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Mirin: identifying microRNA regulatory modules in protein–protein interaction networks

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ABSTRACT

Summary: Exploring microRNA (miRNA) regulations and protein–protein interactions could reveal the molecular mechanisms responsible for complex biological processes. Mirin is a web-based application suitable for identifying functional modules from protein–protein interaction networks regulated by aberrant miRNAs under user-defined biological conditions such as cancers. The analysis involves combining miRNA regulations, protein–protein interactions between target genes, as well as mRNA and miRNA expression profiles provided by users. Mirin has successfully uncovered oncomirs and their regulatory networks in various cancers, such as gastric and breast cancer.

Availability and implementation: Mirin is freely available at http://mirin.ym.edu.tw/.

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Supplementary information: Supplementary data are available at *Bioinformatics* online.

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1 INTRODUCTION

MicroRNAs (miRNA) are short non-coding RNA molecules that repress target gene expression at the post-transcriptional level. miRNAs regulate critical biological processes such as cell growth, tissue differentiation and embryonic development. Therefore, corrupted miRNA and dysfunctional miRNA biogenesis may lead to various disorders, such as cancer. Most miRNAs regulate a large number of genes, and so it is difficult to determine the primary function of a given miRNA. Previous studies report that complexity of miRNA regulation and topological characteristics of protein–protein interaction networks (PINs) are correlated (Hsu *et al.*, 2008; Liang and Li, 2007; Lin *et al.*, 2012). Therefore, the interacting proteins targeted by a given miRNA can reveal its function. Additionally, because PINs are dynamic in cellular systems, they have frequently been integrated with mRNA expression profiles to expose conditional network modules under a biological state (Chen *et al.*, 2014). Consequently, integrating miRNA regulations with PINs and expression profiles of miRNAs and mRNAs could provide opportunities to identify miRNA-regulated PINs and their function under specific biological conditions, such as cancer versus normal samples (Lee *et al.*, 2010; Lin *et al.*, 2012; Tseng *et al.*, 2011).

Here, we designed a web application, Mirin, to identify disturbed miRNA regulatory subnetworks and their functions under user-specified biological conditions. Mirin takes advantage of miRNA targets and protein–protein interactions (PPIs), as well as incorporating gene expression data, to create condition-specific miRNA-mediated PINs. Although there already exists a similar tool, mirConnX (Huang *et al.*, 2011), it does not incorporate PPIs. The core analytic procedure of Mirin has been used to successfully identify cancer-associated miRNA-regulated PIN modules in gastric (Tseng *et al.*, 2011) and breast cancer (Lee *et al.*, 2013). Besides exploring individual miRNA-regulated subnetworks, Mirin also takes the co-regulations of multiple miRNAs into consideration to conduct a more comprehensive analysis of miRNA regulatory networks.

2 OVERVIEW OF MIRIN

2.1 Implementation

Mirin offers user-friendly interfaces for researchers to construct and explore miRNA regulatory networks. The graphic interface was built in PHP program with JavaScript to enhance user experience, and the analysis pipeline was implemented in back-end Perl and R scripts. The network modules were visualized by Cytoscape web (Lopes *et al.*, 2010).

2.2 Input data

To construct condition-specific miRNA regulatory networks, Mirin requires miRNA and mRNA expression profiles obtained in terms of expression intensity by microarray or read count by next-generation sequencing techniques. After receiving expression data, Mirin offers several normalization methods (Supplementary Table S1), if necessary, and executes statistical tests to identify differentially expressed (DE) miRNAs and mRNAs between two user-defined conditions. Alternatively, users can upload DE miRNA and mRNA lists.

Mirin collects various predicted miRNA target (Supplementary Table S2) and protein–protein interaction databases (Supplementary Table S3); users can then choose their preferred ones. Additionally, users can choose multiple miRNA target databases and use a criterion to filter out low-confidence targets.

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†The authors wish it to be known that, in their opinion, the first three authors should be regarded as Joint First Authors.

2.3 Construction of miRNA-regulated modules

We described the core analysis pipeline in a previous study (Tseng *et al.*, 2011). For each DE miRNA, Mirin extracts coherent DE mRNA targeted by the given miRNA, i.e. expression of the miRNA and a target is negatively correlated, and together with the PPIs among these DE targets. Mirin calls miRNA-target genes 'L0' genes. To better reveal the regulatory functions of the miRNA, Mirin expands the network to include interacting partners (called 'L1') of L0 genes. Moreover, users can set several criteria to construct more reliable and biologically meaningful networks by filtering out the L1 genes with fewer L0 partners and/or including non-DE L1 genes.

2.4 Investigation of miRNA-regulated modules

Mirin offers several ways to investigate the miRNA-mediated network modules. Firstly, to determine if a module is active under a specific condition, Mirin assesses the significance of the proportion of coexpressed PPIs in the given module based on the expression profiles uploaded by users (Supplementary Methods). Secondly, to reveal the relevant biological processes, Mirin performs enrichment analysis to identify the enriched Gene Ontology (GO) terms for each module. Enrichment analysis in Mirin provides node-based (i.e. conventional gene-set analysis) and edge-based (i.e. extending GO annotation to network edges, proposed by Lin *et al.*, 2010) methods. From the ranked list of GO terms significantly overrepresented for each module, users can select terms to view function-specific modules. These could assist users to infer plausible molecular mechanisms. Finally, users can investigate the co-regulation or cross talk among miRNAs by incorporating more modules into the network visualization. The construction of co-regulation module is based on the common components of network modules of the miRNAs selected by users. The major functions of Mirin are summarized in Figure 1.

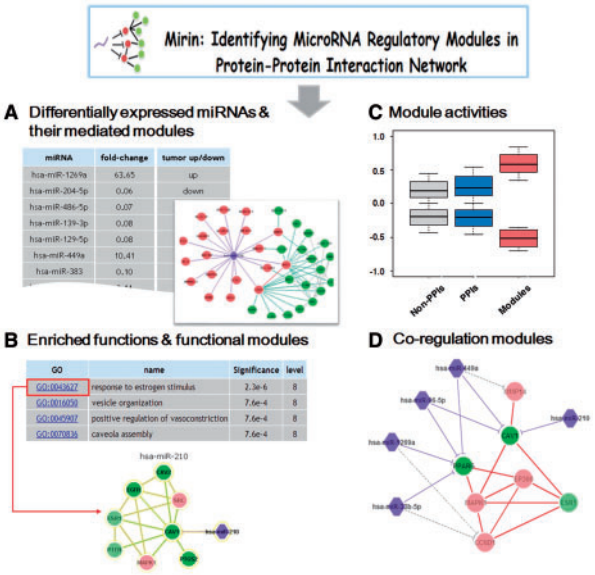

Fig. 1. Overview of Mirin functions. Through integrating user-uploaded expression profiles with built-in miRNA regulation and PIN information, Mirin can identify miRNA regulatory networks and provide (**A**) summary tables describing statistical information about DE miRNAs and their regulatory networks, (**B**) results from functional enrichment analysis that visualize functional modules and summarize enriched functions, (**C**) the activities of miRNA regulatory modules under specific biological conditions and (**D**) co-regulations between DE miRNAs and their co-regulatory networks/modules

3 DISCUSSION

To demonstrate the capabilities of Mirin, we applied it to invasive carcinoma of the breast. Expression profiles of miRNAs and mRNAs were obtained from The Cancer Genome Atlas (Supplementary Tables S4 and S5). After executing the analysis pipeline of Mirin, we identified the top 20 DE miRNAs (9 downregulated and 11 upregulated) in breast cancer tissue (Supplementary Table S6). According to miR2Disease (Jiang *et al.*, 2009), 6 and 10 of the 20 miRNAs are associated with breast and other cancers, respectively. We focused on miR-210 regulatory modules and found some interesting GO terms, such as 'response to estrogen stimulus' and 'positive regulation of canonical wingless receptor signaling pathway', which are associated with the carcinogenesis (Supplementary Table S7). From a network view, we can see that miR-210 might inhibit caveolin 1 and modulate estrogen receptor 1, which are estrogen-responsive genes described as tumor suppressors in breast cancer (Fenne *et al.*, 2013) (Supplementary Fig. S1). Furthermore, by using Gene expression-based Outcome for Breast cancer Online (Ringner *et al.*, 2011), we found that the expression levels of genes involved in the miRNA regulatory networks identified by Mirin can significantly affect breast cancer patient survival rates (Supplementary Fig. S2). This case study exhibits how Mirin could be used to help identify miRNA regulatory networks associated with user-specified conditions.

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Conflict of Interest: none declared.

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