Dissection of human MiRNA regulatory influence to subpathway

Xia Li*, Wei Jiang*, Wei Li*, Baofeng Lian*, Shuyuan Wang, Mingzhi Liao, Xiaowen Chen, Yanqiu Wang, Yingli Lv, Shiyuan Wang and Lei Yang

Submitted: 18th March 2011; Received (in revised form): 14th June 2011

Abstract

The global insight into the relationships between miRNAs and their regulatory influences remains poorly understood. And most of complex diseases may be attributed to certain local areas of pathway (subpathway) instead of the entire pathway. Here, we reviewed the studies on miRNA regulations to pathways and constructed a bipartite miRNAs and subpathways network for systematic analyzing the miRNA regulatory influences to subpathways. We found that a small fraction of miRNAs were global regulators, environmental information processing pathways were preferentially regulated by miRNAs, and miRNAs had synergistic effect on regulating group of subpathways with similar function. Integrating the disease states of miRNAs, we also found that disease miRNAs regulated more subpathways than nondisease miRNAs, and for all miRNAs, the number of regulated subpathways was not in proportion to the number of the related diseases. Therefore, the study not only provided a global view on the relationships among disease, miRNA and subpathway, but also uncovered the function aspects of miRNA regulations and potential pathogenesis of complex diseases. A web server to query, visualize and download for all the data can be freely accessed at http://bioinfo.hrbmu.edu.cn/miR2Subpath.

Keywords: synergistic effect; microRNA; network; subpathway

INTRODUCTION

The mechanism of post-transcriptional regulation of coding genes is rising as one of the new challenges in systems biology. miRNAs are single-stranded RNAs, which regulate gene expression by translation inhibition or degradation of mRNAs in post-transcriptional level [1]. Some investigations have been reported that miRNAs take part in a lot of important biological functions and a broad spectrum of human diseases, such as cell proliferation, differentiation, apoptosis [2–4], immune response [5], tumor development [6], cardiac diseases [7] and so on.

More and more studies have demonstrated that one miRNA can regulate several hundred genes on average [8]. Currently, the functional interpretation
of miRNAs mainly relies on the functions of their target genes. Several studies predicted the functions of miRNAs based on the enrichment analysis of their target genes from a number of functional categories. For example, miRGator [9] and DIANA-mirPath [10] provided the statistically enriched Gene Ontology functions, KEGG/GenMAPP/BioCarta pathways, or diseases from Ingenuity Pathway Analysis [11]. Another comprehensive analysis established a dictionary on miRNAs and their putative target pathways and found that differentially expressed genes in cancer were statistically significant enriched with targets of certain miRNAs [12]. They are effective tools for study on miRNAs and pathways, but they do not pay more attention to local areas of pathway and the properties of miRNA regulations to pathways, especially in human diseases. Recently, we have demonstrated that subpathway (local area of the entire biological pathway)-based analysis may give us much more detailed explanations of type-specific functions for pathology of complex diseases, because the focused genes may not be significantly enriched in the entire pathway but the subpathways [13].

In this study, we constructed the bipartite graph of miRNA–subpathway interactions to explore the rules of miRNA regulatory influence on subpathway. The results indicated that miRNAs have synergistic effect to regulate a group of subpathways with similar function. Through integrating the disease information of miRNAs, the characteristics of disease miRNAs regulation were also uncovered. All the findings can help us to understand the detail mechanisms of miRNAs regulations and identify novel disordered miRNAs or subpathways in human diseases.

**MATERIALS AND METHODS**

**Data Source**

**MiRNA target genes**

We acquired human miRNA target genes from seven miRNA target predicting tools, which were PicTar [14], RNAhybrid [15], DIANA-microT [16], RNA22 [17], miRBase Targets [18], miRanda [19], TargetScan [20]. In order to improve the reliability of the predicted miRNA regulations, we only extracted the regulations that were predicted by at least two tools. In the final, we obtained 776 miRNAs, 15185 miRNA target genes and 289,469 miRNA regulations.

**Disease information of miRNAs**

We downloaded the miR2Disease database (August 2009) [21], which contained the disease-miRNA relationships extracted from literatures. Disease miRNAs are defined by miRNAs themselves deregulation in various human diseases. In total, there are 123 diseases, 414 miRNAs and 2047 miRNA-disease pairs in the whole data file. We classified diseases according to the rules in the online book of Genes and Disease (http://www.ncbi.nlm.nih.gov/books/NBK22183/). In the final, the 123 diseases were grouped into 15 disease categories. The concrete classes were Hematological (Hem), Cancer, Chromosomal (Chr), Ophthamological (Oph), Immunological (Imm), Ear nose and throat (ENT), Female special (FS), Psychiatric (Psy), Skeletal and muscular (SM), Nutritional and metabolic (NM), Respiratory (Res), Dermatological and connective tissue (DCT), Digestive (Dig), Cardiovascular and blood vessel (CBV) and Neurological (Neu).

**Subpathway data**

We used the SubpathwayMiner package of R [13] to find the subpathways in all Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. According to the pathway classification in the KEGG [22], all subpathways were grouped into Environmental Processing (EP), Cellular Processes (CP), Human Diseases (HD) and Metabolism (Met).

**Methods**

**Enrichment analysis**

Enrichment analysis was performed by using cumulative hypergeometric distribution. The formula was as follows:

\[
P = \sum_{j=k}^{m} \frac{(m-j/n-i)(j/i)}{m/n}
\]

On the one hand, the enrichment analysis was used in the identification of subpathways, in which miRNA target genes were significantly enriched. Here, we supposed that the human whole genome had \(n\) genes, and among that \(i\) genes were included in the subpathway. The number of target genes of one miRNA is \(m\), and \(j\) genes out of the \(m\) targets were involved in the subpathway. On the other hand, the enrichment analysis was also performed in the construction of miRNA–miRNA network (MMN) and subpathway–subpathway network (SSN), for each miRNA pair (or each subpathway pair), \(n\) denoted the total number of subpathways.
that were regulated by all miRNAs (or the total number of miRNAs that regulated subpathways), \( m \) represented the number of subpathways that were regulated by one miRNA (or the number of miRNAs that regulated one subpathway), \( i \) denoted the number of subpathways that were regulated by the other miRNA (or the number of miRNAs that regulated the other subpathway), \( j \) represented the number of overlapped subpathways that were regulated by the two miRNAs (or the number of overlapped miRNAs that regulated the two subpathways).

**Randomization tests**
We randomly shuffled the MSN for 1000 times, while keeping the degree of each node unchanged. From these networks, we constructed the randomized MMN and SSN by using the above enrichment analysis.

**Interaction strength**
The interaction strength was a measure to evaluate the intensity of inter-class interaction and intra-class interaction in the MMN and SSN. The intra-class interaction strength was defined as:

\[
IS = N/C_{x_1}^2, \quad \text{where } N \text{ was the observed number of edges in a single miRNA or subpathway category, } x_1 \text{ was the total number of nodes in the category.}
\]

The inter-class interaction strength was computed by \( IS' \) in the two categories. We assumed that \( N \) was the observed number of edges (without overlap) in the two categories, \( x_1 \) and \( x_2 \) were the number of nodes in the two classes, respectively. The \( IS' \) was given formula:

\[
IS' = N/C_{x_1}^1 C_{x_2}^1.
\]

**Cliques and communities search**
The software of Cfinder was used to find cliques and communities in MMN and SSN based on the Clique Percolation Method (CPM) [23]. A clique in the MMN (or SSN) was a complete subgraph of its miRNAs (or subpathways) such that every two miRNAs (or subpathways) in the subgraph were connected by an edge. Each clique must be the biggest fully connected subgraph. A \( k \)-clique community was a union of all \( k \)-cliques (complete subgraph of size \( k \)), and these \( k \)-cliques could be reached from each other through adjacent \( k \)-cliques which shared \( k-1 \) nodes [24].

## RESULTS

**Global properties of miRNA regulation to subpathway**
We constructed miRNA–subpathway network (MSN) based on enrichment analysis by SubpathwayMiner [13]. In the MSN, miRNAs and subpathways were connected if miRNA target genes were significantly enriched in subpathways (\( P < 0.001 \)) (Figure 1A). As a result, we got 3539 significant miRNA–subpathway links between 942 nodes (480 miRNAs and 462 subpathways). The disease information of miRNAs were extracted from miR2Disease database (August 2009) [21]. Of the whole 480 miRNAs, 218 miRNAs had been reported in at least one disease.

First, we paid close attention to the miRNA degree distribution. As shown in Figure 1B and Supplementary Table S1, a small number of miRNAs had high connectivity, which meant that these miRNAs regulated many subpathways. For example, has-miR-16 had the highest degree, which had been reported to be a tumor suppressor [26]. The different expression of has-miR-16 had great influence on cell survival, growth arrest, apoptosis, cell proliferation and invasion [26]. In human leukemia, has-miR-16 could directly or indirectly regulate \( \sim 14\% \) of total genes in human genome [27, 28]. Has-miR-135a had the second highest degree, which had been found to be disordered in many kinds of disease, such as colorectal cancer [29], heart failure [30], prostate cancer [31] and so on.
Therefore, some certain miRNAs acted as global regulators, which could regulate broad biological functions.

Then, we calculated the degree distribution of disease miRNAs and nondisease miRNAs (as shown in Figure 1C). The differences were highly statistically significant by the Wilcoxon rank sum test ($P = 3.332 \times 10^{-12}$). The average degree of disease miRNAs was 10.495, whereas nondisease miRNAs average degree was 4.775. The statistical result indicated that disease miRNAs regulated much more subpathways than nondisease miRNAs.

In addition, we also calculated the degree distribution of the subpathways (Figure 1D). Intuitively, we found that most of the subpathways were associated with a small number of miRNAs; in contrast, only a few of subpathways were related to a mass of miRNAs. Moreover, we evaluated which kind of subpathway was preferentially regulated by miRNAs. We grouped all the subpathways into the categories of Met, EP, CP and HD, according to the rule of pathway classification in KEGG. The average degrees of the four subpathway groups were 1.088, 9.410, 7.110 and 1.780 (Figure 1D), and the degree distinction was significant ($P<0.001$) by one-way ANOVA test. Therefore, the environmental information processing subpathways had the most availability of being regulated by miRNAs, such as the MAPK signal subpathways and the Wnt signaling subpathways. This finding was consistent with the
previous study that miRNA preferred to regulate signal proteins [32]. In another research, signals of toxicity or cellular necrosis were shown to activate miRNAs, and the activated miRNAs could quickly regulate downstream pathways to keep the cell balance in body [33]. This discovery indicated that the rapid and effected regulations of miRNAs may have important function to keep cell steady state in response to different environment stimulus.

Furthermore, the subpathways that were only regulated by disease miRNAs were defined as Dmir-subpathways, which were marked with yellow shade in Figure 1A. Similarly, the subpathways that were only regulated by nondisease miRNAs were defined as Ndmir-subpathways, which were marked with purple shade in the Figure 1A. Next, in order to investigate, if the Dmir-subpathways (or Ndmir-subpathways) are true disease pathways (or normal pathways) defined by KEGG, we downloaded the disease and normal pathways in KEGG as a standard. The detail statistic number of each group is listed in Table 1. We did the Chi-square test and got χ² = 0.1448 (P = 0.704). The result indicated that the Dmir-subpathways, comparing with the Ndmir-subpathways, did not have any hobbies to be actual disease pathways or normal pathways. There might be two causes. First, the category of human disease pathway in KEGG only contained multi-factorial diseases and infectious diseases, such as cancers, immune system diseases, neurodegenerative diseases, cardiovascular diseases and metabolic diseases and others. For single-gene diseases, the perturbed pathway maps were not defined as disease pathway in KEGG, but only mapped the causative genes to normal pathway. Second, with the development of research on diseases and pathways, there would be much more disease pathways added into KEGG.

As described above, disease miRNAs regulated much more subpathways than nondisease miRNAs. For further analyzing the characteristics of miRNAs regulations to subpathways in human diseases, we only extracted disease miRNAs and their regulated subpathways. There were 218 unique disease miRNAs, 379 subpathways and 2288 significant miRNA–subpathway associations. So we calculated the number of subpathways that regulated by disease miRNAs in 15 disease categories separately. As shown in Figure 1E, miRNAs related to respiratory diseases regulated much more subpathways, which was consistent with the previous study. Respiratory disease is caused by the cooperation of environmental, genetic and epigenetic components. If the infections of respiratory disease happened, systemic inflammation would be switched on, causing some miRNAs differently expressed and many inflammation related pathways disordered [34].

Synergistic effect of miRNA regulation to subpathway

From the MSN, two biologically relevant networks projections, MMN and SSN, were constructed. In the MMN, two miRNAs were connected if they significantly co-regulated common subpathways, in which both two miRNAs target genes were significantly enriched. In the SSN, the subpathways were connected if they were significantly co-regulated by common miRNAs. They provided the complementary in miRNA regulatory influences to subpathways. It was the first time to construct miRNA–miRNA and subpathway–subpathway network based on miRNA regulations. These two kinds of networks were mapped and colored according to the communities, which were found by Cfinder [23], in Figures 2A and 3A, respectively.

Next, we randomly permuted the MSN 1000 times and constructed random MMN and random SSN. The sizes of both random networks were significantly smaller than that of the actual networks (as shown in Figure 2B and Supplementary Figure S1; Figure 3B and Supplementary Figure S2, respectively). Comparing with the total 2025 edges and 304 miRNAs in the MMN, the average number of edges was 213.177, and the average number of nodes was 178.780 in the random MMNs. The differences were significant (P < 0.001). In the SSN, there were total 2000 edges and 305 subpathways. The average number of edges was 331.136, and the average

<table>
<thead>
<tr>
<th>KEGG</th>
<th>MSN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Disease subpathway</td>
</tr>
<tr>
<td>Dmir-subpathway</td>
<td>21</td>
</tr>
<tr>
<td>Ndmir-subpathway</td>
<td>24</td>
</tr>
<tr>
<td>Total</td>
<td>45</td>
</tr>
</tbody>
</table>

Table 1: The number of disease and normal subpathways according to the status of their related miRNAs and the classifications of the KEGG.
number of nodes was 203.016 in random SSNs. The differences were both significant ($P < 0.001$).

Owning to the minor functions of miRNAs, the synergistic functions of miRNAs came into the attention of researchers. We demonstrated the miRNA synergisms in the previous study based on Gene Ontology and Protein–protein interaction network [35]. Moreover, the combination of several miRNAs may work together to affect multiple target genes in the same or different biological pathways [36]. In the MMN and SSN, the most important findings were the miRNA cliques, subpathway cliques and their interactions. All miRNAs in one clique were fully connected with each other. According to the approach of constructing MMN, any two connected miRNAs regulated common subpathways significantly.

For example, a miRNA clique 164 is shown in Figure 2C. All of the 10 miRNAs were all cancer related and mainly from two miRNA families (let-7 miRNA family and miR-29 family). All these miRNAs in this clique co-regulated three subpathways of ECM receptor interaction pathways. The miRNA clique 338. Nine of the ten miRNAs in this clique are disordered in cancer, and all these miRNAs co-regulate four subpathways of long-term potentiation pathway.

Figure 2: The MMN and its properties. (A) The MMN. In the MMN, a link is placed between miRNAs if the two miRNAs significantly co-regulate subpathways ($P < 0.001$). All the communities are shown by relatively isolated structures, and all miRNAs that are not involved in any community, are arranged in row at the bottom of the figure. The color meanings are shown in the legend. (B) The distribution of miRNA-miRNA interactions in the random MMNs. The red arrow represents the number of edges in the MMN. (C) The miRNA clique 164. All miRNAs in this clique are disordered in cancer and co-regulate three subpathways of ECM receptor interaction pathways. (D) The miRNA clique 338. Nine of the ten miRNAs in this clique are disordered in cancer, and all these miRNAs co-regulate four subpathways of long-term potentiation pathway.
miR2Disease, except miR-196a and miR-98. Owning to the related function in one clique, we considered that the two miRNAs may be related to lung cancer, which was supported by a recent study that miR-98 overexpressed in small cell lung cancer to negatively regulate FUS1 tumor suppressor function [38].

Another miRNA clique is shown in Figure 2D, which co-regulated four long-term potentiation (LTP) subpathways. Pervious studies demonstrated that LTP pathway could affect synaptic transmission and neuronal cell death in the hippocampus and cerebral cortex. This process would lead to loss of memory function and dysfunction of global cognition [39]. All miRNAs in this clique were neurologic disease related except miR-140-5p. Thus, we predicted that miR-140-5p was potentially related to neurologic disease, which was supported by recent study that miR-140-5p was downregulated in Alzheimer’s disease [40, 41]. All miRNA cliques were listed in Supplementary Table S2.

Furthermore, we identified subpathway cliques in the SSN. In Figure 3C, this subpathway clique was co-regulated by has-miR-185 and has-miR-453. All subpathways were fully connected and belonged to the same pathway (regulation of action cytoskeleton), which took part in many cell functions, such as cell motion, cell division, cell adhesion and cell phagocytosis. Another example was shown in Figure 3D, in which has-miR-148b and has-miR-135a were disordered in cancer, and all subpathways were associated with the human disease category in...
KEGG except the insulin signaling pathway. Based on the hypothesis that one clique may participate in similar function, we investigated the biological significance of the relationship between insulin signaling subpathways and cancer. Through literature review [42], we found that insulin receptor can pass through the IGF receptor channels, such as AKT and mTOR, triggering mitogenic and anti-apoptotic role in promoting tumorigenesis. Insulin signal plays an important role in the mediation both in Akt (P13K-Akt) pathway and in the P13K-Akt signal transduction pathway. The cascade reaction of Ras-Raf-ERK-MAPK is a considerable part, inducing tumor cell proliferation. While the P13K-Akt signal transduction pathway is related with the independent fixed growth of cell proliferation, cell cycle phase conversion from Gl to S phase and inhibition of apoptosis. All subpathway cliques were listed in Supplementary Table S3.

We further analyzed the topological properties of the MMN and SSN. The average betweenness and degree of disease miRNAs were much higher, and the average shortest path distance was much shorter than those of nondisease miRNAs (Table 2), which indicated that disease miRNAs tend to be important within the wider context of the entire network and have synergy and quick communication. These results were consistent with our previous study, and miRNAs in the same disease were much more closer [35]. The average betweenness and degree of human disease subpathways were the highest, and the average shortest path distance of metabolism subpathways were the highest (Table 3). Then, we calculated the intra- and inter-classes interaction strength in the MMN and SSN separately. We grouped all the disease miRNAs into 15 categories (refer to ‘Materials and Methods’ section) and subpathways into four classes (according to KEGG). As a result, in both the two kinds of network, the average interaction strength of intra-class was significantly larger than that of inter-classes ($P < 0.001$; Wilcoxon rank sum test). The difference indicated that miRNAs related to the same disease tend to regulate common subpathways, and the subpathways with similar function tend to be regulated by common miRNAs.

### DISCUSSION

MiRNAs are important post-transcriptional regulators of gene expression and participate in various biological processes, such as proliferation, differentiation, development and cell death. MiRNAs also have the power to regulate diverse function and pathways simultaneously because each of them can target a large amount of genes. However, miRNA regulation is far from full understanding especially in human diseases. In order to detect the detail relationship between miRNAs and subpathways in human diseases, we only extracted disease miRNAs and their regulated subpathways from the MSN. First, we calculated the number of target genes of disease miRNAs and nondisease miRNAs. The average

| Table 2: The topological properties of disease and non-disease miRNAs in MMN |
|---------------------------------|-------------------------------|-----------------------------|
| **Topological properties**      | **Group**                     | **P-value**                 |
|                                 | **Mean of total miRNAs**      | **Mean of disease miRNAs**  | **Mean of non-disease miRNAs** |
| Betweenness                     | 636.309                       | 784.301                     | 467474                        | 6.660E-05 |
| Degree                          | 13.322                        | 15.630                      | 10.690                        | 1.880E-06 |
| Shortest path distance          | 3.313                         | 3.045                       | 3.478                         | 1.090E-09 |

| Table 3: The topological properties of the subpathway classes in the SSN |
|---------------------------------|-------------------------------|-----------------------------|
| **Topological properties**      | **Group**                     | **P-value**                 |
|                                 | **Mean of total subpathways** | **Mean of CP class**        | **Mean of EP class**          | **Mean of HD class**          | **Mean of Met class**          |
| Betweenness                     | 439.810                       | 393.208                     | 524.318                       | 564.118                       | 57736                           | 1.332E-02 |
| Shortest path distance          | 2.764                         | 2.947                       | 3.053                         | 2.849                         | 1.162                           | 3.67E-21 |
number of disease miRNAs target genes was 684.812, whereas that of nondisease miRNAs target genes was 327.748. The differences were highly statistical significance by the Wilcoxon rank sum test ($P = 2.643 \times 10^{-28}$). As a result, the target genes of disease miRNAs were much more than that of nondisease miRNAs. Moreover, the relationship was explored between the number of miRNAs targets and the number of subpathways, which regulated by miRNA (Figure 4A). The positive correlation was found between them by Pearson’s correlation ($r = 0.558$, $P = 4.382 \times 10^{-40}$). This result indicated that the more targets of one miRNA, the more subpathways it regulated. Because one miRNA may not be dysfunction in only one disease, we further investigated whether the number of regulated subpathways was increased with the number of related diseases or disease categories for each miRNA. As a result, we did not obtain any linear correlation in both cases (Figure 4B and C). For a deeper analysis, we chose some disease categories, which contained more than five diseases to find whether this relationship existed in each disease category. We got nothing tendency either (Figure 4D). For example, miR-199a was related with 17 diseases, but it only regulated one subpathway; miR-155 was found in 32 diseases, unlike its related diseases, miR-155 only took part in 4 subpathways in total. On the contrary, miR-135a was disordered only in the melanoma, although it participated in 66 subpathways; miR-503 was related with 2 kinds of diseases (prostate cancer and retinoblastoma), but 50 subpathways. In the final analysis, all the results showed that for each miRNA the number of regulated subpathways is not in proportion to the number of the related diseases.

In addition, our previous study demonstrated that subpathway-based approach was more precise and flexible in annotation and identification of pathways.

Figure 4: Quantitative relationships among miRNAs, miRNA targets and subpathways. (A) The relationship between the number of miRNA’s targets and the number of subpathway, which regulated by miRNA. Each node represents one miRNA, and the positive correlation is shown between miRNAs’ targets and their regulated subpathways. (B) The relationship between the number of regulated subpathways and the number of related diseases. (C) The relationship between the number of regulated subpathways and the number of related disease categories. (D) The relationship between the number of regulated subpathways and the number of related diseases in Cancer category and SM category, which involve more than five diseases.
Thus, this study explored the rules of miRNA regulatory influences to subpathways by analyzing the topology of the miRNA–subpathway network. We found that a small fraction of miRNAs were global regulators for broad subpathways. The environmental information processing pathways are preferentially regulated by miRNAs because of the rapid and effected miRNA regulations in response to different environment stimulus. Integrating the disease states of miRNAs, we also found that disease miRNAs regulated more subpathways than nondisease miRNAs and the number of regulated subpathways was not in proportion to the number of related diseases for each miRNA. Besides, the results indicated that some miRNAs had synergistic effects on a group of subpathways. According to the principle of this finding, novel disease miRNAs or subpathways could be discovered. Currently, experiment and computational analysis provide the evidences for coordinate miRNA regulations [43–45]. For example, multiple miRNAs that target on a common gene could act together to enhance mRNA decay rate [43]. Four miRNAs cooperative effect on (mir-155, mir-222, mir-424 and mir-503) their target genes repression was found in monocyte differentiation. Their combination could induce additional changes not seen by any individual miRNA [44]. An experimental study on the common targets of miR-143 and miR-145, which suggested the common targets of the two cooperative miRNAs are involved in cytoskeletal-related pathways [45]. In addition, some miRNA synergisms were verified in our previous study that constructed functional synergistic network by significant negative regulation to Gene Ontology terms [35]. In the previous study, miRNA synergism was investigated based on Gene Ontology and Protein–Protein interaction network. In this study, biological pathways were employed to extract subpathway and then to dissect synergistic effect of miRNA regulation. The KEGG pathway is a collection of manually drawn pathway maps representing knowledge on the molecular interaction and reaction networks of metabolism, environmental information processing, cell processes and human diseases. These studies focused on different function levels. In the future, we will devote to deeply dissect the miRNA synergisms from multiple levels, such as regulatory motif and miRNA expression. The subpathway–based miRNAs synergisms could help us to reveal the detail underlying biological mechanisms. Finally, this study provided deep insights into the function implementation of miRNA regulations and may present a global perspective on complex disease research for biomedical scientists, clinicians, geneticists and some other researchers. A user-friendly web server, called miR2Subpath, to query, visualize and download for all the data in our research can be freely accessed at http://bioinfo.hrbmu.edu.cn/miR2Subpath.

This study constructed functional spectrum of miRNAs in genome-wide and dissected the characteristics of miRNA regulations especially in human diseases. However, all these data in our study are far from complete, such as the lack of disease related miRNAs, the false positive targets of miRNAs, etc. In this study, we only extracted literature-cited disease miRNAs in the miR2Disease database. Meanwhile, we integrated seven widely used prediction tools of miRNA targets to alleviate false positives of miRNA targets. We believe that the miRNA–subpathway network will be more comprehensive with the information about disease miRNAs, miRNA targets and subpathway increasing. In conclusion, the miRNA and subpathway study provides not only a global and powerful way to investigate the regulatory functions of miRNAs, but also the possibilities of discovering novel disease-related miRNAs, disease-related subpathways, the co-regulating miRNA groups or some function-related subpathways in the future disease studies.

SUPPLEMENTARY DATA
Supplementary data are available online at http://bib.oxfordjournals.org/.

Key Points
- Many researches have focused on the function of miRNA or subpathway separately. In this study, we construct human miRNA–subpathway network to investigate properties of human miRNA regulatory influence to subpathway.
- We find that miRNAs can synergistically regulate group of subpathways, and describe some of them in detail.
- We find that a few of miRNAs have global regulatory influence, and disease miRNAs regulate much more subpathways than nondisease miRNAs.
- Rigorous numerical analyses show that, for all miRNAs, the number of regulated subpathways is not proportional to the number of related diseases.

FUNDING
This work was supported by the National Natural Science Foundation of China (30871394,
References


43. Hu Z. Insight into microRNA regulation by analyzing the characteristics of their targets in humans. BMC Genomics 2009;10:594.
