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Targetscore used to Reveal Potential Targets of Mir-26a in Osteoporosis by Integrating MicroRNA Over-expression and Microarray Data

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ABSTRACT The objective of this paper was to predict the potential targets of miR-26a in osteoporosis based on the TargetScore combined miRNA over-expression information and sequence data. First, TargetScores were calculated by combining log fold-change and sequence scores obtained from TargetScan context score, probabilities of conserved targeting, and derived the distribution of targetScores. The potential target genes (PTGS) for miR-26a were predicted based on the targetScore threshold. To reveal the functions of miR-26a, the researchers implemented pathway enrichment analyses for the targets of miR-26a. Based on TargetScore>0.45, 246 PTGS for miR-26a were identified for osteoporosis, such as CEP55, CD200 and SYDE2. Besides, pathway enrichment results showed that 9 significant pathways were identified under the thresholds of P<0.05 and Counte”5, especially for Hippo, Glucagon and PI3K-Akt signaling pathway. The researchers have identified potential targets for miR-26a using TargetScore method, which might provide signatures in osteoporosis progression.

INTRODUCTION

Osteoporosis is an emerging medical and socio-economic threat characterized by a systemic impairment of bone mass, strength, and micro-architecture which increases the propensity of fragility fractures (Compston et al. 2017; Tarantino et al. 2017). Bone mass in the skeleton is dependent on the coordinated activities of bone-forming osteoblasts and bone-resorbing osteoclasts in discrete bone multi-cellular units (Mcdonald et al. 2017). Furthermore, fragility fractures may lead to substantial pain and suffering, disability and even death for affected patients and substantial costs to society (Harvey et al. 2017). It is estimated that one in three women and one in five men over the age of 50 worldwide will sustain an osteoporotic fracture (Kim et al. 2017). Therefore, an effective prevention, diagnose and treatment before the occurrence of fractures for this disease is on urgent need.

During past decades, substantial advances have been made in uncovering the pathogenesis of osteoporosis. What’s more, microarray technology is a powerful way for monitoring expression level of thousands of genes simultaneously and provides a variety of other basic applications including tumor classification, molecular pathway modeling and functional genomics (Castillo et al. 2017; Sultankulova et al. 2017). Based on gene expression data, several genes have been detected as potential signatures for osteoporosis patients (Zhang et al. 2018). Meanwhile, microRNAs (miRNAs) play important roles in physiology and disease, and present tremendous therapeutic potential (Ding et al. 2017). Previous studies have revealed key roles for miRNAs in the biology of osteoporosis (Jiménez-Ortega et al. 2017). Particularly, Krzeszinski et al. (2014) had suggested that miR-34a was down-regulated during osteoclast differentiation, and then blocked osteoporosis through inhibiting osteoclastogenesis and transforming

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growth factor-â-induced factor 2 (TGIF2). Moreover, miR-133a was determined as a potential biomarker and regulatory element in circulating monocytes for postmenopausal osteoporosis (Wang et al. 2012). Most important, it had been demonstrated that miR-26a was a promising therapeutic candidate to enhance bone formation in osteoporosis and to promote bone regeneration in osteoporotic fracture healing (Li et al. 2015). Significantly, miR-26a was an oncogene in glioma (Huse et al. 2009) but as a tumor suppressor in liver cancer (Kota et al. 2009) and nasopharyngeal carcinoma (Lu et al. 2011). Currently, great efforts have been made to investigate significant roles of this miRNA in cancers, but a few researchers pay their attention on its functions in osteoporosis. Consequently, biological functions and molecular mechanism of miR-26a in osteoporosis still remains unclear.

Therefore, in this paper, the researchers proposed to uncover potential miR-26a targets and functions for osteoporosis patients based on a probabilistic scoring method called TargetScore, which infers miRNA targets as the transformed fold-changes weighted by the Bayesian posteriors given observed target features (Li et al. 2014). Specifically, based on miRNA over-expression data and miR-26a, log fold-change (logFC) was counted for all genes. Subsequently, the researchers computed the TargetScores by integrating logFC and sequence scores obtained from TargetScan context score (TSCS), probabilities of conserved targeting (PCT), and derived the distribution of TargetScores to predict potential target genes for miR-26a. Ultimately, functional enrichment analysis was conducted on predicted miRNA targets, respectively.

METHODOLOGY

The TargetScore, a probabilistic method for miRNA target prediction problem, integrated miRNA over-expression data and sequence-based scores from the other prediction methods (Li et al. 2014). In brief, every score feature is considered an independent variable as input to a Variational Bayesian-Gaussian Mixture Model (VB-GMM). A Bayesian was selected over a maximum likelihood method to avert over fitting. Concretely, with regard to the expression fold-change, three-component VB-GMM was utilized to infer down-regulated targets accounting for genes with little or positive fold-change (because of off-target effects) (Khan et al. 2009). If not, the researchers employed two-component VB-GMM to unsigned sequence scores. Of which, Variational Bayesian Expectation-Maximization (VB-EM) algorithm was utilized to optimize the parameters of the VB-GMM. The mixture component with the largest absolute averages of negative fold-change or sequence score was correlated to miRNA targets and termed as "target component". Additionally, the other components correspond to the "background component". As a result, inferring miRNA-mRNA interactions was equivalent to speculating the posterior distribution of the miRNA targets. The TargetScore was calculated as the transformed fold-change weighted by the mean posteriors of target components over logFC, TSCS, and PCT.

Preparing miRNA Over-expression Data

In the present paper, the researchers collected miRNA over-expression data corresponding to 1 Gene Expression Omnibus (GEO) set, 26 human samples, and 1 distinct miRNAs (miR-26a). The GEO dataset (GSE7158) comprised of 26 samples including 12 osteoporosis samples and 14 normal controls, was downloaded from the NCBI-GEO (http://www.ncbi.nlm.nih.gov/geo/) based on AFFY-44-Affymetrix GeneChip Human Genome U133 Plus 2.0 [HG-U133_Plus_2] Platform. For purpose of controlling the quality of GSE7158, the data were preprocessed in sequence of background correction, quartile normalization, probe match, and expression summarization utilizing the Affy package (Gautier et al. 2004; Irizarry et al. 2003). As a result, 20,514 genes were obtained in the pre-treated dataset.

As mentioned above, a logFC value of each gene was computed, which referred to different changes at the expression levels across osteoporosis patients and normal controls. For mRNAs interrogated by multiple probes in a single experiment, the researchers took the average of the fold-changes. Consequently, logFC values for 20,514 genes were prepared for further exploitation.

Bayesian Mixture Model

Supposing that there were N genes in the gene expression dataset and x stood for one of

the independent variables without loss of generality, the posterior distribution $p(z|x)$ of the latent variable z was needed to identify to deduce the target genes for a miRNA given x, where K= 3 (K=2) for modeling signed (unsigned) scores including logarithmic fold-changes (sequence scores). Subsequently, the standard Bayesian-GMM was conducted according to Bishop with minor modifications. In detail, the latent variables z were sampled at probabilities π (mixing coefficient), which followed a Dirichlet prior $D(\pi|\alpha_0)$, with hyperparameters $\alpha_0 = (\alpha_{0,1},\ldots,\alpha_{0,K})$. With an attempt to interpret the relative frequency of targets and non-targets for any given miRNA, the researchers set the $\alpha_{0,1}$ (connected with the target component) to aN and other $\alpha_{0,k}$ $=^{(1-a)}$ x $N/_{(K-1),}$ in which a= 0.01 (by default). Ultimately, assuming that x followed a Gaussian distribution $\Gamma(x|\mu, \Lambda^{-1})$, of which Λ (precision matrix) was the inverse covariance matrix. Consequently, $p(\mu, \Lambda)$ obeyed a Gaussian-Wishart prior $\prod_k^K \Gamma(\mu_k|m_0,(\beta_0\Lambda)^{-1}) \boxtimes (\Lambda_k|W_0,\nu_0)$ where the hyperparameters $\{m_0,\beta_0, W_0, \nu_0\} = \{\hat{\mu}, 1, I_{D\times D}, D+1\}$.

VB-EM Analysis

During this step, the marginal log likelihood was written in terms of lower bound $L(q)$ (first term) as well as Kullback-Leibler divergence $KL(q||p)$ (second term):

$$ln\, p\,(x) = \int q\,(\theta)\, ln \frac{p\,(x,\theta)}{q\,(\theta)} + \int q\,(\theta)\, ln \frac{p\,(\theta)}{p\,(\theta|x)}$$

Of which, $\theta = \{z, \pi, \mu, \Lambda\}$, $q(\theta)$ represented a proposed distribution for $p(\theta|x)$. Because $\ln p(x)$ was a constant, maximizing $L(q)$ brought to minimizing $KL(q||p)$. The optimal solution $\ln q_j^*(\theta_j)$ was the expectation of variable j *w.r.t* other variables, $E_{i\neq j}[\ln p(x, \theta)]$. Particularly, the researchs set that $q(z, \pi, \mu, \Lambda) = q(z)q(\pi)q(\mu, \Lambda)$. The expectations for the three terms (at log scale), namely $\ln q^*(z)$, $\ln q^*(\pi)$, $\ln q^*(\mu)$, had the same forms as the initial distributions due to the conjugacy of the priors. However, they require evaluation of the parameters $\{z, \pi, \mu, \Lambda\}$, which in turn all were dependent on the expectations of z or the posterior of interest. The inter-dependence of the expectations and model parameters fell naturally into an EM framework, named as VB-EM.

TargetScore

To the best of the researchers knowledge, the Targetscore is a measure of the mean effect of all neighbors serving as the targets, which ranges from 0 to 1 (Wilson et al. 1986). Besides, the higher the TargetScore was, the greater the accuracy in identifying known targets was. Hence, in this work, the TargetScore for each gene was calculated based on logFC, TSCS, and PCT values to further extract the potential targets for miR-26a. Here, TSCS is a sequence-based score for single target site computed by TargetScan (Garcia et al. 2011), and PCT is the probability of conserved targeting for single target site (Friedman et al. 2009). TSCS and PCT were available from TargetScan website (http://www.targetscan.org/). The TargetScore, an integrative probabilistic score of a gene being the targets of a miRNA, was computed as following formula:

$$TargetScore = \frac{1}{1+exp(log\ FC)}\left(\frac{1}{K+1}\sum_x p(z|x)\right)$$

The distribution of TargetScore for validated and non-validated targets of all the miRNA-mRNA interactions each owning at least 1 validated targets was analyzed. The researchers defined the pre-defined δ as the cut off-criteria for potential miRNA targets.

Pathway Enrichment Analysis

To explore functional biological processes associated with target genes of miR-26a, the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis was carried out based on the Database for Annotation, Visualization, and Integrated Discovery (DAVID, https://david.ncifcrf.gov/home.jsp) (Huang et al. 2008). Importantly, KEGG (http://www. genome.jp/kegg/pathway) is a knowledge base to systematically study gene functions, for understanding cellular processes via the process of pathway aligning (Kanehisa et al. 2000). Pathways with P<0.05 were selected using Expression Analysis Systematic Explored (EASE) test applied in DAVID. EASE analysis of the regulated genes indicated molecular functions and biological processes unique to each category (Ford et al. 2006). In addition, the threshold of minimum number of genes was the corresponding term $\geq$ 5 were considered significant for a category.

RESULTS

LogFC Values for Genes

In the present study, a total of 20,514 genes were obtained in the gene expression data of

osteoporosis after standard preprocessing. The logFC values for 20,514 genes were counted, and the result showed that most genes distributed between -0.4 and 0.3. If one gene interrogated by multiple probes in a single experiment, the researchers would take the average of the fold-changes.

Potential Targets for miR-26a

As described above, TargetScores were calculated between miR-26a and 20,514 genes by combining logFC and sequence scores obtained from TSCS and PCT. As a result, the researchers obtained the TargetScores, and then derived their distribution. When setting the threshold for confident targets as 0.45, 246 potential targets were predicted totally. Specifically, the top three targets with higher TargetScores were CEP55 (TargetScore = 0.703), CD200 (TargetScore = 0.698) and SYDE2 (TargetScore = 0.691). In order to illustrate the interactions between miR-26a and 246 targets more clearly, all data were input to Cystoscape software (http://www.cytoscape.org/), and a network for them was visualized as shown in Figure 1. There were 247 nodes and 246 edges in the network. Among 246 interactions, 54 of them (about 22 percent) had the TargetScore>0.6, while only one interaction (miR-26a and CEP55) possessed TargetScore> 0.7.

Pathway Analysis for Potential Targets

Result of KEGG pathway enrichment analysis showed that 246 potential targets of miR-26a were enriched in 9 significant pathways under the thresholds of P<0.05 and Count >5 (Table 1). Particularly, Hippo signaling pathway (P = 5.07E-04 and Count = 8), Glucagon signaling pathway (P = 1.30E-02 and Count = 5) and PI3K-Akt signaling pathway (P = 1.33E-02 and Count = 9) were the most significant ones. Interestingly, 7 of the 9 significant pathways (about 77.78%) were signaling pathways, which suggested that signaling pathways played critical roles in the progression of osteoporosis.

Table 1: Significant pathways with P < 0.05 and Count >5

Pathway	*P value*	*Count*
Hippo signaling pathway	5.07E-04	8
Glucagon signaling pathway	1.30E-02	5
PI3K-Akt signaling pathway	1.33E-02	9
Insulin resistance	1.92E-02	5
AMPK signaling pathway	2.77E-02	5
FoxO signaling pathway	3.47E-02	5
Wnt signaling pathway	3.47E-02	5
Insulin signaling pathway	3.90E-02	5
Protein digestion and absorption	4.45E-02	7

DISCUSSION

miRNAs, short non-coding RNA molecules, regulate gene expression generally by destabilizing mRNAs or suppressing translation (Zedan et al. 2017). Additionally, miRNAs have been identified as important biomarkers and regulators in various human diseases such as cancer (Lee et al. 2017), diabetes (Ding et al. 2017) and myocardial disease (Song et al. 2017). Therefore, miRNAs offer a clue to elaborate the complex mechanisms of the diseases. Generally speaking, functional characterizations of miRNAs were detected relying on accurate predictions of their targets. Nevertheless, great challenges have occurred in extracting miRNA targets experimentally. Fortunately, computational predicted technologies provide a rapid alternative tool to uncover putative miRNA targets. Most of these prediction approaches are carried out dependent on sequence complementarity, evolutionary, conservation, and target site accessibility (You et al. 2017). However, precise prediction of miRNA targets remains a problem with less than fifty percent specificity and having poor overlap among them. Of note, miRNA over-expression data combined with mRNA expression profiling has been indicated to be a promising method (Chen et al. 2017). Meanwhile, target prediction can be improved by integrating expression change and sequence information such as context score and other orthogonal sequence-based features such as conservation into a probabilistic score (Sells et al. 2017).

Therefore, in this paper, the researchers implemented the TargetScore method, a Bayesian probabilistic scoring method taking into account of the fold-change, miRNA over-expression and sequence-based information, to identify the potential targets of miR-26a in osteoporosis patients. When comparing with previous expression based target predicted methods, the TargetScore method had advantages in three important aspects. First, the TargetScore method was specifically designed for miRNA over-ex-

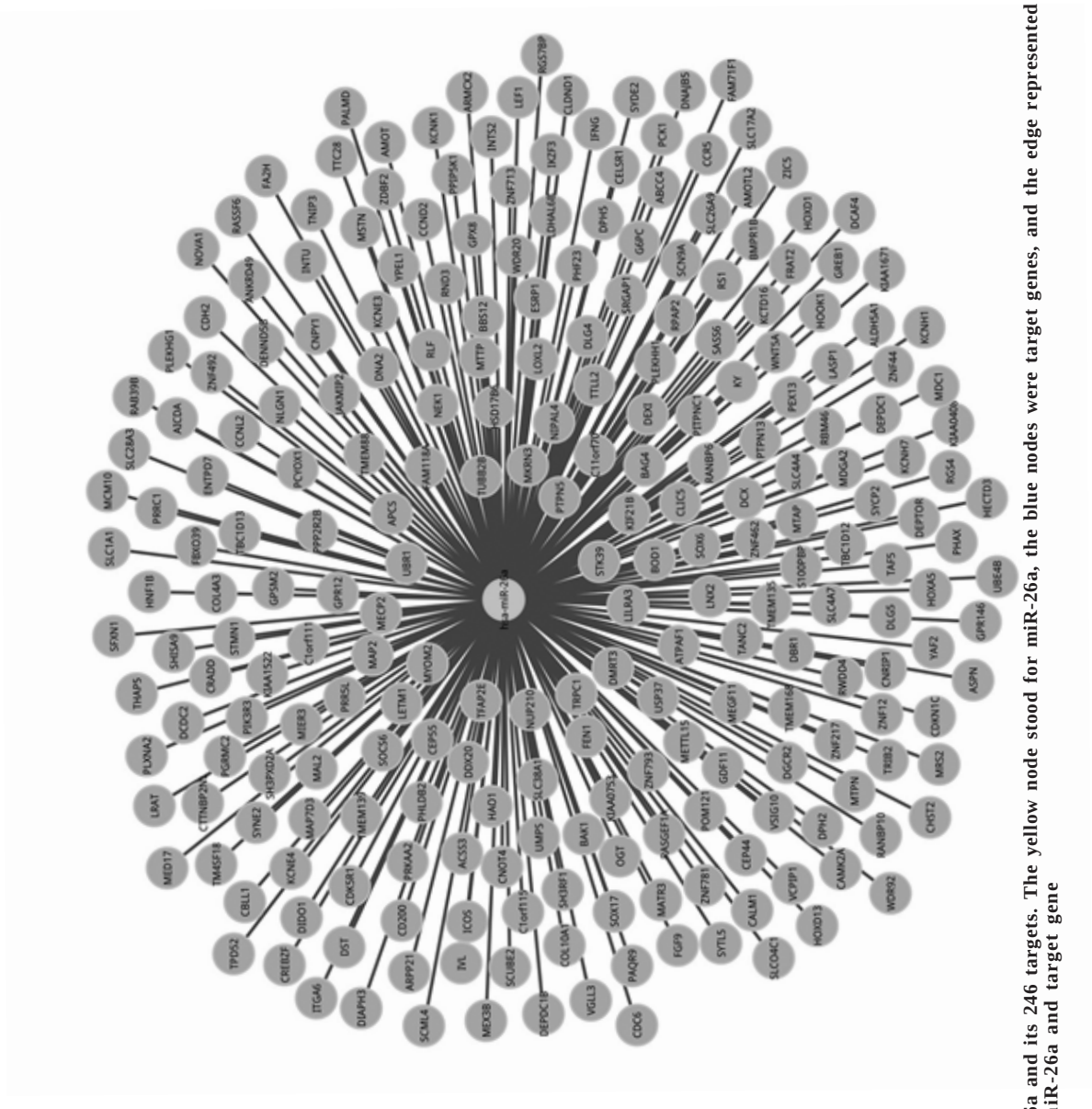

Fig. 1. Network for miR-26a and its 246 targets. The yellow node stood for miR-26a, the blue nodes were target genes, and the edge represented the interaction between miR-26a and target gene

pression data to interrogate targets of a particular miRNA in a specific cell-condition. Second, it inferred miRNA-targets solely based on their distinct high dimensional patterns of expression fold-changes and sequence features. Third, this method operated on the entire gene set to more closely model the overall likelihood rather than only on a subset of genes pre-filtered by the TargetScan score or sample variance (Hai-son et al. 2011; Huang et al. 2007). Thus, the researchers employed the TargetScore method to predict potential targets of miR-26a, and the results showed that a total of 246 potential targets were obtained, such as CEP55, CD200 and SYDE2. Subsequently, pathway enrichment analysis was conducted on target genes to explore functional gene sets and biological processes for osteoporosis patients. Consequently, Hippo signaling pathway, Glucagon signaling pathway and PI3K-Akt signaling pathway were the most significant pathways for osteoporosis when compared with normal controls. Molecular mechanism underlying osteoporosis was inferred and uncovered based on these results.

CEP55 (centrosomal protein 55) is a mitotic phosphoprotein that plays a key role in cytokinesis, the final stage of cell division (Frosk et al. 2017). Additionally, it is a microtubule-bundling protein that associates with centralspindlin to control the midbody integrity and cell abscission (Bondeson et al. 2017). Jeffery et al. (2016) had revealed that CEP55 over-expression was significantly correlated to tumor stage, aggressiveness, metastasis and poor prognosis across multiple tumor types, and even as part of prognostic signatures for cancer. Most important, it is the first time to discover the CEP55 significance in osteoporosis. Meanwhile, CD200 (cluster of differentiation 200), a transmembrane protein, belongs to the immunoglobulin family of proteins and is ubiquitously expressed on a variety of cell types (Curry et al. 2017). Soluble forms of CD200 may prove a useful and rapid means of monitoring subjects at risk of bone loss and accessing the efficacy of treatment regimes designed to counter bone loss (Pontikoglou et al. 2016). In addition, it had been demonstrated that CD200-CD200R interaction controlled osteoclastogenesis and could be a new target to modulate osteoclast function and control bone pathologies such as osteoporosis (Varin et al. 2013). Hence the researchers might infer that potential target of miR-26a CD200 was correlated to osteoporosis closely.

CONCLUSION

In summary, the researchers have successfully identified potential targets of miR-26a in the progression of osteoporosis utilizing the TargetScore method. This research might provide signatures in osteoporosis progression.

RECOMMENDATIONS

However, several limitations must be taken into consideration in the researchers' analysis. Firstly, sample size was small. Secondly, the dataset used in their work were downloaded from the GEO database, not produced by them. Thus, there is a need to conduct a meta-analysis for the relevant datasets of psoriasis in future. Additionally, the results obtained from their work were bioinformatics-based-prediction, but were not confirmed relying on experiments. And so, the researchers do have to implement further experiments to validate the results of the research.

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