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ProteoMirExpress: inferring microRNA-centered regulatory networks from high-throughput proteomic and mRNA expression data

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Running title: inferring miRNA-centered regulatory networks from proteomics data

Abbreviations

mRNA: messenger ribonucleic acid

MicroRNA/miRNA: micro ribonucleic acid

CLIP-Seq: cross linking and immunoprecipitation sequencing

Degradome-Seq: degradome sequencing

UTR: untranslated region

miRISCs: miRNA-induced silencing complexes

TF: transcription factor

SILAC: stable isotope labeling by amino acids in cell culture

PRISM: proteomic investigation strategy for mammals

IS: interaction score

Summary

MicroRNAs (miRNAs) regulate gene expression through translational repression and RNA degradation. Recently developed high-throughput proteomic methods measure gene expression changes at protein levels, and therefore can reveal the direct effects of miRNAs' translational repression. Here, we present a web server, ProteoMirExpress that integrates proteomic and mRNA expression data together to infer miRNA-centered regulatory networks. With both high throughput data from the users, ProteoMirExpress is able to discover not only miRNA targets that have mRNA decreased, but also subgroups of targets whose proteins are suppressed but mRNAs are not significantly changed or whose mRNAs are decreased but proteins are not significantly changed, which were usually ignored by most current methods. Furthermore, both direct and indirect targets of miRNAs can be detected. Therefore ProteoMirExpress provides more comprehensive miRNA-centered regulatory networks. We use several published data to assess the quality of our inferred networks and prove the value of our server. ProteoMirExpress is available at http://jjwanglab.org/ProteoMirExpress, with free access to academic users.

Introduction

MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression by either causing translation inhibition or mRNA decay (1). Posttranscriptional regulation by miRNA is an important level of the complex gene regulatory network and it controls a wide range of biological processes. Deregulation of miRNAs expression can lead to various diseases, including many human cancers (2). Therefore, understanding the regulatory networks of miRNAs in different biological processes is crucial to unraveling their functional importance and to providing a pool of targets for medical therapies.

Several approaches have been proposed to predict miRNA's targets and to construct miRNA-centered regulatory networks. Computational approaches include miRNA target prediction based on binding energy of miRNA-mRNA interactions (3, 4) and on the evolutionary conservation of the seed regions (5-7). Experimental approaches include identification of destabilized mRNAs in the presence of a miRNA, or high-throughput methods to detect mRNAs bound by argonaute proteins and miRNA cleavage sites (8). Databases, such as MiRecords (9) and TarBase (10), collect experimentally validated miRNA targets and starBase (8) collects miRNA-mRNA interaction maps from argonaute CLIP-Seq and Degradome-Seq data.

The anti-correlation between the miRNA and their targets has been widely used to infer miRNA-target relationship. Several web servers have been developed to infer miRNA targets based on the expression profiles of miRNAs and mRNAs from the same set of biological samples (11-16). For example, Generative model for miRNA regulation (GenMir++) uses a Bayesian model to predict miRNA targets based on both target genes' 3' UTR region sequence features

and the correlation between expressions of miRNA and its targets (15). When miRNA expression data is not available, active miRNA and its targets can be inferred by the enrichment of its recognized motifs in the 3' UTRs of suppressed genes in a biological state or process (17-20). Moreover, The condition-specific mRNA-miRNA network integrator (mirConnX) uses transcription factor (TF) binding in the promoter region of miRNAs, as well as mRNA, to construct transcriptional-posttranscriptional regulatory network (21). Besides, miRNA function can also be annotated by its target genes' enrichments in biological pathways, gene ontology (GO) or diseases (11, 22-24).

Despite the great success of these methods, none of them consider the effects of a miRNA on target gene's output at both mRNA and protein levels. In addition to destabilization of mRNA product, translational repression has been proposed to be another major mechanism of miRNA regulation. Many examples have shown that miRNA is able to decrease protein level without changing mRNA abundance (25-27). It is considered that when the mature miRNA pairs with its target perfectly, argonaute protein's endonucleolytic active site will cleave the target mRNA's nucleotides that pair with bases 10 and 11 of the miRNA guide strand (1). In the cases of non-perfect pairing, or argonaute protein lacking of endonucleolytic activity, miRNAs regulate genes through translation repression (1, 28). However, recent study by Brodersen et al. found that translational repression happened irrespective of the degree of complementarity or location of target sites within mRNAs (29). Complete complementary paring miRNA may engage in both mRNA cleavage and translational repression, and consequentially leads to protein product decrease. Several models have been suggested to describe the translation repression mechanism, including competition between miRNA-induced silencing complexes (miRISCs) and

elF4E gene for binding to the mRNA 5' cap structure, deadenylation of the mRNA tail, ribosome drop-off and reduced translation elongation (1). Two recent papers reported that miRNA first inhibited translation initiation and then induced mRNA deadenylation and decay (30, 31). Even though most of the miRNA targets undergo translational repression followed by decay, there is a subgroup of targets primarily regulated by translation repression without significant mRNA decay (30). It is still unclear why some target mRNAs are degraded while others are not.

Due to different mechanisms of miRNA regulation, its targets can either be translationally repressed without significant decrease in mRNA abundance, or translationally repressed with concordant decrease in mRNA abundance, or significantly decreased in mRNA abundance with little protein changes at a certain time point (28, 32, 33). Current methods that use only mRNA abundance to study miRNA regulatory effect may miss many targets that are suppressed at protein level but without detectable mRNA changes, or overestimate miRNA's effect on targets that do not have detectable changes at protein level but with decreases in mRNA. These kinds of false negative and/or false positive links between a miRNA and its targets may lead to misunderstanding of a miRNA's regulatory network, for example, when the miRNA's target is a TF.

Recently, the development of high-throughput quantitative proteomic methods provides us the opportunity to study the effect of miRNA on target's protein outputs (32, 34, 35). However, most of these studies restrict only on one or a few miRNAs or proteins. Furthermore, there is no published tool for inferring miRNA-centered regulatory network from high-throughput proteomic data. To fill the gap, we present here a new web server, ProteoMirExpress, which

integrates mRNA and protein expression data to infer miRNA activities on their direct and indirect targets in the absence or presence of miRNA expression data and construct the regulatory networks controlled by miRNAs. We further use several published data to assess the quality of our inferred networks. The assessment shows that ProteoMirExpressis is able to effectively infer a miRNA-centered regulatory network and identify subgroups of miRNA targets, which are usually ignored by current available tools.

Experimental Procedures

Web Server Integration and Implementation

ProteoMirExpress integrates mRNA and proteomic expression data together to infer miRNA-centered regulatory networks for a specific biological stage or process. The workflow of ProteoMirExpress is briefly described in **Figure 1**. It accepts both high throughput mRNA and proteomic profiling data, and optionally the expression of miRNAs, preferably generated under the same experiment condition (**Figure 1A**). The server has the following functions: 1) In the absence of miRNA expression information, ProteoMirExpress will classify the input genes into different groups according to their mRNA and protein levels, and infer miRNA networks by calculating the overlaps between the potentially suppressed gene sets and the miRNA's target genes (**Figure 1B**). 2) In the presence of miRNA expression information, the targets of active miRNA will be inferred by the anti-correlation between miRNAs and their potential targets in either mRNA or protein level, or both (**Figure 1B**). The miRNA target genes are collected from

multiple sources, including computational predictions, CLIP/Degradome-seq and experimental verifications (**Figure 1C**). The inferred active miRNAs and their targets will be ranked according to their *p*-values (**Figure 1D**). 3) Indirect targets of miRNAs are also predicted by scanning suppressed mRNAs' promoters with the binding site information of miRNA-targeted TFs. 4) The miRNA-centered regulatory network will be visualized on the web page (**Figure 1E**). Users can click on each miRNA to inspect all of its targets, as well as miRNA-target interactions.

Inputs

ProteoMirExpress takes inputs in tab-delimited format containing high-throughput protein and mRNA expressions. With data obtained from the same biological condition, ProteoMirExpress accepts mRNA and protein expression levels from one biological stage, or expression changes from two biological stages. For data from one biological stage in one file, the input file should contain three columns: the identifier of the genes, the corresponding expression values of mRNA and the expression values of the proteins. Protein and mRNA expression data can also be inputted in two files, one for protein and the other for mRNA. Each file should contain two columns: the first one is the identifier of protein or mRNA and the second is the expression values. ProteoMirExpress will match the protein and mRNA from the same gene for the users if the input identifies are in different types. ProteoMirExpress can recognize protein and mRNA identifiers from various databases, such as RefSeq, Ensemble, UCSC, Uniprot, PDB, etc. With samples achieved from two biological stages, like before or after a certain biological treatment, ProteoMirExpress accepts the expression fold change (or $\log_2$(fold change)) of mRNA and

protein in one file or two files with the same format described above. ProteoMirExpress also considers expression change *p*-value from two stage data if the user input the *p*-value in the column next to expression change. Then the input files mentioned above should contain five columns and three columns respectively. The expression data of miRNAs can also be optionally inputted with the same format as mRNA and protein. Furthermore, to serve more users, ProteoMirExpress also accept data from only mRNA or protein for the analysis.

Data analysis procedure

The input genes or miRNAs will be classified into different classes according to their expression levels (or changes), as well as *p*-values if applicable. Users can input a customized expression level (or change) and a *p*-value cutoff for gene classification. For example, if the expression change cutoff is 1 (using Log_2, 1 means the fold change cutoff is 2 folds), then genes whose expression decreases to less than 0.5 fold (Log_2(expression change) < -1) will be classified as "Decreased" (D), while genes whose expression change is more than 2 folds (Log_2(expression change) > 1) will be "Increased" (I) and the rest will be "Unchanged" (U). Genes with low expression or expression suppression in either protein or mRNA level will be regarded as potential targets of miRNA for further analysis. If the *p*-value cutoff is set to be 0.05, for example, only genes whose *p*-value lower than 0.05 will be classified as significant increased or decreased genes.

In the absence of miRNA expression information, ProteoMirExpress will calculate the

significance of overlaps between the potential suppressed gene sets and the predicted target genes of each miRNAs with hypergeometric test or permutation test. With *N* genes in the whole genome, *m* genes in the potential suppressed gene set (set *A*), *n* genes that are the predicted target genes of a miRNA (set *B*), and *k* genes in the overlap of gene set *A* and *B*, hypergeometric *p*-value is calculated by Equation 1.

$$p = \frac{\binom{m}{k}\binom{N-m}{n-k}}{\binom{N}{n}} \qquad \text{(Equation 1)}$$

Permutation test is performed by randomly selecting *m* genes from the genome 1000 times. If the selected genes in *q* of the 1000 times have more than *k* genes that are the predicted targets of a miRNA, the permutation *p*-value is calculated by $p = q/1000$ (36). The cutoff of hypergeometric and permutation *p*-value is set as 0.05 by default, but can be adjusted by the user.

In the presence of miRNA expression information, the targets of active miRNA will be inferred by the anti-correlation between the miRNA expression levels (or changes) and the mRNA or protein levels (or changes) of their potential targets. The information of miRNAs is collected from miRBase (37), and information of their target genes is collected from multiple databases. Computational prediction databases include TargetScan (6), miRanda (5), PicTar (7), PITA (4) and experimental databases include starBase (8), miRecords (9) and TarBase (10). starBase contains miRNA targets identified by high throughput method CLIP/Degradome-seq, while miRecords and TarBase contain experimental verified targets. To integrate multiple databases from heterogenous sources, we use an Interaction Score (IS) to represent the confidence of the

link between a miRNA and its target. IS is calculated as the sum of weighted proportion of the target databases containing the gene with at least one miRNA target site in the three groups of databases (21). We assign different weights for the three different groups of databases according to reliability, with experimental validated targets a weight of 4, high throughput methods a weight of 2, and computational prediction a weight of 1 (Equation 2).

$$IS = \frac{\sum_{i \in I} S_i}{4} + 2 \times S_{starBase} + 4 \times \frac{\sum_{i \in J} S_i}{2}, \quad \text{(Equation 2)}$$

where *I* = (TargetScan, miRanda, PicTar, PITA), *J* = (miRecords, TarBase), and S_i=1 if the target is present in databases *I* or *J* and S_i=0 otherwise. The users can input the cutoff to filter off targets with low score. For example, if cutoff is 0.5, then only targets reported in at least two of four computational predictions, or any one of CLIP/Degradome-seq database and experiment collection databases, will be selected for the analysis.

Besides of direct targets, indirect targets of each miRNA are further predicted. When miRNAs suppress the protein abundance of a TF, targets of the TF may also be suppressed indirectly. Thus, after miRNA-targeted TFs are identified, the promoters of genes with suppressed mRNA levels are scanned for putative binding sites of the TFs with the method described by Qin et al. (38). Indirect targets of the miRNAs are defined as genes with decreased mRNA abundance and putative binding sites of miRNA-targeted TFs, whose protein abundance is decreased. Users can adjust several parameters for the binding site scanning, including the size of promoter region, the statistical *p*-value cutoff of binding site significance, conservation cutoff and the TF information sources.

Output

The inferred miRNA-centered regulatory network will be visualized on a Cytoscape page (39). The network contains regulatory relationships between miRNAs and their targets. Each node is a miRNA or a target gene, and each edge is an arrow pointing out from a miRNA or a TF to its target, which indicates the miRNA's directly or indirectly suppressing regulatory function. The weights of the lines are proportional to the IS scores of the miRNA-target pairs. Targets from different expression gene classes are labeled in different colors (**Figure 2**). For instance, the class "UD", which is colored as blue notes, represents genes that have mRNA levels unchanged (U) but protein levels decreased (D); while class "ML", which is colored as light purple notes, represents genes with medium expression level in mRNA (M) but low expression level in protein (L).

On the second tap, the inferred active miRNAs are ranked according to their *p*-values in potential target gene set. When the users input miRNA expression data, the server will report two lists of significant miRNAs, one is listed in the "Inputted miRNA" tap which contains the miRNAs that have high expression or significant expression changes according to the inputted data, the other is in the "All enriched miRNA" tap which also includes other miRNAs that are not inputted miRNAs but have enriched target genes with expression changes. Users can select a sub-group of miRNAs of their interests from one or both miRNA lists to redraw the regulatory network. Edges between a miRNA from "Inputted miRNA" list and its targets will be shown as purple lines, while edges between other enriched miRNAs and their targets will be grey lines. Indirect targets are also shown in the network. Arrows pointing to an indirect target are in sage

green. Click on a miRNA node, a list of its targets will pop up. Hovering over a target node, users can find its inputted identifier and gene symbol. Click on it, detail information of the gene will be found in its web page from National Center for Biotechnology Information (NCBI).

In addition to targets with mRNA suppressed, ProteoMirExpress also outputs targets with only proteins suppressed but mRNAs unchanged. MiRNAs, enriched in different gene sets classified according to mRNA and protein levels, can be viewed by clicking on different "Type" buttons. Click on each miRNA in the network or tables, a list of its target will be shown in a pop-in window. The miRNA-mRNA interaction site information, hybridization structure and their sources can be viewed by clicking on the hyperlinks in the "Interaction" and "RNAHybrid" columns.

Results

To evaluate the performance of ProteoMirExpress, we run several example tests with data from Baek et al. (35). In the first example, miR-124 was overexpressed in Hela cell, and the global mRNA and protein expressions were quantified after 24 h and 48 h respectively. With the inputs of mRNA and protein expression profiles, and miR-124 as the known miRNA with expression change, ProteoMirExpress generates a list of predicted active miRNAs and their targets (**Table 1**). As expected, miR-124 is ranked on the top with the most significant hypergeometric *p*-value (1.19E-15) in the potential target gene set, in which genes are down-regulated in either protein or mRNA level. According to protein and mRNA expression changes, these potential targets are further classified into three gene sets: "DD" contains genes that both mRNA and protein are down-regulated, "UD" contains genes that mRNA levels are

unchanged but protein levels are decreased, and “DU” contains genes that mRNA levels are decreased but protein levels are unchanged. In “DD” and “DU” gene sets, the hypergeometric *p*-values of miR-124 also rank as the first. In “UD”, even though miR-124 is not the most significant miRNA, the targets of miR-124 are also significantly enriched. Thus, ProteoMirExpress is able to find not only targets with decreased mRNA abundance but also those whose protein abundance is decreased.

Three miR-124-targeted TFs, SP1, TFAP4 and TEAD1, are found to have predicted targets whose mRNA expressions are suppressed in the presence of miR-124 (**Figure 2**). Since only TFs, whose protein abundance is decreased, will be analyzed to predict indirect targets, all of the three TFs have reduced protein levels. However, only TEAD1 has mRNA significantly decreased as well. The mRNA abundance of SP1 and TFAP4 is not significantly changed (92% and 89% of control respectively), even though their protein abundance is less than 50% of control (29% and 43% respectively). SP1 and TFAP4 have 9 and 7 targets in the miR-124 controlled network respectively, which indicates they may be important downstream regulators for the function of miR-124. However, these TFs may not be reported as miR-124’s targets by other tools that use only mRNA expression data for the analysis.

Other miRNAs with significantly enriched targets in the result list are possibly functional-related to or co-expressed with miR-124 (**Table 1**). For example, hsa-miR-506, belonging to the same miRNA family, has very similar expression profiles with miR-124 in lung carcinogenesis (40, 41), as well as breast cancer samples (42). And miR-124 is known as a neural-specific miRNA and is suppressed in Huntington’s disease, while hsa-miR-760, hsa-miR-432 and hsa-miR-1301 are also

found to be down-regulated in the same disease (43). The miR-Ontology Database, miRò, also reports that hsa-miR-124, hsa-miR-760 and hsa-miR-432 are all associated to brain tissues; and other enriched miRNAs, such as hsa-miR-943, hsa-miR-548m and hsa-miR-1301 etc., are associated with Alzheimer's disease (44). The regulatory network controlled by the top 5 enriched miRNAs is shown in **Figure 2**. More enriched miRNAs are listed in supplementary file **Table S1**. Both hypergeometric *p*-value and permutation *p*-value of each miRNA are shown, and the Pearson's correlation coefficient between two *p*-values is as high as 0.89.

To further test the performance of ProteoMirExpress in inferring co-expressed miRNA, we run a second example, in which miR-223 was knocked out in mouse neutrophils, and the mRNA and protein expression were quantified in day 8 from progenitor differentiation (35). Again, miR-223 is reported as the most significantly active miRNA by ProteoMirExpress with the inputs of mRNA and protein expression changes ($\log_2$(wild type/knockout)) (**Table S2**). Of 57 miRNAs in 20 families that co-expressed with miR-223, 44 miRNAs in 15 families are shown in the significant miRNA list generated by ProteoMirExpress (**Table S2**). The co-expression of miRNAs and the co-occurrence of targeting sites on 3'-UTR are highly correlated (*p*-value <2.2E-16 in Fisher's exact test). Regulatory network with all co-expressed miRNAs gives an overview on the collaborative regulation of these miRNAs on their targets (**Figure 3**). It can be seen that ProteoMirExpress is able to infer active miRNAs in a biological process successfully. In both cases, ProteoMirExpress finishes the analyses within minutes. By integrating protein expression data, ProteoMirExpress takes three subgroups of targets into consideration to construct the miRNA centered regulatory networks, which provides a more comprehensive understanding on the targets and regulatory functions of miRNAs.

Discussion

Identification of miRNA targets and construction of miRNA regulatory network are two major steps in studying the function of miRNA in the complex gene regulatory system. Perturbation on the expression of a miRNA is commonly used to infer the targets of a miRNA. Genes with significant changes in mRNA or protein levels after the perturbation are usually considered as the targets of the miRNA. However in our first case study, miR-124 overexpression causes 105 genes with expression significantly down-regulated, but only 56 of them are direct or indirect targets of miR-124. This leaves 46% of the down-regulated genes unexplained. Thus we also report other miRNAs whose targets are enriched in the differentially expressed gene set, which provides the user hints about other miRNAs that may co-expressed with or be regulated by miR-124. These regulatory links are shown as grey lines to distinguish from those from miR-124 (**Figure 2**). With both analyses on input miRNAs and other enriched miRNAs, as well as the prediction of indirect targets, ProteoMirExpress provides a more complete view of miRNA effects on the suppressed genes, which covers 92% down-regulated genes. Besides of perturbation experiments of miRNAs, our web server can also be applied to other biological studies, such as comparison between different developmental stages or disease status, inferring a miRNA-centered regulatory network that controls the gene expression changes between different biological conditions.

With recent development of high-throughput proteomic methods, such as SILAC (stable isotope labeling by amino acids in cell culture) (45, 46), PRISM (proteomic investigation strategy for

mammals) (47) and label-free quantitative methods (48), researchers are able to quantify protein expression in a large scale. More and more high-throughput proteomic data has been generated to study a variety of biological processes (49-55). This facilitates the studies on gene regulation at posttranscriptional level. The complexity of miRNA function mechanism makes it difficult to infer which level miRNA uses to control each individual target. Integrating high-throughput mRNA and protein data provides an opportunity to solve this problem. ProteoMirExpress, taking multiple miRNA function mechanisms into consideration, studies the effect of miRNA on both mRNAs and proteins. ProteoMirExpress reports not only miRNA targets that mRNA level are decreased, but also subgroups of targets whose proteins are suppressed but mRNAs are not significantly changed or mRNAs are decreased but proteins are not significantly changed, which is made possible only with high-throughput proteomic data. Current tools commonly use only mRNA expression data to construct miRNA regulatory networks. With these tools the subgroups of miRNA targets with proteins suppressed but mRNAs little changed would be completely lost in the network. In the case study of miR-124, TFs, SP1 and TFAP4, are regulated by miR-124 but their mRNA abundance is not significantly changed. Without proteomic data, they may not be detected as targets of miR-124 in the network even though both of them have several downstream targets that indirectly regulated by the miRNA. Moreover, when a TF is in the subgroups of miRNA targets with mRNA decreased but protein little affected, genes with putative binding sites of this TF would be reported as indirect targets of miRNAs by tools using only mRNA expression data. However, since the protein abundance of the TF is not significantly change, its effects on the downstream targets in the reported network may not be true. The same problem would also occur when the

miRNA-regulated proteins control downstream pathways, which lead to misunderstanding the functions of these miRNA-regulated proteins in the network.

In the two case studies analyzed above, out of 51 direct target genes that are significantly suppressed (either mRNA is less than 67% of control or protein is less than 50%, since protein level is measures one day later than mRNA level, and at least one miR-124 site) by the overexpression of miR-124 in Hela cell, 12 genes (23%) are suppressed in both mRNA and protein level, 9 genes (18%) are suppressed in only protein level but not mRNA level and 30 genes (59%) are suppressed in only mRNA level but not protein level. While out of 35 direct target genes that are significantly suppressed by miR-223 (either mRNA in miR-223 knockdown is less than 67% of control neutrophil cell or protein is less than 67%, and at least one miR-223 site), 6 genes (17%) are suppressed in both mRNA and protein level, 24 genes (69%) are suppressed in only protein level but not mRNA level and 5 genes (14%) are suppressed in only mRNA level but not protein level. It seems that the proportion of each subgroup of targets can be different for different miRNAs or maybe different cells. Data collection time is also thought to affect the proportion of different subgroups, since short time courses after miRNA perturbation but before deadenylation may lead to more observations of “UD” group (30, 31, 56, 57). On the other hand, long time courses after miRNA perturbation, when mRNA deadenylation and decay shows strong effects, may report more genes from “DD” group (58, 59). However, in an experiment with long time scales, where the miRNA effects are steady, less direct targets but more indirect targets will be detected. Thus, the determination of data collection time is an important issue in miRNA targets identification studies.

It has been reported that miRNA affected target gene's expression through both translation inhibition and RNA degradation, and the former effect is relatively mild compared with the later (32). This implies that multiple strategies are used by miRNA to refine the control on their targets in a quantitative manner. With the analysis of ProteoMirExpress, we find a subgroup of targets whose mRNAs are suppressed but protein levels are not significantly changed. It indicates that degradation of mRNA may not immediately suppress the protein level. This may be caused by the low protein degradation rate of these targets. Thus for this group of targets, the effect of miRNA seems to stop the increase of their protein level but not to immediately decrease them. The three groups of targets detected by ProteoMirExpress demonstrate the limitation of using either mRNA or protein data alone to study the effect of miRNA. Either method may lose a subgroup of targets. Thus with the integrative approach, ProteoMirExpress provides the users a more complete and detail regulatory network controlled by miRNAs. Further analyses on the functions, binding site sequences and expression details of different subgroups of miRNA targets will improve our understanding on the strategy that a miRNA uses to preciously control thousands of targets.

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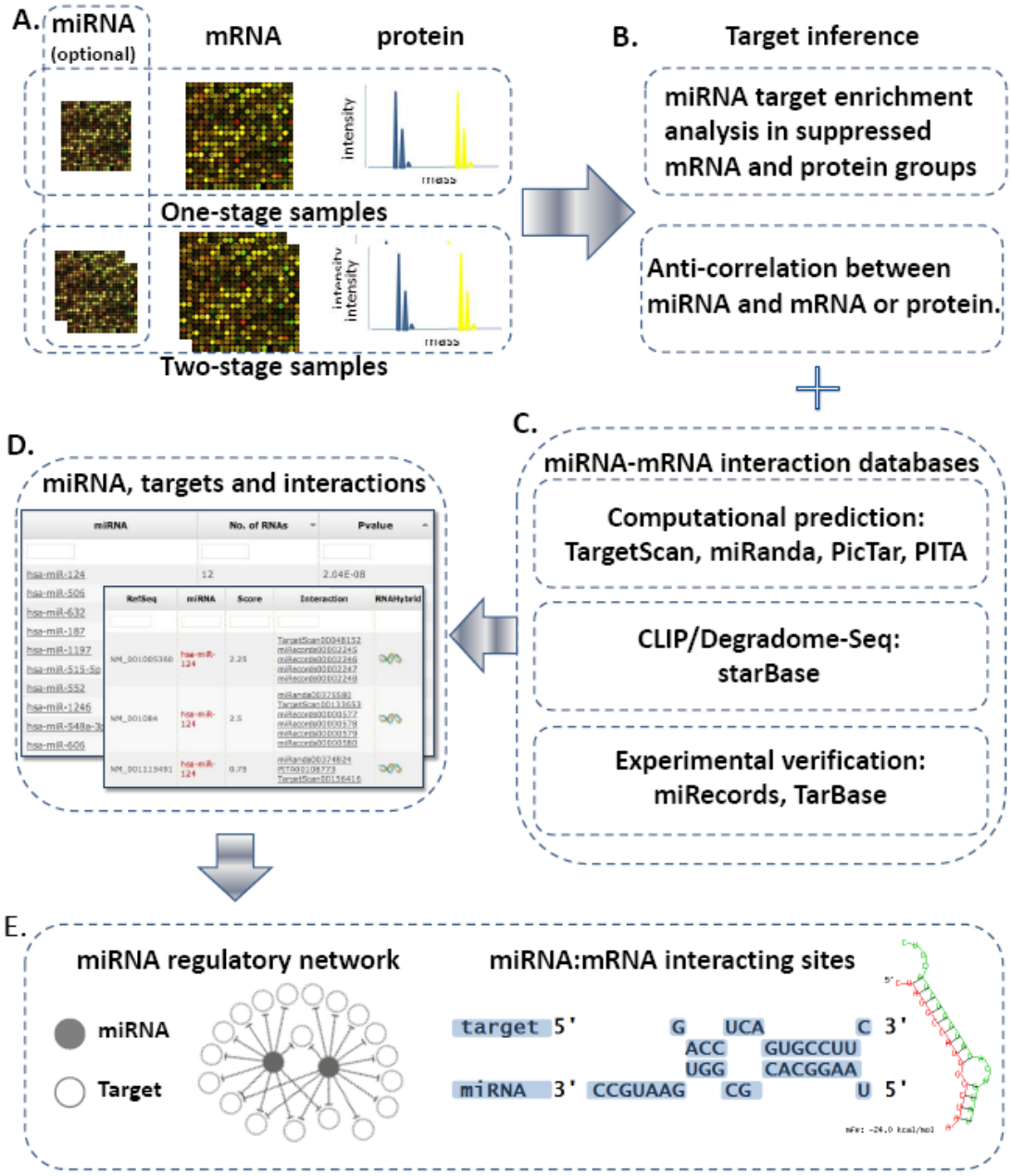
Figure Legends

Figure 1. Overview of ProteoMirExpress workflow. A: input of high throughput mRNA and proteomic profiling data, expression of miRNAs input is optional; B: data analysis of ProteoMirExpress; C: the information sources of miRNAtarget; D: table outputs; E: miRNA-centered posttranscriptional regulatory network andmiRNA-target interactions.

Figure 2.miRNA regulatory network with the top 10 enriched miRNAs in Hela cells with miR-124 overexpression(*p*-value < 1E-4). Each node is either a miRNA or a gene. Each edge is an arrow pointing out from a miRNA to its target, indicating its suppressing role. The weights of the lines are proportional to the IS scores of the miRNA-target pairs. Edges in purple line: connection between a miRNA from "Inputted miRNA" list and its targets; Edges in grey line: connection between other enriched miRNAs and their targets Nodes in blue: genes in "UD" class, having mRNA levels unchanged (U) but protein levels decreased (D); Nodes in green: genes in "ML" class, having medium expression level in mRNA (M) but low expression level in protein (L). Nodes in pink: genes in "D" class, having decreased expression in mRNA or protein when only mRNA or protein data is inputted but not both.

Figure 3. miRNA regulatory network with the co-expressed miRNAs in miR-223 expressed mouse neutrophils. Refer to Figure 2 legend for detail description of the graph.

Figure 1.


A.
miRNA
(optional)
mRNA
protein
intensity
mass
One-stage samples
intensity
intensity
mass
Two-stage samples
B.
Target inference
miRNA target enrichment analysis in suppressed mRNA and protein groups
Anti-correlation between miRNA and mRNA or protein.
C.
miRNA-mRNA interaction databases
Computational prediction: TargetScan, miRanda, PicTar, PITA
CLIP/Degradome-Seq: starBase
Experimental verification: miRecords, TarBase
D.
miRNA, targets and interactions
miRNA
No. of RNAs
Pvalue
E.
miRNA regulatory network
miRNA
Target
miRNA:mRNA interacting sites
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miRNA 3'
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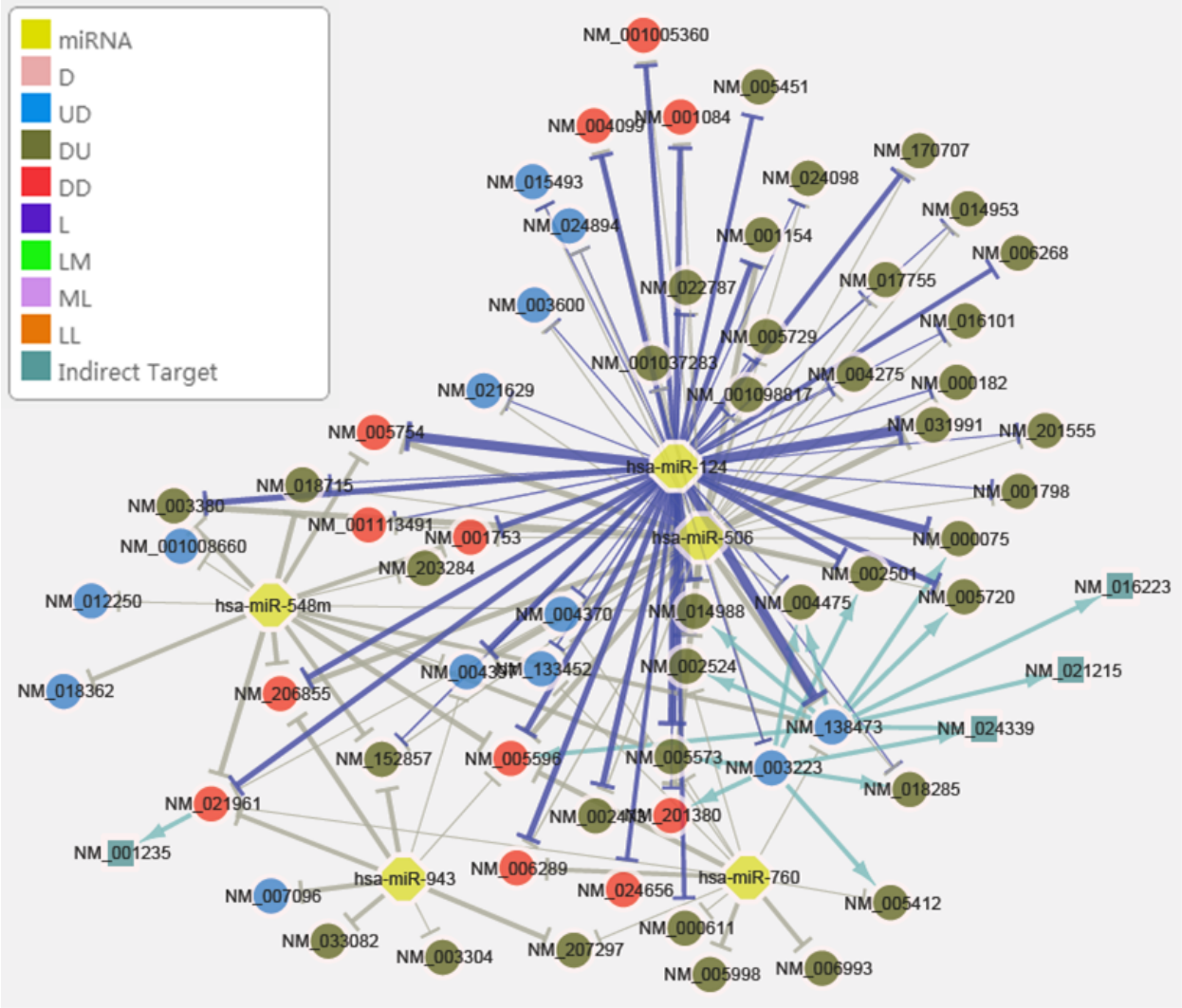
UGG CACGGAA
CCGUAAG CG U
3'
5'

Figure 2.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DU
DD
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ML
LL
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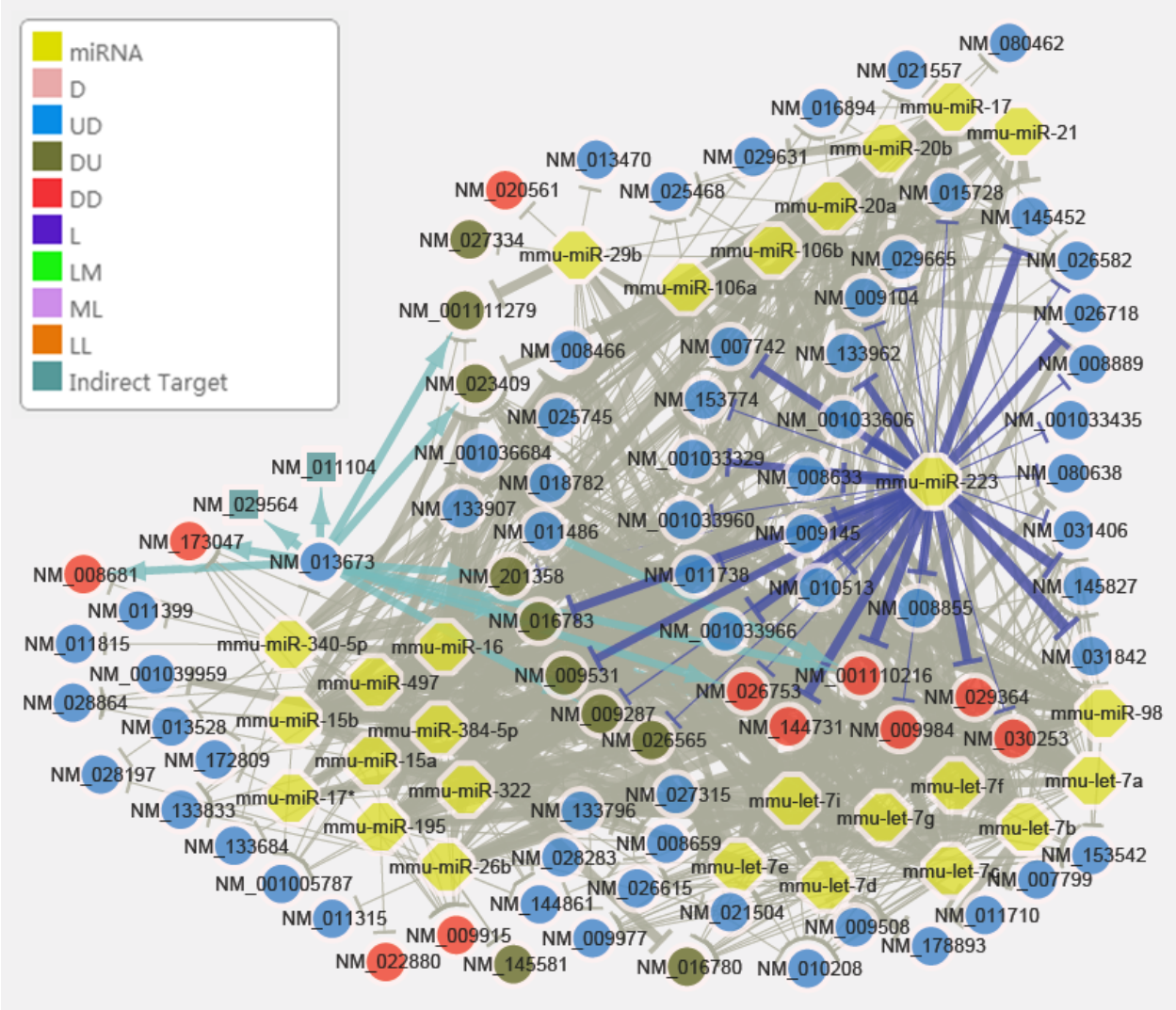
Figure 3.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DU
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mmu-let-7d
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NM_016780
NM_010208

Table 1. Inferred active miRNAs by ProteoMirExpress in Hela cells with miR-124 overexpression (*p*-value < 1E-4).

miRNA	No. of targets	*p*-value	No. in DD*	*p*-value in DD*	No. in UD*	*p*-value in UD*	No. in DU*	*p*-value in DU*
hsa-miR-124	51	1.19E-15	12	2.04E-08	9	1.83E-03	30	2.62E-08
hsa-miR-506	46	4.96E-13	11	2.49E-07	9	1.28E-03	26	2.03E-06
hsa-miR-760	13	3.09E-06	3	5.41E-03	3	1.44E-02	7	1.82E-03
hsa-miR-943	9	1.78E-05	3	1.01E-03	2	3.20E-02	4	1.45E-02
hsa-miR-548m	16	2.19E-05	5	3.47E-04	5	1.99E-03	6	6.31E-02
hsa-miR-545	18	2.94E-05	3	4.21E-02	5	5.24E-03	10	5.11E-03
hsa-miR-525-3p	7	4.94E-05	2	7.43E-03	0	-	5	4.21E-04
hsa-miR-802	14	6.03E-05	4	1.99E-03	3	4.03E-02	7	1.49E-02
hsa-miR-1252	16	1.15E-04	5	6.69E-04	6	5.45E-04	5	1.50E-01
hsa-miR-432	14	1.17E-04	2	1.05E-01	5	1.38E-03	7	1.98E-02
hsa-miR-564	5	1.48E-04	1	6.58E-02	1	9.12E-02	3	4.33E-03
hsa-miR-323b-5p	10	2.50E-04	2	4.52E-02	3	1.30E-02	5	2.27E-02
hsa-miR-513c	10	4.32E-04	3	5.90E-03	2	8.99E-02	5	2.89E-02
hsa-miR-544	30	4.46E-04	6	1.50E-02	6	6.47E-02	18	1.21E-02
hsa-miR-548p	18	5.42E-04	4	1.69E-02	5	1.36E-02	9	4.25E-02
hsa-miR-922	13	5.53E-04	2	1.10E-01	2	1.73E-01	9	2.65E-03
hsa-miR-1301	10	6.76E-04	2	5.64E-02	2	9.83E-02	6	1.11E-02
hsa-miR-518b	5	7.05E-04	1	9.08E-02	2	8.42E-03	2	7.02E-02
hsa-miR-1197	8	7.33E-04	4	1.26E-04	3	6.30E-03	1	3.55E-01
hsa-miR-518c*	6	7.37E-04	0	-	2	1.85E-02	4	5.40E-03
hsa-miR-381	33	7.81E-04	6	3.07E-02	8	2.08E-02	19	2.58E-02
hsa-miR-765	11	8.46E-04	4	1.22E-03	3	2.95E-02	4	1.31E-01
hsa-miR-1227	6	8.75E-04	1	1.37E-01	1	1.82E-01	4	6.05E-03
hsa-miR-1266	8	9.01E-04	1	2.19E-01	2	5.50E-02	5	9.63E-03
hsa-miR-661	8	9.66E-04	3	2.57E-03	3	7.10E-03	2	2.44E-01

*Gene classes “DD” contains genes that both mRNA and protein are down-regulated, “UD” contains genes that mRNA levels are unchanged but protein levels are decreased, and “DU” contains genes that mRNA levels are decreased but protein levels are unchanged.