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Predicting Potential Targets of miR-130a in Venous Thromboembolism Based on a Target Score Method

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ABSTRACT This paper proposed to predict potential targets of miR-130a in venous thromboembolism (VTE) utilizing a TargetScore method, and to further uncover the functions of miR-130a in VTE. miRNA-overexpression data were collected and log Fold Change (logFC) value was computed for every gene in the dataset. Subsequently, sequence scores were calculated based on Variational Bayesian-Gaussian Mixture Model and Variational Bayesian Expectation-Maximization algorithm. Ultimately, the target score between miR-130a and each gene was counted by integrating the logFC and sequence scores, to investigate potential targets for miR-130a. Based on the targets, pathway enrichment analysis was conducted to explore significant gene sets in VTE patients. 225 potential targets in VTE were predicted for miR-130a, and 16 significant pathways with $P < 0.05$ were identified for VTE patients after enrichment analysis. MiR-130a was critical for the development of VTE, partially through regulating expressions of potential targets and their gene sets.

INTRODUCTION

Venous thromboembolism (VTE), with an annual incidence of 1 to 2 cases per 1000 persons in the general population, is the third most common cause of vascular death after myocardial infarction and stroke (Zabrocka et al. 2018), resulting from deep vein thrombosis, pulmonary embolism, or both (Beckman et al. 2010). VTE, as an independent and major risk factor of cancer, is reported in up to twenty percent of patients with cancer (Konigsbrugge et al. 2014). Hence, the prevention and treatment of VTE patients represent major challenges in daily practice. Currently, conventional therapy includes a parenteral anticoagulant, such as warfarin and enoxaparin (Kearon et al. 2012). However, the regimen still exists shortness since warfarin therapy need to coagulate monitoring and dose adjustment, and enoxaparin requires daily subcutaneous injections (Agnelli et al. 2013).

MicroRNAs (miRNAs), as a class of small non-coding RNA molecules, are highly conserved across species and exert important functions as regulators of gene expression through promoting mRNA degradation and repressing translation (Miya Shaik et al. 2018). It has been reported that as much as sixty percent of human protein coding genes are managed by miRNAs estimably. Substantially, they modulate the levels of targeted genes post-transcriptionally based complementary sequences in the 3'/5'-untranslated regions or in the open reading frames of the mRNAs (Hammond 2015; Simonson and Das 2015). Besides, miRNAs have significant power to regulate biological processes (Schonrock et al. 2012). Thus, miRNAs offer a clue to elaborate the complex mechanisms and physiology, and present tremendous therapeutic potential of diseases (Chivukula and Mendell 2008; Kasinski and Slack 2011). As a consequence, investigating miRNA targets offers an excellent manner to elucidate the molecular mechanisms underlying malignant diseases, and gives a hand to the design of drugs for its treatments.

Generally, 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 quick select tool to uncover putative miRNA targets.

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Most prediction methods are carried out dependent on target site accessibility, sequence complementarity, convervation, and evolutionary (Friedman et al. 2009; Kertesz et al. 2007; Lewis et al. 2003). However, precise prediction of miRNA targets still has a problem with no more than 50 percent specificity and poor overlap ratio. Of note, miRNA over-expression data combined with mRNA expression profiling has been indicated to be a great potential method (Arvey et al. 2010; Lim et al. 2005). Meanwhile, some methods can improve the target prediction, which mainly include the integration of expression change and sequence information (for example, context score) and other orthogonal sequence-based features for example, conservation into a probabilistic score) (Friedman et al. 2009).

Objective

In the present paper, a probabilistic scoring method named TargetScore was implemented, which can infer miRNA targets as the transformed fold-changes weighted by the Bayesian posteriors given observed target features (Li et al. 2014), to predict the potential targets of miR-130a in VTE patients. Significantly, the TargetScore method had advantages in three important aspects compared with the other methods. First, it was particularly designed for miRNA over-expression data to interrogate targets of a particular miRNA in a specific cell-condition. Second, miRNA-targets were inferred using the TargetScore method according to their distinct high dimensional patterns of expression fold-changes and sequence features. Third, this method was used to analyze the entire gene set so as to more closely model the overall likelihood rather than just on a subset of genes prefiltered via sample variance or the TargetScan score (Huang et al. 2007; Tensorer et al. 2011).

METHODOLOGY

Summarization

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). Briefly, every score feature is regarded as an independent variable, and is considered 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 speculate down-regulated targets based on genes with little or positive fold-change (because of off-target effects) (Khan et al. 2009). If not, two-component VB-GMM was applied to unsigned sequence scores. In order to optimize the parameters of the VB-GMM, Variational Bayesian Expectation-Maximization (VB-EM) algorithm was used. The mixture component with the maximum absolute averages of negative fold-change or sequence value was connected with the targets of miRNA and determined as "target component". However, the other components correspond to the "background component". Consequently, inferring miRNA-mRNA pairs was equal to speculating the posterior distribution of the miRNA targets.

TargetScore was calculated as the transformed fold-change, and its prediction for targets of miR-130a in VTE patients was comprised of 3 steps. First of all, miRNA-over-expression data were from the online Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/) database, and the log fold change (logFC) value was computed for every gene in the dataset. Subsequently, sequence scores were calculated based on the VB-GMM and VB-EM algorithm. Ultimately, the target score between miR-130a and each gene was counted by integrating the logFC and sequence scores, to investigate potential targets for miR-130a. Based on these targets, pathway enrichment analysis was conducted to explore significant pathways in VTE.

MiRNA Overexpression Data Collection

In this paper, the dataset of miRNA overexpression was recruited corresponding to 1 GEO set (GSE48000 (Lewis et al. 2015)), 1 platform (Illumina HumanHT-12 V4.0 expression beadchip), 132 samples (107 VTE patients and 25 normal controls), and 1 distinct miRNA (miR-130a). Here, the GEO database is an international public repository, which freely distributes and archives high-throughput gene expression and other functional genomics data sets (Clough and Barrett 2016). With an attempt to control the quality of GSE48000, the researchers performed

standard pre-treatments through the Affy package, including background correction, quartile normalization, probe match and expression summarization (Gautier et al. 2004; Irizarry et al. 2003). Consequently, a total of 21,032 genes were identified in the gene expression data.

Subsequently, a logFC value was assigned to every gene of 21,032 genes, since it represented the different changes at the expression levels between VTE patients and normal controls. As mRNAs were interrogated by multiple probes in a single experiment, the average of the fold-changes were obtained. Finally, logFC values of 21,032 genes were gained for further exploitation.

Bayesian Mixture Model

Supposing there were M genes, the researchers defined $x = (x_1, \ldots, x_M)^T$ as the expression of logFC (x_f) or sequence scores (x_s). Therefore, for L sets of sequence scores, $x \in \{x_f, x_1, \ldots, x_L\}$. With the goal of simplifying the following formulas, the researchers utilized x to stand for one of the independent variables without loss of generality. In order to deduce the target genes for a miRNA given x, the posterior distribution $p(z|x)$ of the latent variable $z \in \{z_1, \ldots, z_K\}$ was needed to obtain, in which $K = 3$ ($K = 2$) for modeling signed (unsigned) scores including logarithmic fold-changes (sequence scores). The standard Bayesian-GMM was conducted in accordance with the Bishop with minor modifications. And then 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 $\alpha_{o,K} = {}^{(1-a) \times N}/_{(K-1)}$, in which $\alpha = 0.01$ (by default). Ultimately, assuming x followed a Gaussian distribution $\Gamma(x|\mu, \Lambda^{-1})$, of which Λ (precision matrix) was the inverse covariance matrix. As a result, $p(\mu, \Lambda)$ obeyed a Gaussian-Wishart prior $\prod^{K}_{k} \Gamma(\mu_k|m_0, (\beta_0\Lambda)^{-1}) W(\Lambda_k|W_0, v_0)$, where the hyperparameters $\{m_0, \beta_0, W_0, v_0\} = \{\hat{\mu}, 1, I_{D\times D}, D + 1\}$.

VB-EM Analysis

During this step, the marginal log likelihood was presented 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, $q(z, \pi, \mu, \Lambda) = q(z)q(\pi)q(\mu, \Lambda)$ was set. 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 that are attributed to the conjugacy of the priors. Nevertheless, the parameters $\{z, \pi, \mu, \Lambda\}$ need to be evaluated, 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 and Keddy 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 dependent on the logFC, TargetScan context score (TSCS), and probabilities of conserved targeting (PCT) to further extract the potential targets for miR-130a. Here, TSCS is a sequence-based score for single target site computed using 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 that is an integrative probabilistic score of a gene being the target s 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

For purpose of exploring functional gene sets for targets of miR-130a, the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis was conducted by the Database for Annotation, Visualization, and Integrated Discovery (DAVID, https://david.ncifcrf.gov/home.jsp) tool (Huang da et al. 2009). Most important, the 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 and Goto 2000). Pathways with P < 0.05 were selected using the Expression Analysis Systematic Explored (EASE) test applied in DAVID. The regulated genes analyzed by EASE demonstrated 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 > 5, which were regarded significant for a category.

RESULTS

Calculation of logFC Values

After conducting quality control and normalization on gene expression data of VTE patients, a total of 21,032 genes were obtained for further analysis. The logFC values for 21,032 genes were computed, and the result showed that most genes distributed between -0.5 and 0.5. If one gene interrogated by multiple probes in a single experiment, the average of the fold-changes would be collected.

Identification of the Potential Targets for miR-130a

As mentioned above, target scores were computed between each gene and miR-130a by integrating logFC values and sequence scores obtained from TSCS and PCT. Subsequently, the distribution of target scores were derived and ä > 0.5 was defined as the cut-off for potential targets of miR-130a. Accordingly, a total of 225 targets were predicted, especially for ARHGEF37 (target score = 0.746), FNBP4 (target score = 0.685), and ABHD3 (target score = 0.612). To illustrate these interactions between miR-26a and 225 targets more clearly, all data were input to the Cystoscape software (http://www .cytoscape.org/), and a network for them was visualized as shown in Figure 1. Among these interactions, 4 targets (ATXN1 (Lee et al. 2008), HOXA5 (Chen and Gorski 2008), MAFB (Garzon et al. 2006) and MEOX2 (Chen and Gorski 2008) were verified experimentally.

Significant Pathways for Potential Targets

For purpose of exploring significant gene sets for 225 target genes, pathway enrichment analysis was conducted on them. Consequently, a total of 12 significant pathways were gained when setting the thresholds as P < 0.05 and Count > 5, as shown in Table 1. Specifically, FoxO signaling pathway (P = 4.44E-04, Count = 8), MicroRNAs in cancer (P = 3.43E-03, Count = 7), and Prolactin signaling pathway (P = 6.98E-03, Count = 5) were the most significant ones in VTE patients as compared to normal controls. Interestingly, the results showed that 6 of 12 significant pathways were attributed to signaling pathway, whereas 4 of 12 were belonged to cancer related pathways.

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

Pathway	*Count*	*P Value*
FoxO signaling pathway	8	4.44E-04
MicroRNAs in cancer	7	3.43E-03
Prolactin signaling pathway	5	6.98E-03
Chemokine signaling pathway	7	8.81E-03
TGF-beta signaling pathway	5	1.05E-02
Signaling pathways regulating pluripotency of stem cells	6	1.48E-02
Pathways in cancer	10	2.21E-02
Pancreatic cancer	5	3.01E-02
Renal cell carcinoma	5	3.14E-02
Adipocytokine signaling pathway	5	3.93E-02
Circadian rhythm	6	3.99E-02
Regulation of autophagy	7	4.75E-02

DISCUSSION

MiRNAs are associated with post-transcriptional regulation of gene expression in multi-cellular organisms by affecting both the stability and translation of mRNAs, and transcribed by RNA polymerase II as part of capped and polyadenylated primary transcripts that can be either protein-coding or non-coding (Schonrock et al. 2012). Additionally, miRNAs have been identified as important biomarkers and regulators in various human diseases such as cancer (Li et al.

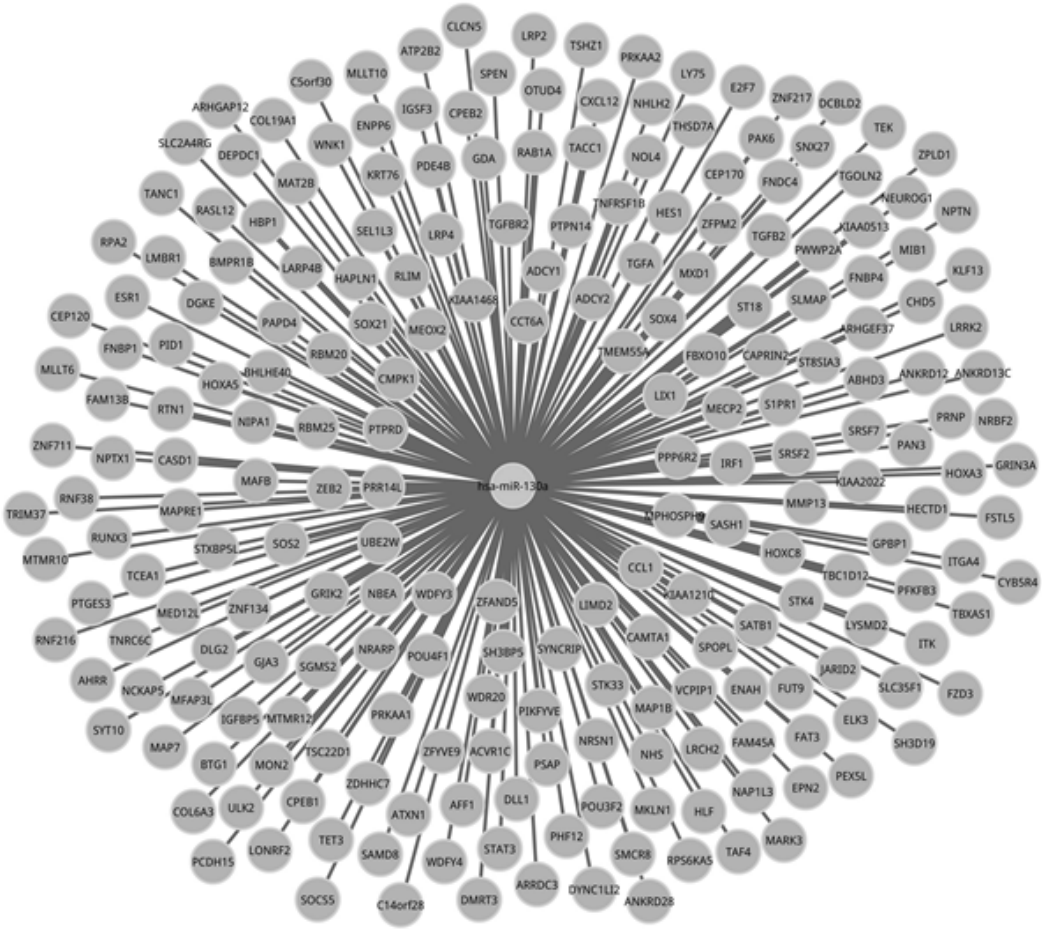

Fig. 1. Network for miR-130a and its target genes. The yellow node stood for miR-130a, the blue nodes were target genes, and the edge represented the interaction between miR-130a and target gene
Source: Author

2010), diabetes (Balasubramanyam et al. 2011) and myocardial disease (Chen et al. 2018) . In particular, miR-130 appears to be vertebrate-specific, and has been predicted or experimentally confirmed in a range of vertebrate species (Li et al. 2011). Therefore, in the paper, potential targets of miR-130a for VTE patients were predicted utilizing a Bayesian probabilistic scoring method, the TargetScore method. As a result, a total of 225 targets were obtained, such as ARHGEF37, FNBP4, and ABHD3. Fortunately, 4 of 225 targets were validated by previous studies, which suggested the feasibility and confidence of the TargetScore method.

ARHGEF37 (Rho guanine nucleotide exchange factor 37) is a number of cytoplasmic proteins family that activate the Ras-like family of Rho proteins through exchanging bound GDP for GTP, and may form a complex with G proteins and stimulate Rho-dependent signals (Guo et al. 2016). Besides, Rho GTPase is critical for various cellular processes that are activated by extracellular stimuli that work through G protein-coupled receptors. This is the first time to uncover the relationship between VTE patients and ARHGEF37. Meanwhile, FNBP4 (formin binding protein 4) plays important roles in regulating cytoskeletal dynamics during cell division and migration, and binds intersection family proteins suggesting a role in the maintenance of membrane curvature at sites of nascent vesicle formation (Das et al. 2015). It has been demonstrat-

ed that FNBP4 interacts with formins and other proteins via its WW domains, and the length of the FNBP4 gene varies greatly among different species (Das et al. 2016). What's more, FNBP4 acted as important role in the progression of breast cancer (Nourashrafeddin et al. 2015), which indicated that this gene correlated to cancer closely. Additionally, VTE was related to cancer tightly. Thus, the present research might indicate that FNBP4 played certain roles in VTE patients to some extent.

KEGG pathway enrichment analysis was conducted to identify biological gene sets and functional processes in VTE patients. In consequence, the 225 targets were enriched in 12 significant pathways, of which 6 were attributed to signaling pathways and 4 belonged to cancer related pathways. In detail, FoxO signaling pathway, MicroRNAs in cancer, and Prolactin signaling pathway were the most significant ones when compared with normal controls. Taking FoxO signaling pathway as an example, the FoxO family of transcription factors regulates genes expression in cellular physiological events mainly associated with longevity, apoptosis, glucose metabolism, cell-cycle control, and oxidative stress resistance. Activation of FoxO factors has been shown to suppress development and reproduction but increase the expression of genes involved in a wide spectrum of stress responses conserved in organisms (van der Vos and Coffer 2011). Hence, this pathway might be critical for VTE patients.

CONCLUSION

The present research revealed potential targets of miR-130a, and their enriched significant pathways based on the TargetScore method. The findings might provide potential biomarkers for treatment and prevention of VTE patients, and even to reveal molecular mechanism in the progression of VTE. Future study should focus on validations of these potential targets of miR-130a.

RECOMMENDATIONS

Results from the current study will provide potential biomarkers for treatment and prevention of VTE patients, and the groundwork for the understanding of VTE pathogenesis.

REFERENCES

Agnelli G, Buller HR, Cohen A, Curto M, Gallus AS, Johnson M, Masiukiewicz U, Pak R, Thompson J, Raskob GE, Weitz JI 2013. Oral apixaban for the treatment of acute venous thromboembolism. *N Engl J Med*, 369: 799-808.

Arvey A, Larsson E, Sander C, Leslie CS, Marks DS 2010. Target mRNA abundance dilutes microRNA and siRNA activity. *Mol Syst Biol*, 6: 363.

Balasubramanyam M, Aravind S, Gokulakrishnan K, Prabu P, Sathishkumar C, Ranjani H, Mohan V 2011. Impaired miR-146a expression links subclinical inflammation and insulin resistance in Type 2 diabetes. *Mol Cell Biochem*, 351: 197-205.

Beckman MG, Hooper WC, Critchley SE, Ortel TL 2010. Venous thromboembolism: A public health concern. *Am J Prev Med*, 38: S495-501.

Chen Y, Gorski DH 2008. Regulation of angiogenesis through a microRNA (miR-130a) that down-regulates antiangiogenic homeobox genes GAX and HOXA5. *Blood*, 111: 1217.

Chen Z, Li C, Lin K, Zhang Q, Chen Y, Rao L 2018. MicroRNAs in acute myocardial infarction: Evident value as novel biomarkers? *Anatol J Cardiol*, 19: 140-147.

Chivukula RR, Mendell JT 2008. Circular reasoning: microRNAs and cell-cycle control. *Trends Biochem Sci*, 33: 474-481.

Clough E, Barrett T 2016. The Gene Expression Omnibus Database. *Methods Mol Biol*, 1418: 93-110.

Das A, Bhattacharya S, Bagchi A, Dasgupta R 2015. In-silico characterization of Formin Binding Protein 4 Family of proteins. *Interdiscip Sci*, 7: 43-64.

Das A, Bhattacharya S, Bhattacharjee S, Bagchi A, Dasgupta R 2016. Computational analyses of the length and compositional variations of the FNBP4 gene across 10 different species. *Gene*, 575: 765-777.

Ford G, Xu Z, Gates A, Jiang J, Ford BD 2006. Expression Analysis Systematic Explorer (EASE) analysis reveals differential gene expression in permanent and transient focal stroke rat models. *Brain Res*, 1071: 226-236.

Friedman RC, Farh KK, Burge CB, Bartel DP 2009. Most mammalian mRNAs are conserved targets of microRNAs. *Genome Res*, 19: 92-105.

Garcia DM, Baek D, Shin C, Bell GW, Grimson A, Bartel DP 2011. Weak seed-pairing stability and high target-site abundance decrease the proficiency of lsy-6 and other microRNAs. *Nat Struct Mol Biol*, 18: 1139-1146.

Garzon R, Pichiorri F, Palumbo T, Iuliano R, Cimmino A, Aqeilan R, Volinia S, Bhatt D, Alder H, Marcucci G, Calin GA, Liu CG, Bloomfield CD, Andreeff M, Croce CM 2006. MicroRNA fingerprints during human megakaryocytopoiesis. *Proc Natl Acad Sci U S A*, 103: 5078-5083.

Gautier L, Cope L, Bolstad BM, Irizarry RA 2004. affy—analysis of Affymetrix GeneChip data at the probe level. *Bioinformatics*, 20: 307-315.

Guo Y, Hu J, Zhu L, Sun J, Xie L, Kong F, Han L, Li F 2016. Physical status and variant analysis of Human Papillomavirus 16 in women from Shanghai. *Gynecol Obstet Invest*, 81: 61-70.

Hammond SM 2015. An overview of microRNAs. *Adv Drug Deliv Rev*, 87: 3-14.

Huang da W, Sherman BT, Lempicki RA 2009. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat Protoc*, 4: 44-57.

Huang JC, Morris QD, Frey BJ 2007. Bayesian inference of MicroRNA targets from sequence and expression data. *J Comput Biol*, 14: 550-563.

Irizarry RA, Hobbs B, Collin F, Beazer-Barclay YD, Antonellis KJ, Scherf U, Speed TP 2003. Exploration, normalization, and summaries of high density oligonucleotide array probe level data. *Biostatistics*, 4: 249-264.

Kanehisa M, Goto S 2000. KEGG: Kyoto Encyclopaedia of Genes and Genomes. *Nucleic Acids Research*, 28: 27-30(24).

Kasinski AL, Slack FJ 2011. Epigenetics and genetics. MicroRNAs en route to the clinic: Progress in validating and targeting microRNAs for cancer therapy. *Nat Rev Cancer*, 11: 849-864.

Kearon C,Akl EA, Comerota AJ, Prandoni P, Bounameaux H, Goldhaber SZ, Nelson ME, Wells PS, Gould MK, Dentali F, Crowther M, Kahn SR 2012. Antithrombotic therapy for VTE disease: Antithrombotic Therapy and Prevention of Thrombosis, 9th Edition. American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. *Chest*, 141: e419S-e496S.

Kertesz M, Iovino N, Unnerstall U, Gaul U, Segal E 2007. The role of site accessibility in microRNA target recognition. *Nat Genet*, 39: 1278-1284.

Khan AA, Betel D, Miller ML, Sander C, Leslie CS, Marks DS 2009. Transfection of small RNAs globally perturbs gene regulation by endogenous microRNAs. *Nat Biotechnol*, 27: 549-555.

Konigsbrugge O, Pabinger I, Ay C 2014. Risk factors for venous thromboembolism in cancer: Novel findings from the Vienna Cancer and Thrombosis Study (CATS). *Thromb Res*, 133 Suppl 2: S39-43.

Lee Y, Samaco RC, Gatchel JR, Thaller C, Orr HT, Zoghbi HY 2008. miR-19, miR-101 and miR-130 co-regulate ATXN1 levels to potentially modulate SCA1 pathogenesis. *Nat Neurosci*, 11: 1137-1139.

Lewis BP, Shih IH, Jones-Rhoades MW, Bartel DP, Burge CB 2003. Prediction of mammalian microRNA targets. *Cell*, 115: 787-798.

Lewis DA, Suchindran S, Beckman MG, Hooper WC, Grant AM, Heit JA, Manco-Johnson M, Moll S, Philipp CS, Kenney K, De Staercke C, Pyle ME, Chi JT, Ortel TL 2015. Whole blood gene expression profiles distinguish clinical phenotypes of venous thromboembolism. *Thromb Res*, 135: 659-665.

Li T, Cao H, Zhuang J, Wan J, Guan M, Yu B, Li X, Zhang W 2011. Identification of miR-130a, miR-27b and miR-210 as serum biomarkers for atherosclerosis obliterans. *Clin Chim Acta*, 412: 66-70.

Li Y, Vandenboom TG, 2nd, Wang Z, Kong D, Ali S, Philip PA, Sarkar FH 2010. miR-146a suppresses invasion of pancreatic cancer cells. *Cancer Res*, 70: 1486-1495.

Li Y, Goldenberg A, Wong KC, Zhang Z 2014. A probabilistic approach to explore human miRNA targetome by integrating miRNA-overexpression data and sequence information. *Bioinformatics*, 30: 621-628.

Lim LP, Lau NC, Garrett-Engele P, Grimson A, Schelter JM, Castle J, Bartel DP, Linsley PS, Johnson JM 2005. Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs. *Nature*, 433: 769-773.

Miya Shaik M, Tamargo IA, Abubakar MB, Kamal MA, Greig NH, Gan SH 2018. The role of microRNAs in Alzheimer's Disease and their therapeutic potentials. *Genes (Basel)*, 9.

Nourashrafeddin S, Aarabi M, Modarressi MH, Rahmati M, Nouri M 2015. The Evaluation of WBP2NL-related genes expression in breast cancer. *Pathol Oncol Res*, 21: 293-300.

Schonrock N, Harvey RP, Mattick JS 2012. Long noncoding RNAs in cardiac development and pathophysiology. *Circ Res*, 111: 1349-1362.

Simonson B, Das S 2015. MicroRNA therapeutics: The Next Magic Bullet? *Mini Rev Med Chem*, 15: 467-474.

Tensorer JML, Falkenstein VV, Tensorer HL, Muhesen S 2011. Inferring interaction networks using the IBP applied to microRNA target prediction. *Advances in Neural Information Processing Systems*, 235-243.

van der Vos KE, Coffer PJ 2011. The extending network of FOXO transcriptional target genes. *Antioxid Redox Signal*, 14: 579-592.

Wilson SD, Keddy PA 1986. Species competitive ability and position along a natural stress/disturbance gradient. *Ecology*, 67: 1236-1242.

Zabrocka E, Wojtukiewicz MZ, Sierko E 2018. Thromboprophylaxis in cancer patients in hospice. *Adv Clin Exp Med*, 27: 283-289.

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