more than 2) and p-value (p value is less than 0.05) were obtained and listed.

Computing the TargetScore Value

TargetScore can be defined as the integrative probabilistic score of a gene that could be the target t for specific miRNA. Supposing there are N genes, the $x=(x_1,x_2,.....x_N)^T$ can be designated as the value of log expression fold-change (x_f) or sequence score (x_l). Consequently, sequence score of L sets can be represented as $x \varepsilon$ (x_f , x_1, x_2,....x_L). Ultimately, the value of targetScore can be calculated from the following formula:

$$targetScore = \sigma(-\log FC)\left(\frac{1}{K+1}\sum_{x\in(x_f,x_1,\ldots,x_L}p(t|x)\right) \quad (1)$$

Where the value of σ (-log FC) can be computed using the following equation

$$\sigma(-\log FC) = \frac{1}{1+\exp(\log FC)} \quad (2)$$

And $p(t|x)$ is the posteriors distribution, which can be comp uted by integrating the prior probabilities and likelihood functions. The TargetScanCS values and TargetScanPCT values as the parameter of prior probabilities were obtained from the experimental data for all the genes. In addition, the maximum likelihood functions can be inferred by using the Variational Bayesian Formula and Gaussian Mixture Model (VB-GMM). The choice of maximum likelihood functions is helpful to avoid overfitting of data (Khan et al. 2009). Furthermore, a Variational Bayesian Expectation-Maximization (VB-EM) approach should be adopted to optimize the parameters of the VB-GMM. The mixture component associated with miRNA among the negative fold-change and sequence score h expressed as "target component". Therefore, the posterior distribution of the target component obtained by observing the variables is regarded as the interaction between miRNAs and target mRNA. The targetScore as the sigmoid-transformed fold-change, can be calculated by weighting the averaged posterior values of all diagnostic target components (Li et al. 2014).

RESULTS

Obtaining Differential Expression Genes

In this work, 18 RA patients and 15 controls were adopted. The gene expression profiling were authenticated by analyzing the peripheral blood mononuclear cells of 33 samples associated with RA disease, and the results can be obtained by importing the number GSE15573 from the GEO database. Ultimately, 19027 gene expression profiling data were acquired. Furthermore, p values and FC values of all genes were calculated using t-test and F-test, and generally defining the screened gene with p value less than 0.05 are statistically significant. Consequently, 725 expression genes with larger differences were screened out by using limma package to compute the differential expression of the obtained genes.

A volcano plot was used to display the relationship between the p values and logFC values of all DE genes. It can be found from Figure 1a that approximately 700 expressed genes with larger difference were screened out, and the obtained DE genes not only have a larger FC value, but also a smaller p value (less than 0.05). In addition, the expression levels of several genes that have significant expression difference by analyzing the p values and logFC values were plotted. It can be seen from Figure 1b that p value after being adjusted is much smaller than 0.05, demonstrating that DE genes screened by limma package are statistically significant.

Analyzing the TargetScore Values and Predicting the Target Genes

The targetScore value of all genes was obtained by importing the results that obtained from the computation of microarray pretreatment. Eventually, there are 33 genes with targetScore value more than 0.7 by comparing the transcendental

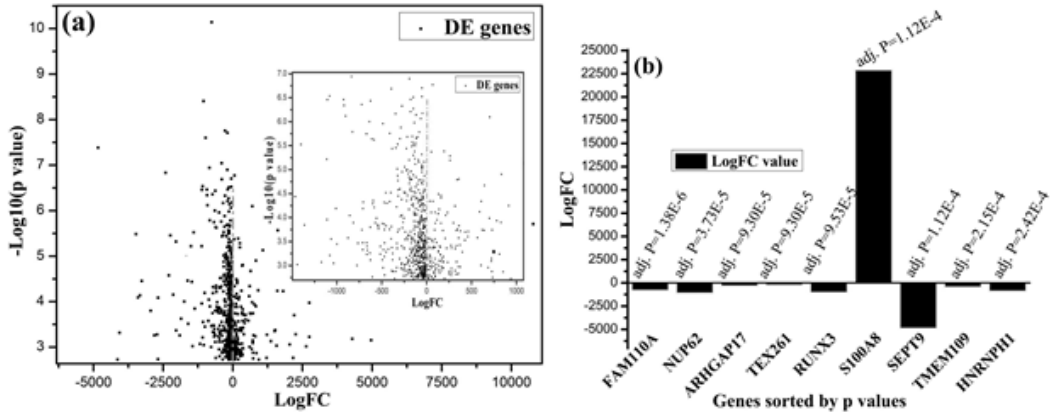

Fig. 1. Volcano plot of 725 differentially expressed genes (a) and (inset) higher magnification. The logFC value of the top 10 differentially expressed genes sorted by p-values (b)
Source: Author

The targetScore value of all genes was obtained by importing the results that obtained from the computation of microarray pretreatmentmeEventually, there are 33 genes with targetScore value more than 0.7 by comparing the transcendental values and the posterior values for miRNA, hsa-miR-223 associated with RA. Similarly, 31 genes with targetScore value more than 0.8 are identified for the hsa-miR-146a, 298 genes with targetScore value more than 0.99 are identified for the hsa-miR-150, and 54 genes with targetScore value more than 0.99 are identified for the hsa-miR-16. It is worth mentioning that the predicted target genes contain some validated and no validated target genes. Screening the target genes that have an intersection in two or more miRNAs by integrating the predicted targets in this four miRNAs, the results of the integration of target genes in Figure 2 showed the superposition of target genes in different miRNAs. Consequently, 35 DE genes were identified from the known genes in the four miRNAs. Then, relevant information of some optimal target genes in Table 1 indicates that the targetScore value of the optimal target genes was similar in different miRNAs.

In general, defining the higher targetScore value has a higher probability on the detected genes becoming the optimal targets. Therefore, when targetScore value is more than 0.7, the prediction of the target gene is feasible. Additionally, the interactions between miRNAs and mRNA target for the four miRNAs suggest that one mRNA target may be regulated by different miRNAs. Additionally, an interaction network in Figure 3 showed the interactions between miRNAs and mRNA target for the four miRNAs associated with RA.

Comparing the Prediction Parameters of Target Genes

Due to the potentially inhibitory action of miRNAs on the translation transcription, the negative logFC of protein outputs resulted by the transfection of miRNAs (hsa-miR-223, hsa-miR-146a, hsa-miR-150 and hsa-miR-16) repre-

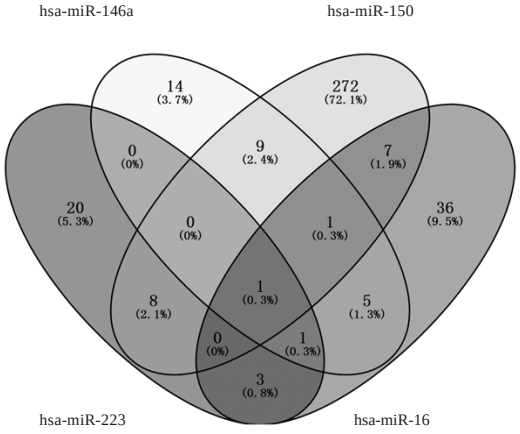

Fig. 2. Intersection of known target genes in the four miRNAs. Different color areas represented different miRNAs, the cross areas meant the overlapping of target genes in two or more miRNAs
Source: Author

Table 1: Relevant analysis results of some optimal target genes associated with rheumatoid arthritis

Target genes	*Relevant miRNAs*	*logFC*	*targetScanCS*	*targetScanPCT*	*targetScore*
TPM3	hsa-miR-150	-472.09567	-0.11	-0.2	1
	hsa-miR-16	-472.09567	-0.239	-0.32	1
	hsa-miR-223	-472.09567	-0.113	-0.19	0.9999999
	hsa-miR-146a	-472.09567	-0.02	-0.15	0.8881021
BTG2	hsa-miR-16	-318.37950	-0.119	-0.63	0.9918493
	hsa-miR-146a	-318.37950	-0.046	-0.16	0.9918486
	hsa-miR-150	-318.37950	-0.102	-0.13	1
CNDP2	hsa-miR-146a	-706.74623	-0.263	-0.15	1
	hsa-miR-150	-706.74623	-0.09	-0.2	1
CYTH1	hsa-miR-146a	-324.67696	-0.067	-0.13	0.9943935
	hsa-miR-150	-324.67696	-0.146	-0.14	1
PRKAR1A	hsa-miR-16	-629.06634	-0.18	-0.1	1
	hsa-miR-223	-629.06634	-0.076	-0.08	1
IL2RB	hsa-miR-16	-1495.8365	-0.072	-0.45	1
	hsa-miR-150	-1495.8365	-0.121	-0.2	1
MXD4	hsa-miR-146a	-903.15045	-0.061	-0.15	1
	hsa-miR-150	-903.15045	-0.054	-0.14	0.9999999
ZFAND5	hsa-miR-16	-632.87908	-0.051	-0.45	0.9999999
	hsa-miR-146a	-632.87908	-0.27	-0.14	1
	hsa-miR-223	-632.87908	-0.01	-0.19	0.7159032
NPC2	hsa-miR-16	-2000.8520	-0.143	-0.08	1
	hsa-miR-146a	-2000.8520	-0.078	-0.16	1
FRMD8	hsa-miR-16	-627.59864	-0.081	-0.07	1
	hsa-miR-146a	-627.59864	-0.163	-0.14	1
SUMO3	hsa-miR-16	-316.2802	-0.149	-0.74	0.99078822
	hsa-miR-146a	-316.2802	-0.129	-0.15	0.99078818
STK38	hsa-miR-16	-2035.2523	-0.161	-0.46	1
	hsa-miR-150	-2035.2523	-0.252	-0.13	1
PIM1	hsa-miR-16	-835.54624	-0.147	-0.75	1
	hsa-miR-150	-835.54624	-0.087	-0.13	1
IL10RA	hsa-miR-16	-444.00108	-0.184	-0.52	0.99999902
	hsa-miR-150	-444.00108	-0.056	-0.14	0.99999999
SAE1	hsa-miR-16	-345.51865	-0.092	-0.08	0.99848130
	hsa-miR-150	-345.51865	-0.214	-0.14	1
TMEM43	hsa-miR-16	-662.87092	-0.172	-0.09	1

sents the down-regulated function of miRNAs on the mRNA. In this work, TargetScore, an effective indicator on identifying the potential miRNA targets is computed by comparing the priori and posteriori values of the obtained genes. Comparing the parameters of target genes in Table 1 that was obtained by intersecting the known genes of the four miRNAs, it could be found that the same target gene in different miRNAs shows a same logFC value, and similar targetScore value whereas for the value of targetScanCS and targetScanPCT in different miRNAs, a large difference can be observed. Furthermore, the expression levels of four targets in different state acquired from the GEO database were plotted. The results in Figure 4 showed that the expression levels were reduced in RA state for the genes ZFAND5, BTG2 and TMEM43, and a significantly differential expression with a high was found in RA disease for the TPM3 gene.

DISCUSSION

RA is a chronic polygenic disease, which is characterized in that autoimmunity and systemic inflammation associated with progressive joint destruction, eventually leading to lifelong disability and increased mortality. It has been reported that changed expression of miRNAs in immune and host cells associated with the pathogenesis of RA disease was conducive to maintaining the pathogenic features of typical RA (Andersson et al. 2017). Since the altered expression of miRNAs associated with RA disease in the synovial fluid, synovial tissue and immunological activated cells has been reported in numerous literatures (Maeda et al. 2017). Accordingly, the miRNAs in peripheral blood or inflamed tissues can be used as potential biomarkers of RA. Studies have shown that miR-223 can regulate the differentiation of osteoclasts, and present up-regulated expression in the sites

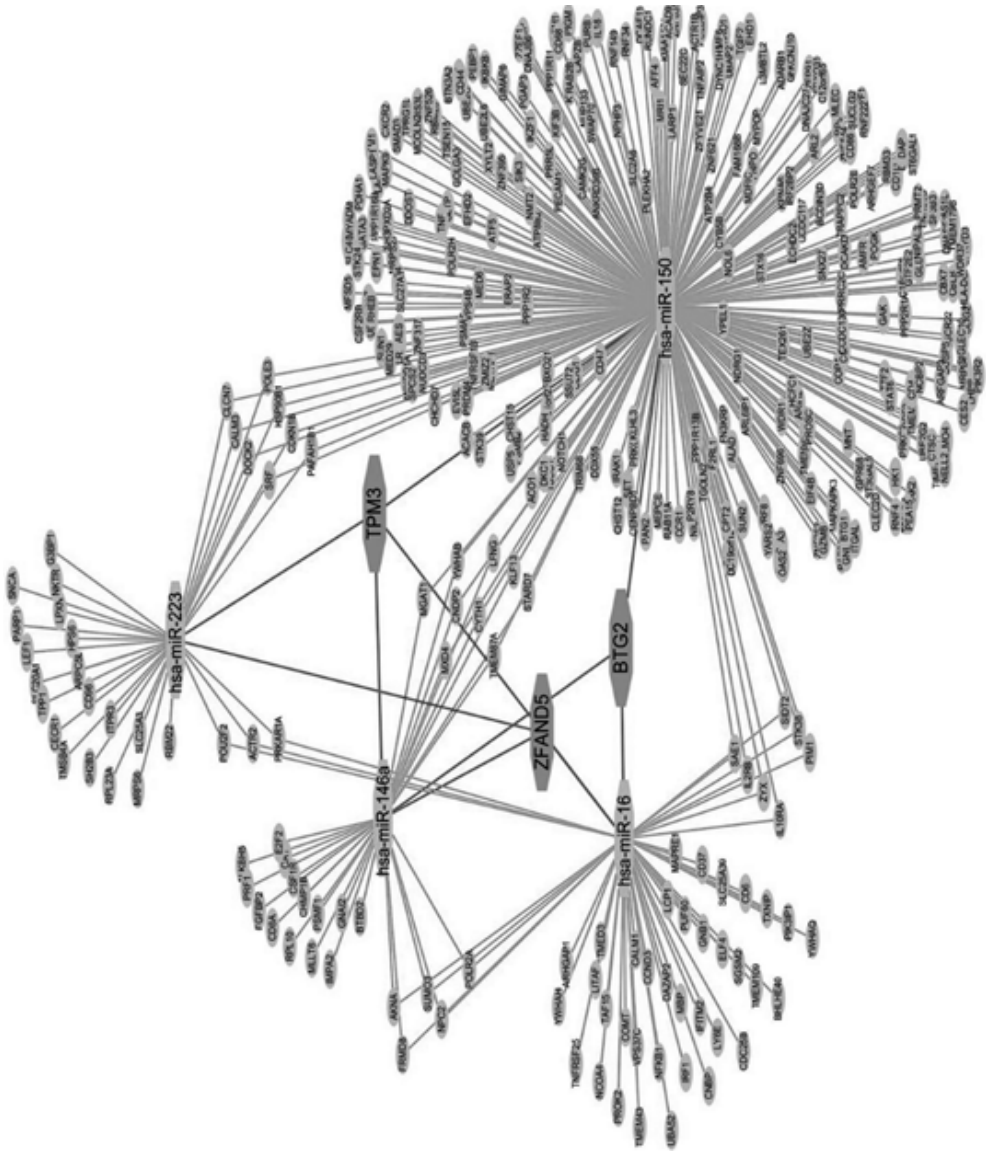

Fig. 3. Interaction between miRNAs and mRNAs associated with rheumatoid arthritis
Source: Author

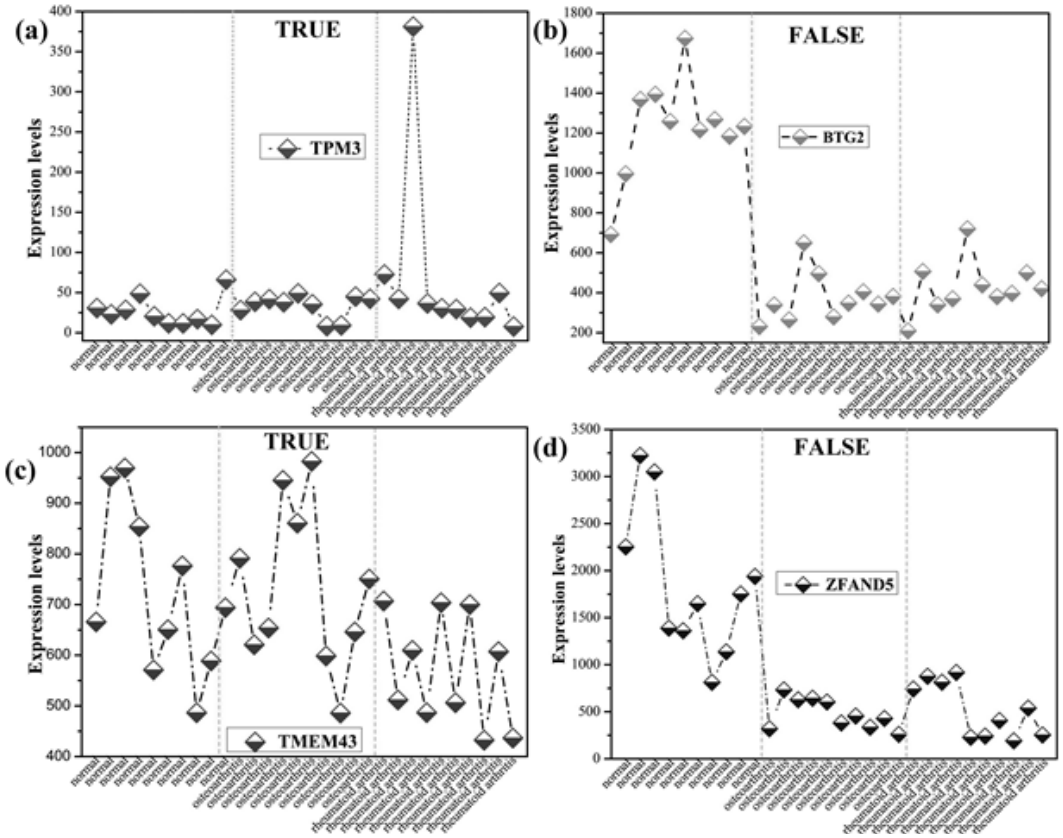

Fig. 4. The expression levels of four selected target genes in normal, osteoarthritis, and rheumatoid arthritis state. (a) and (c) are validated genes, (b) and (d) are not validated genes
Source: Author

of inflammation, peripheral circulation as well as RA plasma (Kriegsmann et al. 2016; Hayatoshi Shibuya et al. 2013). Besides, overexpression of miR-223 in human peripheral blood mononuclear cells plays an important role in inhibiting the generation of osteoclasts, the expression of cathepsin K and osteoclast marker genes (Shibuya et al. 2013). Moreover, expression levels of miRNAs, miR-155 and miR-146a in synovial fibroblasts from peripheral blood of RA patients have been demonstrated to be significantly higher than that patients with osteoarthritis (Kriegsmann et al. 2016; Shumnalieva et al. 2017). It has been reported that miR-16 expression in plasma, and synovial fluid of RA patients was up-regulated, as well as miR-150 was also highly expressed during differentiation of IL-17 producing cells (Wu et al. 2016; Yokoi and Nakajima 2011). Therefore, miRNAs as the biomarker is useful to diagnose the disease. Though mRNAs is the downstream control factor of miRNAs, single miRNA usually regulate hundreds of mRNAs. Additionally, alterations of miRNA expression may be caused by many diseases, resulting in the identification of the disease being unstable. Hence, in order to accurately diagnose the disease, it is necessary to predict the downstream target genes that have specificity to identify the pathogenesis of diseases.

In this work, targetScore, a novel target prediction algorithm was used to predict the targets of the four miRNAs associated with RA. The fold-change induced by miRNA expression and sequence-based information were considered in the use of Bayesian probabilistic scoring approach. Besides, in the targetScore algorithm,

two or three-component VB-GMM were used to simulate the distribution of multiple sets of precalculated or fold-changes and score based on the sequence supplied by the user. All of these are helpful to improve the accuracy of target prediction. Considerable absolute logFC value was observed for targets of the four miRNAs. Generally, the statistically significant is presented for the selected target genes when the absolute logFC is greater than 2. And correlative target genes of miRNAs were obtained by screening genes with targetScore value. The targetScore value represents the consistency between the priori and the posteriori. It is worth mentioning that a compact correlation between the miRNAs and targets was denoted when the targetScore value is 0.7. Thereby, it could be inferred that a close interaction between the predicted 33 genes and miR-223 was shown, indicating that the screened 33 genes may become the optimal targets of miR-223 associated with RA. In particular, larger targetScore values that were greater than 0.99 were used to screen potentially optimal targets of miR-150 and miR-16. Eventually, detecting optimal target genes of miR-150 and miR-16 were 298 and 54, respectively.

Through observing the results in Table 1, a higher similar value can be seen for the logFC value and targetScore value. Besides, considerable absolute value logFC and relatively small p-value was used to compute the targetScore value demonstrating that the target gene predicted by the targetScore algorithm has a higher accuracy. Furthermore, several predicted target genes in this work have been reported in previous studies (Frank et al. 2010; Maney et al. 2017). Ruyssen-Witrand et al. (2014) have investigated the effects of single nucleotide polymorphisms (SNPs) in IL2RB gene on the RA. Moreover, the inhibiting effect and approximately 2.6–fold difference of PRKAR1A gene associated with chronic fatigue syndrome/myalgic encephalomyelitis was detected (Brenu et al. 2012). Similarly, the robust differential expression of PIM1 gene with about 1.6-fold was confirmed (Pratt et al. 2012).

CONCLUSION

In summary, the prediction of miRNAs targets may contribute to diagnose pathogenic mechanism of disease. And in this study, several target genes were highlighted by integrating different miRNAs associated with RA, which could be used to identify the pathogenic mechanism and therapeutic interventions. However, the functional response of the predicted targets to miRNAs in prognosis and therapeutic potential requires further study.

RECOMMENDATIONS

The results summarized from this study were mainly based on the bioinformatic prediction. However, no experiment was performed to verify these results. Therefore, experimental verification is necessary in the next study.

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