Identifying miRNA synergism using multiple-intervention causal inference

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Abstract

Background

Studying multiple microRNAs (miRNAs) synergism in gene regulation could help to understand the regulatory mechanisms of complicated human diseases caused by miRNAs. Several existing methods have been presented to infer miRNA synergism. Most of the current methods assume that miRNAs with shared targets at the sequence level are working synergistically. However, it is unclear if miRNAs with shared targets are working in concert to regulate the targets or they individually regulate the targets at different time points or different biological processes. A standard method to test the synergistic activities is to knock-down multiple miRNAs at the same time and measure the changes in the target genes. However, this approach may not be practical as we would have too many sets of miRNAs to test.

Results

In this paper, we present a novel framework called miRsyn for inferring miRNA synergism by using a causal inference method that mimics the effects in the multiple-intervention experiments, e.g. knock-down multiple miRNAs. Our experimental results show that several miRNA-miRNA pairs that have shared targets at the sequence level are not working synergistically at the expression level. Moreover, the identified miRNA synergistic network is small-world and biologically meaningful, and a number of miRNA synergistic modules are significantly enriched in breast cancer. Our further analyses also reveal that most of synergistic miRNA-miRNA pairs behave the same expression patterns. The comparison results show that miRsyn performs better in identifying miRNA synergistic network.

Conclusions

Taken together, the results imply that miRsyn is a promising framework to identify miRNA synergism, and could enhance the understanding of miRNA synergism in breast cancer.

Keywords

miRNA – miRNA synergistic network – miRNA synergistic module – Multiple intervention causal inference – Breast cancer

Background

MicroRNAs (miRNAs) are a class of short non-coding RNAs with ~23 nucleotides (nts) in length. They play an important regulatory role at the post-transcriptional level by targeting messenger RNAs (mRNAs) for degradation or translation repression [1]. Previous studies have demonstrated that miRNA plays a crucial role in regulating gene expression involved in diverse biological processes including cell proliferation, cell death, cell apoptosis and human cancers [2-4]. Generally, the relationships between miRNAs and target genes are not one-to-one but multiple-to-multiple, indicating cooperative regulation of miRNAs. The combinatorial regulation of miRNAs has been accepted and confirmed by cross-linking and immunoprecipitation technologies, and may be related to human complex diseases [5]. Therefore, studying miRNA synergism can greatly help to understand the synergistic regulation mechanism of miRNAs in human complex diseases.

Until now, a number of computational methods have been proposed to identify miRNA synergism. These methods can be divided into three different categories: (1) sequence-based [6-8], (2) correlation-based [9-14], and (3) causality-based [15]. In the first category, the sequence data mainly includes putative miRNA-target interactions and protein-protein interactions (PPIs). For each candidate miRNA-

miRNA pair, they firstly evaluate the significance of common target genes by using a hypergeometric test. Then, by conducting Gene Ontology (GO) [16] or Kyoto Encyclopedia of Genes and Genomes (KEGG) [17] enrichment analysis of the shared target genes, they determine whether a candidate miRNA-miRNA pair is functionally synergistic or not. The main limitation of the methods in the category is that they only use static data to study miRNA synergism. In fact, the co-regulation between miRNAs is usually dynamic in human cancers [18]. For the second category, they use expression data of miRNAs to identify differentially expressed miRNA synergistic network or integrate matched miRNA and mRNA expression data with sequence data to infer miRNA synergistic network. The identified miRNA synergistic networks or modules using statistic correlation methods may not reveal the causal relationships of gene regulation. To address this issue, a causal discovery based method in the third category is presented to infer miRNA-target causal relationships. However, the method only simulates the causal effect in the single-intervention experiments, e.g. knock-down a miRNA each time. However, the miRNA co-regulation simultaneously involves multiple miRNAs.

In general, the miRNA-miRNA synergistic pairs at the sequence level may not crosstalk with each other to co-regulate target genes at the expression level. Previous study [19] has shown that miRNAs tend to synergistically control expression levels of target genes. Furthermore, the above approaches don’t evaluate the synergistic activities of multiple miRNAs on the target genes, e.g. causal effects of multiple synergistic miRNAs on the shared target genes.

To address the above issues, in this work, we present a framework called miRsyn for inferring miRNA synergism from both sequence-based binding information and expression data by simulating multiple-intervention experiments, e.g. knock-down

multiple miRNAs. We apply the proposed method to The Cancer Genome Atlas (TCGA) breast cancer (BRCA) dataset. Our experimental results show that several miRNAs that have shared targets at the sequence level may not be working synergistically at the expression level, and the miRNA synergistic modules discovered are strongly related to BRCA. Our further analyses also reveal that most of synergistic miRNA-miRNA pairs tend to be co-expressed, which help make a rapid response to external disturbances. Finally, the comparison results demonstrate that miRsyn performs better in studying miRNA synergism.

Methods

Overview of miRsyn

As illustrated in Figure 1, miRsyn is a step-wise method for identifying miRNA synergism. Firstly, given matched miRNA and mRNA expression data, we use feature selection based on Cox regression model [20] to identify expression data of significant miRNAs and mRNAs. Then, by using multiple-intervention causal inference method, we obtain joint causal effects between significant miRNAs and mRNAs. On the other hand, the putative miRNA-target binding information is used to generate binary relationships between significant miRNAs and mRNAs. By integrating joint causal effects and binary relationships between significant miRNAs and mRNAs, we find a set of miRNAs with the maximum joint causal effect on each mRNA. The miRNAs in each set of miRNAs synergistically regulate with each other, and all synergistic miRNA-miRNA pairs are combined to construct miRNA synergistic network. To identify miRNA synergistic modules, we firstly find a set of bi-cliques with at least 2 miRNAs and mRNAs based on putative miRNA-mRNA binding information. For each bi-clique, the subset of bi-clique with the maximum joint causal effect is regarded as a miRNA synergistic module. Finally, we conduct

functional analysis of miRNA synergism at both network and module levels. In the following, we will describe key steps in detail.

Estimating multiple-intervention effects

Let $G=(V,E)$ be a graph including a set of vertices V and a set of edges $E \subseteq V \times V$. Here, $V=\{X_1,...,X_p,Y_1,...,Y_q\}$ is a set of random variables denoting the expression levels of p miRNAs and q mRNAs, and E represents the regulatory relationships between these variables. If a graph G only contains directed edges and no cycles, the graph is a Directed Acyclic Graph (DAG). An equivalence class of DAGs can be described by a *completed partially directed acyclic graph* (CPDAG) which includes both directed edges and undirected edges [21].

Let's assume that we have observational data (e.g. gene expression data) that are multivariate Gaussian and faithful to the true (but unknown) underlying causal DAG without hidden variables. Under the assumption, the Joint-IDA (Joint Intervention calculus when the DAG is Absent) [22] estimates the multiset of possible total joint causal effects of X ($\{X_1,...,X_p\}$) on Y ($\{Y_1,...,Y_q\}$). For example, the joint-intervention effect of X on Y_j ($j \in \{1,...,q\}$) is denoted by $(ef_{1j},...,ef_{pj})$, where (ef_{ij} ($i \in \{1,...,p\}$, $j \in \{1,...,q\}$) represents the causal effect of X_i on Y_j when we knock-down all X_k ($k \neq i$) at the same time. Based on Pearl's *do-calculus* [23], the joint causal effect of X on each of Y_j ($j \in \{1,...,q\}$) is defined as follows:

$$
\begin{aligned}
&(ef_{11},...,ef_{p1}) = (E[Y_1 \mid do(X_1 = x_1+1,...,X_p = x_p)] - E[Y_1 \mid do(X_1 = x_1,...,X_p = x_p)] \\
&,...,E[Y_1 \mid do(X_1 = x_1,...,X_p = x_p+1)] - E[Y_1 \mid do(X_1 = x_1,...,X_p = x_p)]) \\
&\vdots \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad (1) \\
&(ef_{1q},...,ef_{pq}) = (E[Y_q \mid do(X_1 = x_1+1,...,X_p = x_p)] - E[Y_q \mid do(X_1 = x_1,...,X_p = x_p)] \\
&,...,E[Y_q \mid do(X_1 = x_1,...,X_p = x_p+1)] - E[Y_q \mid do(X_1 = x_1,...,X_p = x_p)])
\end{aligned}
$$

In the formula, $do[.]$ is the operation to force X_i to have the value x_i, and $E[.]$ is the operation of evaluating causal effects. Although Joint-IDA implemented in the R package *pcalg* [24] is useful, it is not practical to apply to gene expression datasets with thousands of variables. Thus, we implemented the parallelized Joint- IDA algorithm in the R package *ParallelPC* [25] which uses multiple-core CPU to speed up the runtime of the Joint-IDA algorithm.

Moreover, under the Gaussian assumption, the partial derivatives ef_{ij} in the formula do not depend on the values at which they are evaluated. Therefore, we estimate a multiset of possible total joint causal effects instead of the unique total joint effect, since it is typically impossible to identify the latter when the true underlying causal DAG is unknown (even with an infinite amount of data). The total joint causal effect $(\delta_1,...,\delta_q)$ of X on each of Y_j is defined in the following:

$$\begin{aligned} \delta_1 &= \sum_{i=1}^{p} -average(X_i)ef_{i1} \\ &\text{M} \\ \delta_q &= \sum_{i=1}^{p} -average(X_i)ef_{iq} \end{aligned} \tag{2}$$

In the formula, $average(X_i)$ denotes the average expression level of X_i in the expression data.

Identifying miRNA synergistic network

After feature selection in the matched miRNA and mRNA expression data, we obtain a list of significant p miRNAs and q mRNAs. Given the putative miRNA-target binding information, let $A=\{miR_1,...,miR_p\}$ be a set of significant miRNAs that have binding sites with a significant target mRNA mR_j ($j \in \{1,...,q\}$). Our aim is to find a set of $A^*=\{miR_1,...,miR_{p'}\}$ that has the maximum joint causal effect on the mRNA mR_j. This is the total joint causal effect of A^* on the mRNA mR_j when we knock-down A^*

at the same time. For q mRNAs, we will have q sets of A^* at most. In each set of A^*, the miRNAs synergistically regulate with each other, and all synergistic miRNA-miRNA pairs are integrated to identify miRNA synergistic network. It is noted that our identified miRNA synergistic network is different from that of existing methods, as we draw each A^* as a node by simulating multiple gene knock-down experiments.

Identifying miRNA synergistic modules

In this section, we firstly initialize the miRNA-mRNA bipartite network between significant p miRNAs and q mRNAs by using putative miRNA-target binding information. Then, we use the R package *biclique* [26] to find all of the bi-cliques from the miRNA-mRNA bipartite network. The bi-cliques provide the candidate miRNA synergistic modules for testing the miRNA synergistic activities. For each bi-clique, let $C=\{miR_1,...,miR_k\}$ and $D=\{mR_1,...,mR_l\}$ denote k (≥ 2) miRNAs and l (≥ 2) mRNAs in the bi-clique. Based on the joint causal effects of C on each mRNA of D, we find a set of $C^*=\{miR_1,...,miR_{k'}\}$ and $D^*=\{mR_1,...,mR_{l'}\}$ with the maximum joint causal effect. For each bi-clique, the identified (C^*, D^*) is regarded as a miRNA synergistic module. For n bi-cliques, we will obtain a maximum of n miRNA synergistic modules. For each miRNA synergistic module, the number of miRNAs or mRNAs is at least 2.

Topological and functional analysis of miRNA synergism

The topological analysis of miRNA synergism could help to understand the internal organization of miRNA synergistic network, e.g. power law degree distribution, the average clustering coefficient and the average characteristic path length. If the node degree in a biological network obeys a power law curve (in the form of $y = bx^a$) distribution with high value of R^2, the network is regarded to be scale-free. Here, the R^2 value is a deterministic coefficient to measure the quality of a power curve fit. The

interval of R^2 value is [0 1], and a larger value of it indicates a better power law curve fit. The average clustering coefficient is used to evaluate the dense neighborhood of a biological network. In a small-world biological network, it is much larger than that of random networks [27, 28]. The average characteristic path length indicates the density of a biological network. In a small-world biological network, it is smaller than that of random networks [28].

In this work, we obtain topological features (power law degree distribution, the average clustering coefficient and the average characteristic path length) of the miRNA synergistic network by using *NetworkAnalyzer* plugin [29] in Cytoscape [30]. For generating random networks, we use the duplication model [31] of *RandomNetworks* plugin (https://github.com/svn2github/cytoscape/tree/master/csplugins/trunk/soc/pjmcswee/src/cytoscape/randomnetwork) in Cytoscape. We construct 10000 random instances by randomizing the miRNA synergistic network, and calculate the average clustering coefficient and the average characteristic path length of them.

We conduct functional enrichment analysis to investigate the biological functions of miRNA synergism. For the identified miRNA synergistic network, we use the online tool miEAA [32] to infer the significantly enriched or depleted biological processes, pathways and diseases associated with synergistic miRNAs (p-value < 0.05). For the identified miRNA synergistic modules, we focus on annotating BRCA-related miRNA synergistic modules by conducting BRCA enrichment analysis. Here, we use a hypergeometric test to perform BRCA enrichment analysis. For each miRNA synergistic module, the significance p-value of BRCA genes is calculated as follows.

$$p-value = 1 - \sum_{i=0}^{x-1} \frac{\binom{M}{i}\binom{N-M}{K-i}}{\binom{N}{K}} \quad (3)$$

In the formula, N is the number of significant genes (including miRNAs and mRNAs) after feature selection, M denotes the number of BRCA genes in significant genes, K represents the number of genes in each miRNA synergistic module, and x is the number of BRCA genes in each miRNA synergistic module. The miRNA synergistic modules with p-value < 0.05 are regarded as BRCA-related modules.

Results

Data source

We obtain the matched BRCA expression data of miRNAs and mRNAs and the clinical information of BRCA samples from TCGA [33]. Firstly, we remove all the male samples for BRCA because this is a relative minority event. For the matched miRNA and mRNA expression data, the genes with missing values across the samples (>30%) is removed. The remaining missing values are imputed using the *k*-nearest neighbours (KNN) algorithm from the *impute* R package [34]. Then, we conduct $log_2(x+1)$ transformation and *z*-score normalization for the expression levels of miRNAs and mRNAs. In addition, we use the *miRBaseConverter* R package [35] to convert miRNA names to the latest version of miRBase. Finally, we use the *FSbyCox* function (a feature selection based on Cox regression model) from the *CancerSubtypes* R package [36] to identify significant miRNAs and mRNAs. After the feature selection, we identify expression data of 79 miRNAs and 1314 mRNAs in 753 BRCA samples at a significant level (p-value < 0.05) in total.

For the putative miRNA-target interactions, we use the experimentally validated interactions from miRTarBase v7.0 [37]. A list of BRCA-related miRNAs are from

HMDD v3.0 [38], miR2Disease [39], miRCancer [40] and oncomiRDB [41]. A list of BRCA-related genes is obtained from DisGeNET v5.0 [42] and COSMIC v86 [43].

MiRNA synergistic network is small-world and biologically meaningful

By following the steps of Figure 1, we have identified a list of 702 miRNA-miRNA synergistic pairs between 78 miRNAs (details can be seen in Additional file 1). These miRNA-miRNA synergistic pairs are integrated into a miRNA synergistic network. Out of 78 miRNAs, the number of BRCA-related miRNAs is 39 (red nodes in Figure 2). We define that BRCA-related miRNA-miRNA pairs are those in which the two synergistic parties are BRCA-related miRNAs. As a result, we obtain a list of 269 BRCA-related miRNA-miRNA pairs (details can be seen in Additional file 1).

As shown in Figure 2 (table at the bottom of the figure), the distribution of node degrees of the miRNA synergistic network do not follow power law distribution with $R^2 = 0.192$. This result indicates that the identified miRNA synergistic network is not scale-free. However, the miRNA synergistic network exhibits dense local neighborhoods with the average clustering coefficient of 0.528, which is much larger than that of random networks (0.178 ± 0.037). In addition, the miRNAs in the network are closely connected with the average characteristic path length of 1.837, which is smaller than that of random networks (2.511 ± 0.048). Altogether, the dense local neighborhoods and the small average characteristic path length imply that the miRNA synergistic network is small-world, and can be used to predict miRNA synergism [27, 28].

To investigate the potential biological processes, pathways and diseases related to the synergistic miRNAs, we conduct functional enrichment analysis of them. As shown in Table 1, the synergistic miRNAs are significantly enriched in several biological processes, pathways and diseases associated with BRCA, such as cell cycle

(GO0007050, GO0007093) [44],cell division (GO0051781) [45], cell apoptosis (GO0002903, GO0042981, GO0043065, hsa04210) [46], cell migration (GO0030334, GO0010595, GO0030335,) [47], cell differentiation (GO0045595, GO0045446,) [48], cell proliferation (GO0050678, GO0072091) [49], signaling pathway (P00038, P00056, WP304) [50] and Breast Neoplasms. The detail information of miRNA enrichment analysis results can be seen in Additional file 2. This result demonstrates that the miRNA synergistic network is closely associated with the biological condition of BRCA dataset, and is biologically meaningful.

A number of miRNA synergistic modules are significantly enriched in BRCA

In this section, we have identified 361 miRNA synergistic modules by following the steps in Figure 1 (details in Additional file 3). To understand whether the identified miRNA synergistic modules are closely associated with BRCA, we conduct BRCA enrichment analysis of these modules. As a result, the number of miRNA synergistic modules significantly enriched in BRCA is 72 (p-value < 0.05), indicating that a number of miRNA synergistic modules is closely related to the biological condition of BRCA dataset (details in Additional file 3).

Most of synergistic miRNA-miRNA pairs behave the same expression patterns

In this study, we use Pearson correlation of each synergistic miRNA-miRNA pair to measure the co-expression level. A synergistic miRNA-miRNA pair with significantly positive correlation (p-value < 0.05) is regarded as a co-expressed pair. Out of 702 synergistic miRNA-miRNA pairs, we discover that 499 synergistic miRNA-miRNA pairs are co-expressed (details in Additional file 4). This result indicates that most of synergistic miRNA-miRNA pairs (~71.08%) behave the same expression patterns. It also implies that most miRNAs with the same expression patterns would like to

collaborate with each other to co-regulate target genes. The result is consistent with previous studies [7, 51].

Several synergistic miRNA-mRNA pairs at the sequence level are not working synergistically at the expression level

At the sequence level, we only use putative miRNA-target interactions to construct miRNA synergistic network. In this work, we use the DmirSRN motif in [15] to generate miRNA synergistic regulatory network. Consequently, we find that 1313 miRNA-miRNA pairs can directly regulate the same target by cooperating with each other at the sequence level (details in Additional file 5). Out of 1313 synergistic miRNA-miRNA pairs at the sequence level, 611 miRNA-miRNA pairs are not working synergistically at the expression level by comparing with the miRNA synergistic network generated by miRsyn (details in Additional file 5). This result implies that several miRNA-miRNA pairs that have shared targets at the sequence level may not work synergistically at the expression level.

Comparison results

There are several existing methods to infer miRNA synergistic network. However, to be fair, we use the existing method mirSRN [15] for the comparison. Firstly, mirSRN use the same types of input data (expression data and sequence-based binding information) as our method miRsyn to study miRNA synergism. In addition, mirSRN also uses a causal inference method to identify miRNA synergistic network.

As shown in Figure 3, we compare our method miRsyn with mirSRN in different terms. The detail results of mirSRN can be seen in Additional file 6. In terms of the identified miRNA synergistic pairs (Figure 3A), the number of miRNA synergistic pairs predicted by miRsyn (702) is more than that by mirSRN (239). The majority of the identified miRNA synergistic pairs from mirSRN (163) are predicted by miRsyn. As for the significantly enriched terms (Gene Ontology, Pathways and Diseases)

associated with the identified miRNA synergistic network (Figure 3B), the identified miRNA synergistic network from miRsyn is significantly enriched in more number of functional terms excepting the terms of Diseases.

For the comparison in the percentage of BRCA miRNAs and miRNA synergistic pairs (Figure 3C), the constructed miRNA synergistic network by mirSRN contains higher percentage of BRCA miRNAs. However, the constructed miRNA synergistic network by mirSRN involves higher percentage of BRCA miRNA synergistic pairs. Since the dense local neighborhoods and the small average characteristic path length can be exploited to predict miRNA synergism, Figure 3C implies that miRsyn is more suitable than mirSRN to identify miRNA synergism.

As shown in Figure 3D, most of synergistic miRNA-miRNA pairs identified by both miRsyn (~71.08%, 499 out of 702) and mirSRN (~82.43%, 197 out of 239) all behave the same expression patterns. This comparison result indicates that the results from miRsyn and mirSRN are consistent with each other. Although there is still no ground-truth for validating miRNA-miRNA synergistic pairs, we can use putative high-confidence miRNA-miRNA from the third-party database. In this work, we use the PmmR database [52] to compare the overlap with putative miRNA synergistic pairs between miRSyn and mirSRN. The score (the interval is [0 1]) in the PmmR database indicates the strength of each miRNA-miRNA synergistic pair, and a larger score denotes a stronger strength. Under different score cutoffs (range from 0.50 to 0.70 with a step of 0.05), the overlap of miRsyn is always larger than that of mirSRN (Figure 3E).

In sum, the above comparison results indicate miRsyn is more suitable than mirSRN in studying miRNA synergism.

Conclusions

It is known that human complex diseases including cancers are affected by multiple miRNAs rather than individual miRNA. Therefore, identifying miRNA synergism at a system-wide level is important to understand the regulatory mechanisms of human complex diseases.

In this work, we propose a framework called miRsyn to identify miRNA synergism from both sequence and expression data. By using multiple-intervention causal inference, we simulate the causal effects of multiple knock-down miRNAs on target genes in the multiple-intervention experiments. To study miRNA synergism, we conduct analysis at both network and module levels.

Topological analysis shows that the constructed miRNA synergistic network is not scale-free, but small-world. The small-worldness may help the synergism of miRNAs to quickly adapt to a new biological environment caused by disturbances. In addition, most of synergistic miRNA-miRNA pairs behave the same expression patterns, which allows for a rapid response to external disturbances.

By only using sequence-based binding information for identifying miRNA synergistic network, we discover that a number of miRNA-miRNA pairs at the sequence level are not working synergistically at the expression level. This result implies that it is necessary to study miRNA synergism from heterogeneous data source. To further reveal the potential functions, we conduct functional enrichment analysis of synergistic miRNAs. The miRNA enrichment analysis results display that the identified miRNA synergistic network is directly or indirectly associated with the biological condition of BRCA dataset. Moreover, by conducting BRCA enrichment analysis, we find that several miRNA synergistic modules are significantly enriched in BRCA.

We compare our method miRsyn with mirSRN in different terms, including the number of synergistic miRNA pairs, the number of significantly enriched terms, the percentage of BRCA miRNAs and miRNA synergistic pairs, clustering coefficient and characteristic path length, the number of co-expression and non co-expression miRNA synergistic pairs, and the overlap with putative miRNA synergistic pairs under different score cutoffs. The comparison results show that miRsyn is more suitable to identify miRNA synergism.

Taken altogether, this work provides a simple framework to identify miRNA synergism that can be applied in variable biological fields. The presented results from the proposed method provide insights to understand the synergistic roles of miRNAs in breast cancer. We believe that the presented method is applicable to the study of miRNA synergism associated with other human complex diseases.

Abbreviations

miRNA: microRNA; **nt:** nucleotide; **mRNA:** Messenger RNA; **PPI**: Protein-protein interaction; **GO**: Gene Ontology; **KEGG**: Kyoto Encyclopedia of Genes and Genomes; **TCGA:** The cancer genome atlas; **BRCA:** Breast cancer; **DAG**: Directed Acyclic Graph; **CPDAG:** Completed Partially Directed Acyclic Graph; **Joint-IDA**: Joint Intervention calculus when the DAG is Absent; **KNN:** *k*-nearest neighbours

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets and source code in the current study are available at https://github.com/zhangjunpeng411/miRsyn.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

JZ, JL and TDL conceived the idea of this work. VVHP, LL, TX and BT refined the idea. JZ and VVHP designed and performed the experiments. TX and BT participated in the design of the study and performed the statistical analysis. JZ, LL, JL and TDL drafted the manuscript. All authors revised the manuscript. All authors read and approved the final manuscript.

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Figures

Figure 1 - The workflow of miRsyn.
The process contains three main steps. Firstly, we identify significant miRNAs and mRNAs using feature selection from miRNA and mRNA expression data. Secondly, by integrating expression data of significant miRNAs and mRNAs and putative miRNA-target interactions, we identify miRNA synergistic network and modules. Finally, we make a functional analysis of the identified miRNA synergistic network and modules.

Figure 2 - Visualization of miRNA synergistic network generated by Cytoscape.
The BRCA-related miRNA nodes are colored in red, and the non BRCA-related miRNA nodes are colored in white. The dash lines denote synergistic relationships.

Figure 3 - Comparison results between miRsyn and mirSRN.
(A) The number of miRNA synergistic pairs. (B) The number of significantly enriched terms. (C) The percentage of BRCA miRNAs and miRNA synergistic pairs, clustering coefficient and characteristic path length. (D) The number of co-expression and non co-expression miRNA synergistic pairs. (E) The overlap with putative miRNA synergistic pairs under different score cutoffs.

Tables

Table 1 - A portion of enriched or depleted biological processes, pathways and diseases associated with BRCA by using miRNA enrichment analysis.

Additional files

Additional file 1 –miRNA synergistic network.
The miRNA synergistic network includes 702 miRNA-miRNA synergistic pairs, and 269 BRCA-related miRNA-miRNA pairs.

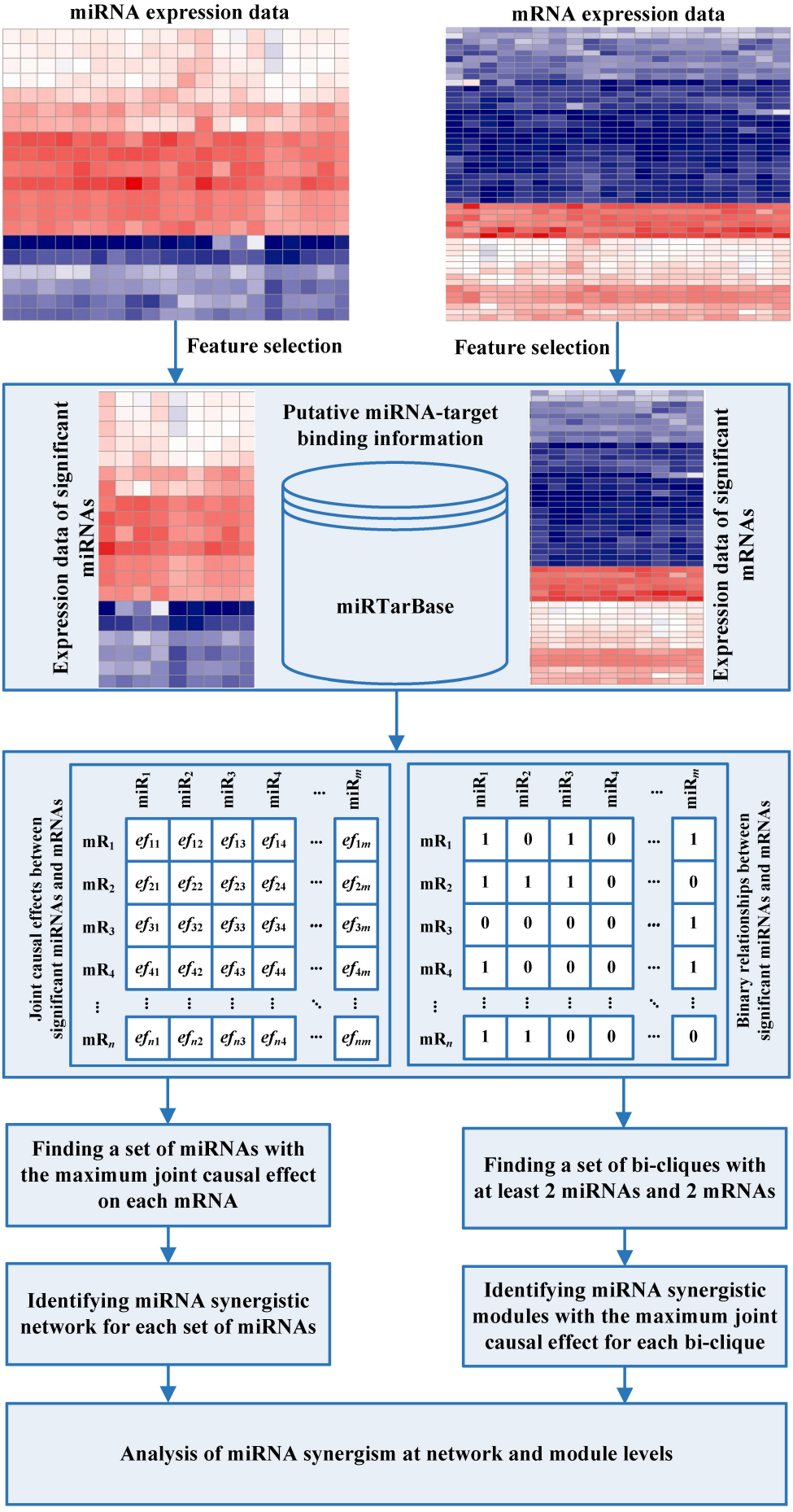
Additional file 2 – miRNA enrichment analysis results of the synergistic miRNAs in the identified miRNA synergistic network.
In total, we obtain 444 Gene Ontology terms, 57 Pathways terms and 6 Diseases terms related to the synergistic miRNAs, respectively.

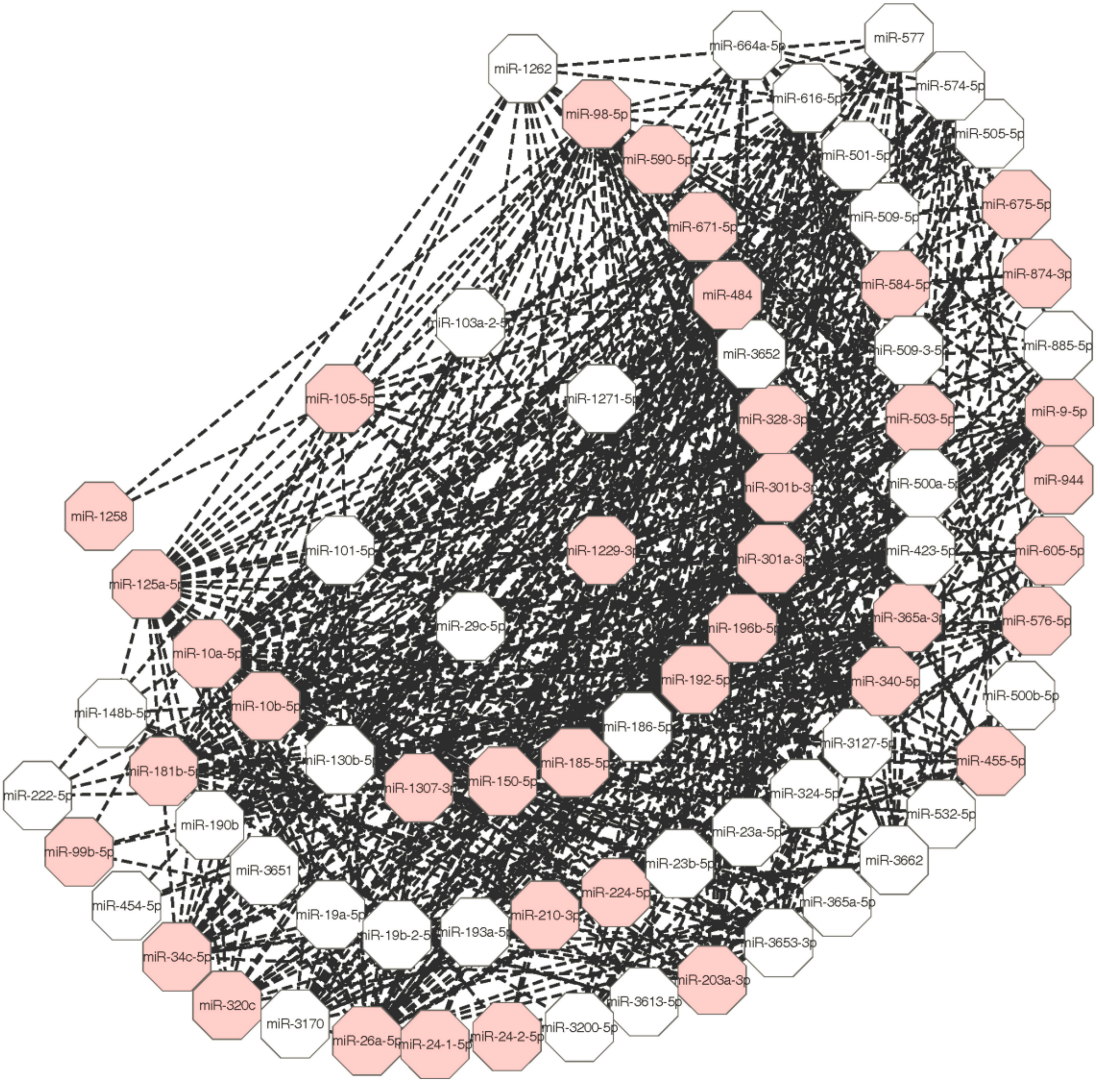
Additional file 3 –miRNA synergistic modules.
The numbers of miRNA synergistic modules and BRCA-related miRNA synergistic modules are 361 and 72, respectively.

Additional file 4 – Co-expression and non co-expression miRNA-miRNA synergistic pairs.
The numbers of co-expression and non co-expression miRNA-miRNA pairs are 499 and 203, respectively.

Additional file 5 – miRNA synergistic network at the sequence level.
The number of miRNA-miRNA synergistic pairs is 1313 at the sequence level, and 611 synergistic miRNA-miRNA pairs of them are not working synergistically at the expression level.

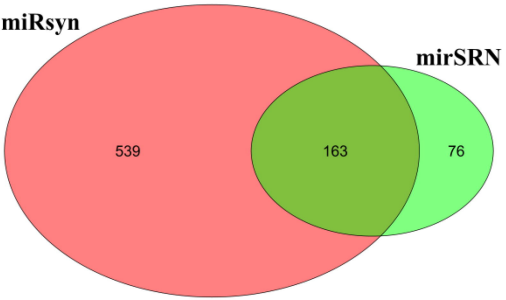
Additional file 6 – The comparison results of the mirSRN method.


miRNA expression data
mRNA expression data
Feature selection
Feature selection
Expression data of significant miRNAs
Putative miRNA-target binding information
miRTarBase
Expression data of significant mRNAs
Joint causal effects between significant miRNAs and mRNAs
miR1 miR2 miR3 miR4 ... miRm
mR1 ef11 ef12 ef13 ef14 ... ef1m
mR2 ef21 ef22 ef23 ef24 ... ef2m
mR3 ef31 ef32 ef33 ef34 ... ef3m
mR4 ef41 ef42 ef43 ef44 ... ef4m
mRn efn1 efn2 efn3 efn4 ... efnm
miR1 miR2 miR3 miR4 ... miRm
mR1 1 0 1 0 ... 1
mR2 1 1 1 0 ... 0
mR3 0 0 0 0 ... 1
mR4 1 0 0 0 ... 1
mRn 1 1 0 0 ... 0
Binary relationships between significant miRNAs and mRNAs
Finding a set of miRNAs with the maximum joint causal effect on each mRNA
Finding a set of bi-cliques with at least 2 miRNAs and 2 mRNAs
Identifying miRNA synergistic network for each set of miRNAs
Identifying miRNA synergistic modules with the maximum joint causal effect for each bi-clique
Analysis of miRNA synergism at network and module levels

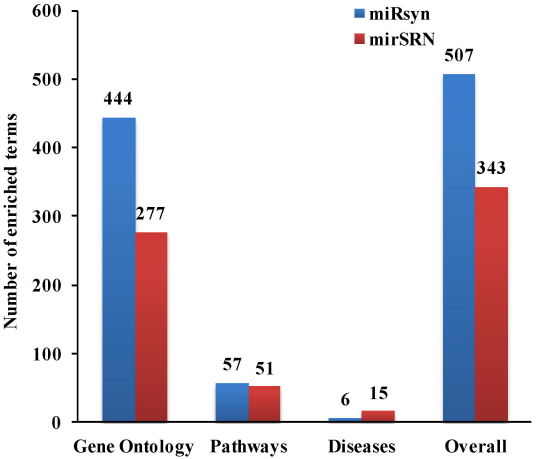


Nodes	Edges	Clustering coefficient	Characteristic path length	$y=bx^{a}$	R^2
78	702	0.528	1.837	$y=4.565x^{-0.299}$	0.192

A



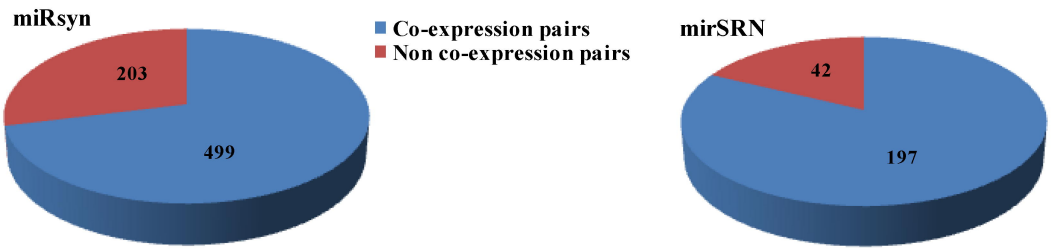
B



C

Method	Percentage of BRCA miRNAs	Percentage of BRCA miRNA pairs	Clustering coefficient	Characteristic path length
miRsyn	50.00%	38.32%	0.528	1.837
mirSRN	51.52%	30.96%	0.282	2.428

D



E

Method	#Score > 0.50	#Score > 0.55	#Score > 0.60	#Score > 0.65	#Score > 0.70
miRsyn	51	28	12	6	2
mirSRN	25	15	6	1	0

Table 1 A portion of enriched or depleted biological processes, pathways and diseases associated with BRCA by using miRNA enrichment analysis.

Category	Subcategory	Enrichment	*p*-value	#miRNAs
Gene Ontology	GO0007050:cell cycle arrest	enriched	3.079E-02	27
	GO0007093:mitotic cell cycle checkpoint	enriched	8.732E-03	10
	GO0051781:positive regulation of cell division	enriched	1.146E-02	16
	GO0002903:negative regulation of b cell apoptotic process	depleted	3.023E-02	3
	GO0042981:regulation of apoptotic process	enriched	2.025E-02	21
	GO0043065:positive regulation of apoptotic process	enriched	7.562E-03	27
	GO0030334:regulation of cell migration	enriched	3.296E-02	14
	GO0010595:positive regulation of endothelial cell migration	enriched	2.851E-02	12
	GO0030335:positive regulation of cell migration	enriched	2.327E-02	20
	GO0045595:regulation of cell differentiation	enriched	3.939E-02	10
	GO0045446:endothelial cell differentiation	depleted	3.330E-04	2
	GO0050678:regulation of epithelial cell proliferation	enriched	3.845E-02	3
	GO0072091:regulation of stem cell proliferation	enriched	3.497E-02	2
	GO0010719:negative regulation of epithelial to mesenchymal transition	depleted	4.996E-02	3
Pathways	hsa04210:Apoptosis	enriched	3.859E-02	22
	P00038:JAK STAT signaling pathway	enriched	4.995E-03	2
	P00056:VEGF signaling pathway	enriched	3.376E-02	15
	WP304:Kit receptor signaling pathway	enriched	7.517E-03	18
Diseases	Breast Neoplasms	enriched	7.184E-03	11