miRTarBase update 2014: an information resource for experimentally validated miRNA-target interactions

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ABSTRACT

MicroRNAs (miRNAs) are small non-coding RNA molecules capable of negatively regulating gene expression to control many cellular mechanisms. The miRTarBase database (http://mirtarbase.mbc.nctu.edu.tw/) provides the most current and comprehensive information of experimentally validated miRNA-target interactions. The database was launched in 2010 with data sources for >100 published studies in the identification of miRNA targets, molecular networks of miRNA targets and systems biology, and the current release (2013, version 4) includes significant expansions and enhancements over the initial release (2010, version 1). This article reports the current status of and recent improvements to the database, including (i) a 14-fold increase to miRNA-target interaction entries, (ii) a miRNA-target network, (iii) expression profile of miRNA and its target gene, (iv) miRNA target-associated diseases and (v) additional utilities including an upgrade reminder and an error reporting/user feedback system.

INTRODUCTION

MicroRNAs (miRNAs) are non-coding RNAs ~19–25 nt in length, which are widely found in organisms such as plants, nematodes, fruit flies and mammals (1). *lin-4* was the first identified miRNA from *Caenorhabditis elegans* and was found to control the timing and progression of the nematode life cycle (2). In humans, miRNAs play important roles in cellular physiology, development and disease by negatively regulating gene expression (3). miRNAs bind to complementary sequences in the 3′ untranslated regions of their target mRNAs and induce mRNA degradation or translation repression (4).

miRNAs play important roles in causing many diseases including various types of cancer, cardiovascular diseases and neurological disorders (5–8). Thus, as shown in Supplementary Figure S1, miRNA and the field of non-coding RNA have attracted increasing research interest. In recent years, many databases related to miRNAs have been developed, providing information about miRNAs and their target genes. miRBase (9) is the central repository for miRNAs nomenclature, sequence data, annotation and target prediction, containing ~24 521 miRNAs entries. Many databases such as microRNA.org (10),

miRGen (11), miRGator (12), miRDB (13) and miRNAMap (14) identify miRNA target interactions (MTIs) using a number of target prediction tools like TargetScan (15,16), miRanda (17), PicTar (18), RNAhybrid (19) and PITA (20). Furthermore, some databases provide evidence for experimentally validated miRNAs and their target genes. TarBase (21) contains a manually curated collection of experimentally tested miRNA targets, in humans, mice, fruit flies, worms and zebrafish. miRecords (22), an integrated resource for animal miRNA-target interaction, hosts a large, high-quality manually curated database of experimentally validated miRNA-target interactions with systematic experimental documentation for each interaction. The HMDD (23) database is the first resource to provide experimentally supported human miRNA and disease associations. miR2Disease (24) provides a brief description of the miRNA–disease relationship along with information about miRNA expression patterns as well as experimentally verified miRNA target genes and literature references. DIANA-LncBase (25) provides comprehensive annotations of miRNA targets on long non-coding RNAs with transcriptome-wide experimentally verified and computationally predicted miRNA recognition elements. miRSel (26), a miRNA–gene association database, combines text-mining results with existing databases and computational predictions. miRWalk (27) presents predicted and validated information on miRNA-target interactions and enables researches to validate new targets of miRNA not only on 3′ untranslated regions but also on other regions of all known genes. By including experimental evidence, these research resources are highly effective in identifying MTIs.

Before proceeding with experimental validation, a number of computational programs are used to predict a putative miRNA binding site within a given mRNA target. Once the predicted miRNA binding sites have been determined, these MTIs are then validated by molecular experiments, including reporter assays and western blots, which are the conventional methods for confirming miRNA and target gene interactions. The rationale for using the reporter assay is that the binding of a given miRNA to its specific mRNA target site will repress reporter protein production thereby reducing expression, so that the inhibited level can be easily compared with control. Experiments like northern blot analysis or quantitative real-time PCR (qPCR) use total RNA isolated from a specific cell type and examine the coexpression of miRNA and mRNA. One typical approach to validate the functional importance of a miRNA/mRNA target pair is the transient overexpression of a given miRNA mimic in a cell type known to repress the putative target protein followed by western blot analysis (28). Recently, genome-wide screening experiments have been developed including microarrays with overexpression or the knockdown of miRNAs, stable isotope labeling with amino acids in culture (SILAC) or pulsed SILAC [pSILAC (29)].

The identification of the roles of miRNAs and their targets in different biological systems raise the need to easily access and frequently update central information repositories. miRTarBase serves as an important repository for experimentally validated MTIs, which are frequently updated by manually surveying research articles. In addition, miRTarBase contains the largest number of validated MTIs with strong evidentiary support, and the collection is more frequently updated than other databases such as TarBase, miRecords and miR2Disease. Table 1 summarizes features added in the latest update.

IMPROVEMENTS

Table 1 lists the advancements and new features supported in the 2014 miRTarBase update. Major improvements include (i) a 14-fold increase in miRNA-target interaction entries as compared with the initial release, (ii) a miRNA-target network, (iii) expression profile of miRNA and its target gene, (iv) miRNA target-associated diseases and (v) additional uses including an upgrade reminder service and an error reporting/user feedback system.

Updated database content

In the 2014 update, 51 460 curated MTIs between 1232 miRNAs and 17 520 target genes were collected from 2636 articles. Table 2 lists the number of collected MTIs in each species. In all, 38 113 human MTIs were collected between 587 miRNAs and 12 194 target genes with experimental support from 2143 articles; in addition, 1778 and 3026 interactions were, respectively, confirmed by western blot and reporter assays. Each research article was carefully reviewed by at least two of our developers to extract the MTIs, which were experimentally confirmed by reporter assay, western blot, qPCR, microarray, pSILAC or NGS (CLIP-seq or Degradome-seq). The 2014 update included a large increase in the number of MTIs supported by strong experimental evidence (as validated by reporter assay, western blot or qPCR; Figure 1).

Experimental validation method—addition of the 'NGS' support type

Experimental approaches for identifying MTIs (e.g. the reporter assay) are time-consuming and incapable of handling large-scale screenings. Recent studies have demonstrated that MTIs can be uncovered via high-throughput screening using next-generation sequencing (NGS) technology. Ultraviolet cross-linking and immunoprecipitation (CLIP) was first developed to identify specific Nova RNA–protein complexes in mouse brain tissue (30). Chi *et al.* (31) pioneered the use of cross-linking and immunoprecipitation approach, combined with NGS technologies (CLIP-seq or HITS-CLIP) to identify MTIs to obtain Argonaure-miRNA-protein complexes in mouse brain tissue. Furthermore, Hafner *et al.* (32) modified the CLIP-seq method [as the photoactivatable-ribonucleoside-enhanced cross-linking and immunoprecipitation (PAR-CLIP)] to enhance the Argonaure-miRNA-protein complex resolution of the original CLIP-seq method. German *et al.* (33) also developed an NGS approach to detect MTIs by identifying mRNA cleavage products through parallel analysis of

Table 1. The comparison of data and function between miRTarBase version 1.0 and miRTarBase version 4.0

Features	miRTarBase version 1.0	miRTarBase version 4.0
Known miRNA entry	miRBase (version 14)	miRBase (version 20)
Species	17 species	18 species
Curated miRNA-target interactions	3576 MTIs	51 460 MTIs
MTIs were validated by experimental technology	Reporter assay, western blot, northern blot, qRT-PCR, microarray, pSILAC	Reporter assay, western blot, northern blot, qRT-PCR, microarray, pSILAC, NGS (CLIP-seq, Degradome-seq and CLASH-seq)
Number of MTIs validated by 'reporter assay' or "western blot"	2207 MTIs	4572 MTIs
miRNA expression profile	None	NCBI GEO (21 data sets)
Target gene expression profile	None	NCBI GEO (21 data sets)
Relationship between miRNA and target genes	None	Analysis of the expression profiles between miRNA and its target genes
miRNA regulatory network	None	First-order neighbors of miRNA and target gene.
Disease information	None	558 diseases
Upgrade reminder service	None	Yes
Error report system	None	Yes
Graphical visualization	miRNA, secondary structure, known and novel miRNA target sites, functional and non-functional MTIs, experimental conditions	miRNA, secondary structure, known and novel miRNA target sites, functional and non-functional MTIs, experimental conditions, miRNA-target network, miRNA-target expression profile, miRNA disease, upgrade reminder service, error report system, user feedback service

Table 2. The statistics of miRTarBase entries

Species	No. of miRNA-target interactions	No. of miRNAs	No. of target genes	No. of articles collected[a]	No. of miRNA-target interactions experimentally validated by				
					Strong evidence		Less strong evidence		
					Western blot	Reporter assay	pSILAC	Microarray	NGS
Human	38 113	587	12 194	2143	1778	3026	495	11 704	20 492
Mouse	9378	217	4443	409	439	676	2	636	8054
Rat	363	126	165	108	129	122	0	178	0
Chicken	30	12	21	15	7	21	0	2	0
Cattle	7	5	5	3	0	3	0	0	0
Sheep	2	1	2	3	0	0	0	0	0
Frogs	6	3	6	7	0	2	0	0	0
Japanese medaka	1	1	1	1	0	1	0	0	0
Zebrafish	114	30	82	40	36	90	0	2	0
Fruit fly	129	39	77	37	9	127	0	12	0
Silkworm	2	2	1	1	0	2	0	0	0
Nematode	3216	154	452	27	1	39	0	0	3171
Thale cress	92	40	66	17	7	2	0	13	20
Viruses	7	15	5	16	1	7	0	0	0
Total	51 460	1232	17 520	2636	2407	4118	497	12 547	31 737

[a]Articles may report various miRNA-target interactions in different species.

RNA ends (PARE), also known as degradome-seq. Because the RNA-induced silencing complex (RISC)-mediated cleavage is not the major mechanism of miRNA regulation in mammals, this approach is mainly used in plants such as Arabidopsis (33,34) and rice (35). In addition, only limited records are available for mammals (36–38). Several databases have been developed to compile publicly available CLIP-seq, PAR-CLIP and degradome-seq data sets for analysis, such as CLIPZ (39), starBase (40), doRiNA (41) and TarBase 6.0. In contrast, CLASH (cross linking, ligation and sequencing of hybrids) was recently developed to directly map the miRNA–mRNA binding sites without using the target prediction (42,43).

The database was populated with entries derived from manually curated articles. The curators noted the miRNA, the related target gene and information regarding the experiment such as the cell line or tissue used. Besides MTIs included in the initial miRTarBase release and validated using reporter assays, western blots, qPCR, microarrays and pSILAC, the updated version supports NGS data
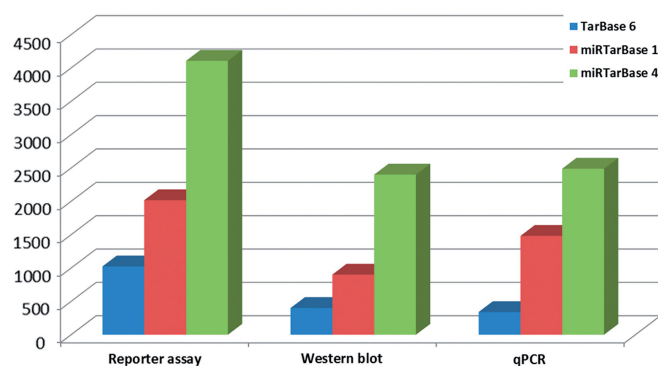
Figure 1. Number of miRNA-target interactions with 'strong experimental evidence' in TarBase version 6, miRTarBase version 1 and miRTarBase version 4.

(NGS: CLIP-seq, Degradome-seq and CLASH-seq). Here we incorporate the 21 human CLIP-seq data set, 5 mouse CLIP-seq data set, 6 nematode data set and one human CLASH-seq data set into miRTarBase. CLASH-seq data set provides the large number of miRNA binding sites including canonical and non-canonical miRNA-target interactions, which make the information of miRNA-target interactions more complete.

miRNA target-associated diseases

miRNA-related dysfunctions are associated with a broad spectrum of diseases, including various types of cancer, cardiovascular diseases and neurological disorders, and miRNAs have emerged as a novel class of potential biomarkers or targets for disease diagnosis and therapy. To provide more information about miRNA-associated diseases and the relationship between miRNA-target interactions and disease, the data contents of HMDD version 2.0 and miR2Disease are integrated in miRTarBase. In addition to the integration of external disease databases, experimentally verified miRNA-associated diseases are manually curated through literature surveys.

miRNA-target network

Interactions between a given MTI and other miRNAs/ mRNAs in the miRTarBase can be visualized through an interactive network web interface by integrating CytoscapeWeb (44). This network visualization can help researchers understand complicated miRNA-target regulation. For example, given an MTI (hsa-miR-26a-5p and EZH2), Figure 2 shows a miRNA-target regulatory network consisting of the first-order neighbors of this MTI. This network visualization clearly demonstrates that miR-26a and miR-217 regulate EZH2 and PTEN, which is a complicated regulation. Furthermore, we examined the functions of these target genes involved in miRNA-target interactions collected in the database by performing Gene Ontology (GO) and KEGG pathway enrichment annotation using the DAVID gene annotation tool (45).

Expression profile of miRNA and its target gene

Many previous studies have integrated miRNA and mRNA expression profiles to predict MTIs with lower false positives and more biologically meaningful targets (12,46–50). The correlation between miRNA and mRNA provides an important indication of miRNA direct targets. In addition, the miRNA and mRNA expression data provides dynamic information for MTIs, which can help biologists investigating phenotype-specific miRNA regulatory pathways. Thus, we collected human miRNA and mRNA matched expression data to provide phenotype-specific miRNA-mRNA correlation analysis.

Once a user finds an interesting MTI in miRTarBase, the next step is to determine what phenotype condition can make activate the MTI. Some MTIs are active in various conditions but others are only active in a specific phenotype. To address this issue, we provided phenotype-specific MTI coexpression profiles for many data sets with various phenotypes. We selected 21 human data sets from the NCBI GEO database with at least 9 matched mRNA and miRNA samples (51), producing 1596 samples. These data sets were originally generated to study both mRNA and miRNA profiles under various and complex phenotypes, e.g. mRNA and miRNA expression profiles of the renal cortex for hypertensive and normotensive patients (GSE28260); and mRNA and miRNA expression profiles of breast cancer cells treated with the Novel Histone Deacetylase Inhibitor CG-1521 (GSE25844). Supplementary Table S1 shows the data set title, platform and the number of miRNA/mRNA samples. As GSE19783 has 216 samples including the estrogen receptor negative (ER−) and estrogen receptor positive (ER+) samples, we divided GSE19783 into two separate data sets: GSE19783 ER− and GSE19783 ER+.

We integrated human gene expression data from multiple platforms including 11 mRNA and 8 miRNA platforms. In the mRNA expression data, all data sets were log-transformed and median-centered per sample, and standard deviations were normalized to one per sample. In addition, if one gene has several probes, we calculate the mean expression value for the gene. For each miRNA and mRNA pair, we calculated the Pearson correlation between miRNA and mRNA expression profiles for each data set. To estimate the significance of the MTI using expression profiles, we transferred the Pearson correlation to a *P*-value for each MTI as follows. Given an MTI with a Pearson correlation r and a sample size n, we calculated the *t*-value (52) as follows:

$$t = \frac{r}{\sqrt{\frac{1-r^2}{n-2}}}$$

The *P*-value was calculated using the *t*-value and t-distribution with a degree of freedom of $n-2$.

Given a human MTI, miRTarBase will show the expression table with a data set-specific correlation and *P*-value for all expression data sets (Figure 3A), which indicates how likely the MTI is to be active in a certain phenotype. Furthermore, when the user selects a data set, miRTarBase will draw the expression profiles of miRNA and mRNA of the MTI (Figure 3B). To facilitate the comparison of miRNA and mRNA profiles, we normalized both miRNA and mRNA profiles to a mean of 0 and a standard deviation of 1.
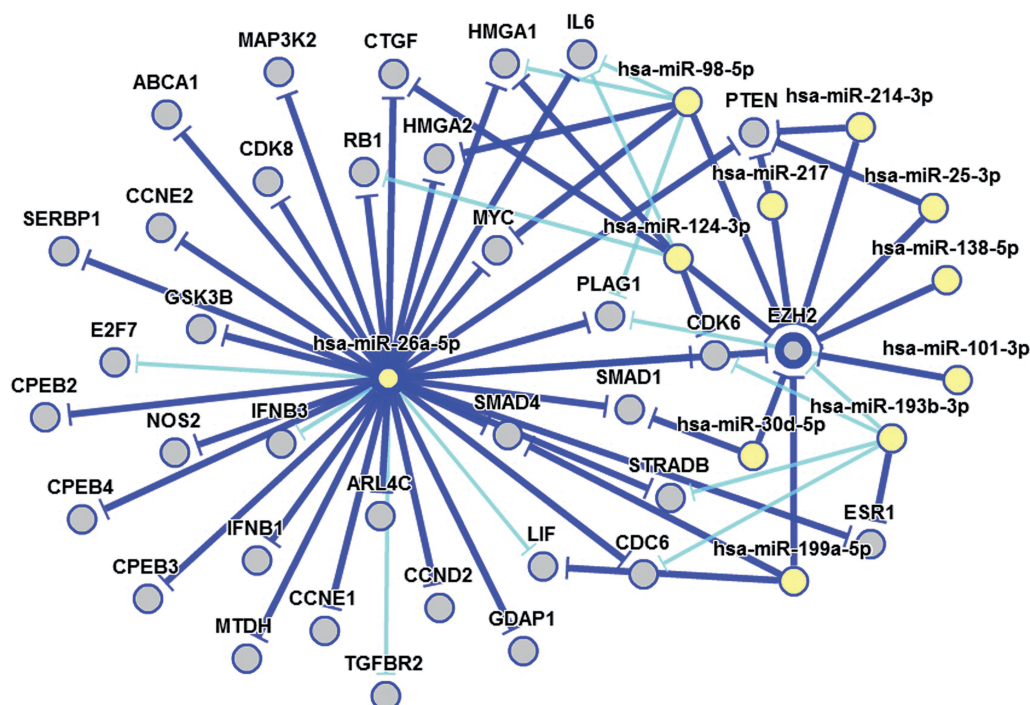
Figure 2. miRNA-target regulatory network. Given an MTI hsa-miR-26a-5p and EZH2, miRTarBase shows a miRNA-target regulatory network consisting of all the target genes of hsa-miR-26a-5p and all the miRNAs that target EZH2.

To demonstrate the phenotype-specific correlation analysis, Figure 3 shows miRNA/mRNA expression profiles for miR-26a and EZH2. Three data sets have significantly negative correlations between miR-26a and EZH2 ($P < 0.001$), and the top two data sets are breast cancers (Figure 3A). Interestingly, Zhang *et al.* reported that miR-26a is a tumor suppressor and targets EZH2 in breast cancer (53), which is consistent with our results.

ENHANCED INTERFACE

To facilitate access to data and further analyses to support research on miRNA-target interactions, various query interfaces and graphical visualization pages were redesigned and enhanced. The miRTarBase provides two modes for querying specific MTI's information—the species browser and search utility. Users can browse through numerous miRNA targets and explore the outcomes of hundreds of experimental studies in a way which is both simple and intuitive. The user can perform basic MTI searches by miRNA, target gene symbol, validation method or PubMed ID. The search box suggests plausible keywords and supports autocomplete mode as users type in the search field. Each MTI output page consists of basic information including miRNA and target gene information, miRNA disease, experimental evidence support with literature references, miRNA-target expression profiles and miRNA-target regulatory network. To visualize the interactions between MTI and other miRNAs/mRNAs, we support the interactive network web interface to present a regulatory network consisting of the MTI's first-order neighbors. In

addition, the upgrade integrates an upgrade reminder, and error reporting/user feedback mechanism. User feedback is extremely valuable to further improve the database as a comprehensive and user-friendly tool for exploring miRNA-target interactions.

CONCLUSIONS AND PERSPECTIVES

The current update represents a 10-fold increase of MTIs as compared to the initial miRTarBase release, and includes a significant extension of specific research-oriented features. miRTarBase 4 is designed to serve as a multifaceted tool for providing extensive experimental support in all miRNA-related research. Moving forward, the database will be updated at 2 month intervals to capture the growing number of publications covering novel targets. To summarize complicated miRNA-target regulations, we provide a visualization of the miRNA-target regulatory network consisting of the MTI's first-order neighbors. To support dynamic MTI information, we collected human miRNA/mRNA matched expression data sets to provide phenotype-specific correlations between miRNA and mRNA expression profiles.

The latest release incorporates human miRNA target expression profiles from 21 GEO data sets, which were all done using microarray platforms. Future work will extend our functional and expression analyses to other species as miRNA-target expression profiles of these species become available, so that miRTarBase will eventually provide sufficient information to support any miRNA-related work.
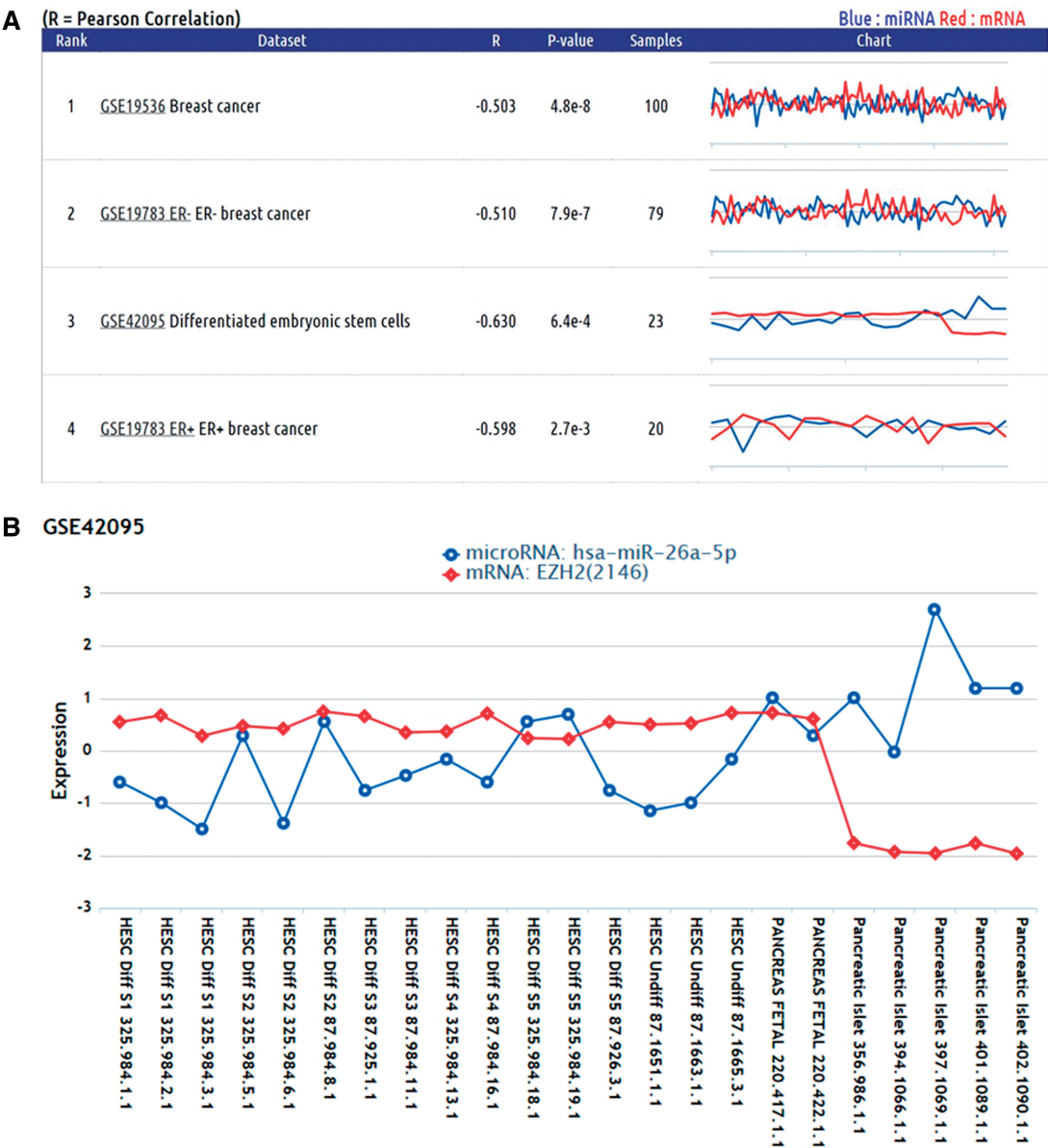
Figure 3. Phenotype-specific correlation analysis. (**A**) Given an MTI hsa-miR-26a-5p and EZH2, miRTarBase shows the Pearson correlation, *P*-value and miRNA/mRNA expression profile for each data set. This table shows the top 4 data sets with significant *P*-value. (**B**) When the user selects a data set, miRTarBase shows the detailed profiles for hsa-miR-26a-5p and EZH2 in this data set.

AVAILABILITY

The miRTarBase content will be continuously maintained and updated every 2 months. The database is now publicly accessible at http://miRTarBase.mbc.nctu.edu.tw/.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online, including [54–69].

ACKNOWLEDGEMENTS

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