dbDEMC 2.0: updated database of differentially expressed miRNAs in human cancers

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ABSTRACT

MicroRNAs (miRNAs) are often deregulated in cancer and are thought to play an important role in cancer development. Large amount of differentially expressed miRNAs have been identified in various cancers by using high-throughput methods. It is therefore quite important to make a comprehensive collection of these miRNAs and to decipher their roles in oncogenesis and tumor progression. In 2010, we presented the first release of dbDEMC, representing a database for collection of differentially expressed miRNAs in human cancers obtained from microarray data. Here we describe an update of the database. dbDEMC 2.0 documents 209 expression profiling data sets across 36 cancer types and 73 subtypes, and a total of 2224 differentially expressed miRNAs were identified. An easy-to-use web interface was constructed that allows users to make a quick search of the differentially expressed miRNAs in certain cancer types. In addition, a new function of 'meta-profiling' was added to view differential expression events according to user-defined miRNAs and cancer types. We expect this database to continue to serve as a valuable source for cancer investigation and potential clinical application related to miRNAs. dbDEMC 2.0 is freely available at http://www.picb.ac.cn/dbDEMC.

INTRODUCTION

As the leading cause of human death, cancer continues to represent a huge economic and social burden to society. It currently accounts for >8 million deaths according to World Cancer Report 2014 (1), and the incidence and mortality rates of many cancer types have been increasing over the last years. It is therefore urgent to understand the molecular mechanisms underlying cancer development and to develop reliable biomarkers for early detection, diagnosis and treatment of cancer.

In recent years, large numbers of studies have indicated that microRNAs (miRNAs), a class of small noncoding RNAs (∼18–23 nt in length), are highly associated with initiation and progression of cancer. MiRNAs mainly function to regulate mRNA expression through sequence specific interaction with 3'-untranslated regions (3'-UTRs) (2,3). To date, more than 2500 miRNAs have been identified in the human genome according to latest release of miRBase (4). Studies have indicated that miRNAs play important roles in a wide range of physiological and biological processes, such as: cell division, proliferation, differentiation, development, metabolism and apoptosis (5–8). Hence, many miRNAs could function as oncogenes or tumor suppressors by regulating different cancer associated genes. Over the last decade, associations between alterations in miRNA expression and the occurrence of cancer has been the subject of intense investigation (9). MiRNAs have emerged as an important kind of diagnostic, prognostic and predictive biomarker for different types of cancer, and earned a promising role for cancer biology (10,11). In addition, miRNAs offer potential new avenues for cancer treatment, for instance by using miRNA mimics or antogomirs (12,13).

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In the past years, genome wide detection methods including microarray and next generation sequencing have been developed to identify miRNA profiles in a variety of cancer types (14,15). Huge amount of miRNA expression profiling data for cancers was released by public resources, such as the Gene Expression Omnibus (GEO) and other cancer projects including The Cancer Genome Atlas (TCGA), and the International Cancer Genome Consortium (ICGC). These valuable resources provide an opportunity to investigate cancer associated miRNAs from large amount of samples. However, these miRNA–cancer relationships are buried in thousands of published studies. It is still a challenging task to translate this huge amount of miRNA expression data into a systematically annotated and documented database for ease of interpretation.

Previously, we developed dbDEMC (16), a database of miRNA expression changes in different cancer types. It differs from other databases that used mainly manually collection or text mining methods, such as miR2Disease (17), HMDD (18), miR2cancer (19), TUMIR (20) or OncomiRDB (21), to identify cancer associated miRNAs, in that dbDEMC identifies these differentially expressed miRNAs from *de-novo* analysis of high-throughput expression data. The first version of dbDEMC provides researchers an easy to use resource to retrieve cancer related miRNAs in fourteen cancer types. Each miRNA entry provides differential expression pattern seen in these cancer types, and also the annotation from different experiments. Since its first release in 2010, many more cancer miRNA expression profiling studies have been published. It is therefore paramount to update the database to keep a pace with the rate of data accrual. Here we introduce dbDEMC 2.0, an updated and significantly expanded version of this database. The second version of dbDEMC documents a total of 2224 differentially expressed miRNAs from 36 cancer types through the processing of >200 expression data sets. In addition to the expanded data volume, the search and browser functions are retained and enhanced, and new features have been added for better usage of the database. We expect this updated database could facilitate the identification of cancer associated miRNAs and benefit the investigation of their roles in physiological and pathological processes of cancer development. All the information in dbDEMC 2.0 is freely available to the public domain through http://www.picb.ac.cn/dbDEMC.

DATA COLLECTION AND PROCESSING

We conducted a systematic data search for cancer related miRNA expression profiles by using cancer-related keywords, such as: 'cancer', 'tumor', 'carcinoma' and 'neoplasm', in combination with 'microRNA' or 'miRNA' from GEO. All the data sets used are limited to human studies published before June 2016. In addition, we also collected high quality miRNA expression profiles for 22 cancer types generated by miRNA-seq from TCGA. More than 400 data sets were collected initially. The data processing procedure is as follows:

i. For data quality control, we made a rigorous manual review for each data set to screen those meeting the aim of this study initially. To ensure that only high quality data sets were included in our database, only the data sets have enough samples for both case and control (at least three) groups were used. After this quality control step, a total of 209 miRNA expression data sets remained.

ii. For each dataset, the expression values were logarithmically transformed (base 2) and quantile normalized. For miRNA expression data sets get from TCGA, we used miRNA isoforms expression data as it provides the mature miRNA expression information. The maximum expression value was selected if there were multiple isoforms for a given miRNA in each sample. The limma (Linear Models for Microarray and RNA-seq Data) package (22) embedded in R (http://www.r-project.org/) was used to select miRNAs whose mean expression level is significantly different between case and control samples. The population level control and one factor analysis were used. Those miRNAs with FDR adjusted *P*-value <0.05 were extracted as candidates that have significant different expression.

iii. The inconsistency of the miRNA name annotated in different updates from miRBase could result in ambiguities for the miRNA names from different expression platforms. To overcome this problem, we used miRBase-tracker (23) to unify all the miRNA names to those annotated in the most recent release of the miRBase. In addition, the miRNA IDs were also uniformly mapped to the HUGO Gene Nomenclature Committee (HGNC) (24), Entrez Gene ID (25) and Ensemble Gene ID (26). The sequences for mature and precursor miRNAs from miRBase were integrated. Furthermore, the cancer names were also unified into 36 different cancer tissues: for example, 'lung squamous cell carcinoma', 'lung adenocarcinoma', 'small cell lung cancer' and 'large cell lung cancer' were grouped into the 'lung cancer', but decimated by the 'cancer subtype'.

iv. In addition to the miRNA expression data generated from microarray and miRNA-seq, low-throughput data in the original articles such as real-time PCR and northern blot, etc. were manually collected. These types of information were also integrated into database as validation of results obtained from high-throughput methods and represented separately in the database. The flowchart for data collection and database construction is shown in Supplementary Figure S1.

DATABASE CONSTRUCTION

For dbDEMC 2.0, all the data are managed by a relational database implemented with MySQL. The dynamic web interface was developed using PHP and JavaScript. The data processing and the tool modules implementation for the database were implemented by in-house R scripts. Apache was used for the http server.

DATABASE CONTENTS

In this current release, dbDEMC documents 209 miRNA expression data sets from 143 peer-reviewed publications and also those from TCGA (Supplementary Table S1). It now contains 49 202 miRNA–cancer associations for 2224

differentially expressed miRNAs identified from 436 experiments, and a total of 36 cancer types and 73 cancer subtypes were covered (Supplementary Table S2). The number of differentially expressed miRNAs in this version accounts for 86% of the miRNAs identified in the human genome (miRBase Release 21). Figure 1A illustrates the number of differentially expressed miRNAs for each cancer type. Take the colon cancer, the one with highest number of miRNAs, as an example, a total of 2116 miRNAs were identified to be differentially expressed, among which 1116 are upregulated and 1000 are downregulated. In addition, gastric cancer and pancreatic cancer are also among the top ranked cancer types. The number of miRNAs identified for breast cancer, esophageal cancer, lung cancer and hepatocellular carcinoma are also increased dramatically over the first release. The number of differentially expressed miRNAs that validated by low-throughput methods across major cancer types is depicted as Figure 1B, brain cancer, colon cancer and breast cancer are top ranked cancer types.

NEW FEATURES AND DATABASE UTILITY

Improved experimental description

For each of the microarray data sets presented, we reviewed the samples profiled and classified the experiments for differential expression as one of the following categories: cancer versus respective normal tissue, high grade cancer versus low grade cancer, metastasis versus primary cancer, subtype1 versus subtype2 (include histological subtypes comparison or molecular subtypes comparison), poor outcome versus good outcome (includes recurrence versus nonrecurrence, long-term versus short-term survival and caner-specific death versus alive), blood samples from cancer patient versus blood samples from normal person, and also drug-treatment versus non-treatment sample. After the assignment of samples to different classes, each miRNA was assessed for differential expression with the limma package. For each experiment, a detail information page was constructed to delineate the related publication reference, GEO expression profile description, experimental design, cancer type and subtypes, sample information, miRNA quantification procedure and the total number of miRNAs identified. The percentage of top ranked cancer types for all the experiments was depicted as Figure 1C, the breast cancer constitute the largest part of 13% of the total experiments, then followed by lung cancer and kidney cancer. Whereas for the experimental design, cancer vs. normal comparison and high grade versus low grade comparison account for the most of the total experiments (Figure 1D).

Database query and searching tools

We provide several ways to allow database query. First, users can perform a quick search in dbDEMC 2.0 by using miRNA names from the 'Search' page (Figure 2A). By imputing the interested miRNA name in the textbox as the keyword, the search engine will search all the items that contain the query miRNA in database. The search result page briefly lists the associated GEO ID, cancer types, subtype, experimental design, and log Fold Change between case and control samples (Figure 2B). Multiple miRNAs can be submitted at a time. Secondly, we also provide the sequence similarity search tools implemented by using BLAST, which allow user to determine whether an unknown miRNA is overlapped with the existing miRNAs by using the miRNA sequence. In addition, users can also select particular cancer type or subtype and browse all related experiments from the 'Browse' page (Figure 2C). The experiment ID, experimental design, case sample, control sample and the number of upregulated and downregulated miRNAs will be listed (Figure 2D). After the experiment list obtained, users can select particular experiment and click the 'view miRNAs' button to navigate the differentially expressed miRNA list.

Enhanced miRNA page

By clicking the hyperlink of a particular miRNA ID, users can view the detailed expression information of a specific miRNA (Figure 2E). In this updated version, the detailed expression information page has also been enhanced. This page mainly consists of four sections: miRNA Summary, Expression Profile and Expression Detail and Validation. In the 'Summary' section, miRNA ID, miRBase accession number, sequences for both mature miRNA and the precursor miRNA are listed. The hyperlinks to external databases including HUGO, Entrez gene and Eensembl are provided, In addition, this page also provides the links to predicted miRNA target databases including TargetScan (27), DIANA-microT-CDS (28) and RNA22v2 (29). The 'Expression Profile' section demonstrates six different heatmaps of the differential expression profile across six types of the experimental design. The heatmap indicates the number of experiments to support the conclusion of upregulation and downregulation in each cancer type. Here, the expression profiling heatmap for drug treatment samples analysis were not included due to the heterogeneity of different studies, such as different small molecule or various experimental conditions used. In the 'Expression Detail' section, a list of experiment ID, cancer types and subtypes, experimental design and the differential expression results getting from limma, such as the log Fold Change, t-statistics, P-value, FDR adjusted P-value, profiles of the miRNA were displayed so that the degree of deregulated information can be evaluated. In the 'Validation' section, the expression information for this miRNA retrieved from low-throughput experiments were presented if it is available.

Meta-profiling tool

dbDEMC 2.0 adds a new function of meta-profiling that allow users to draw differential expression profile for user defined miRNAs among a specific set of cancer types. Users can input a list of miRNAs, pick one of the six types of experimental designs and select the cancer types of interested (Figure 3A). The meta-profiling tool will return a heatmap depicting the expression change for queried miRNAs across multiple cancer types (Figure 3B). The up- and downregulated expression status is represented by red and green colors according to a confidence score', that is calculated as the number of studies on certain types of cancer and experimental design supporting the differential expression status.
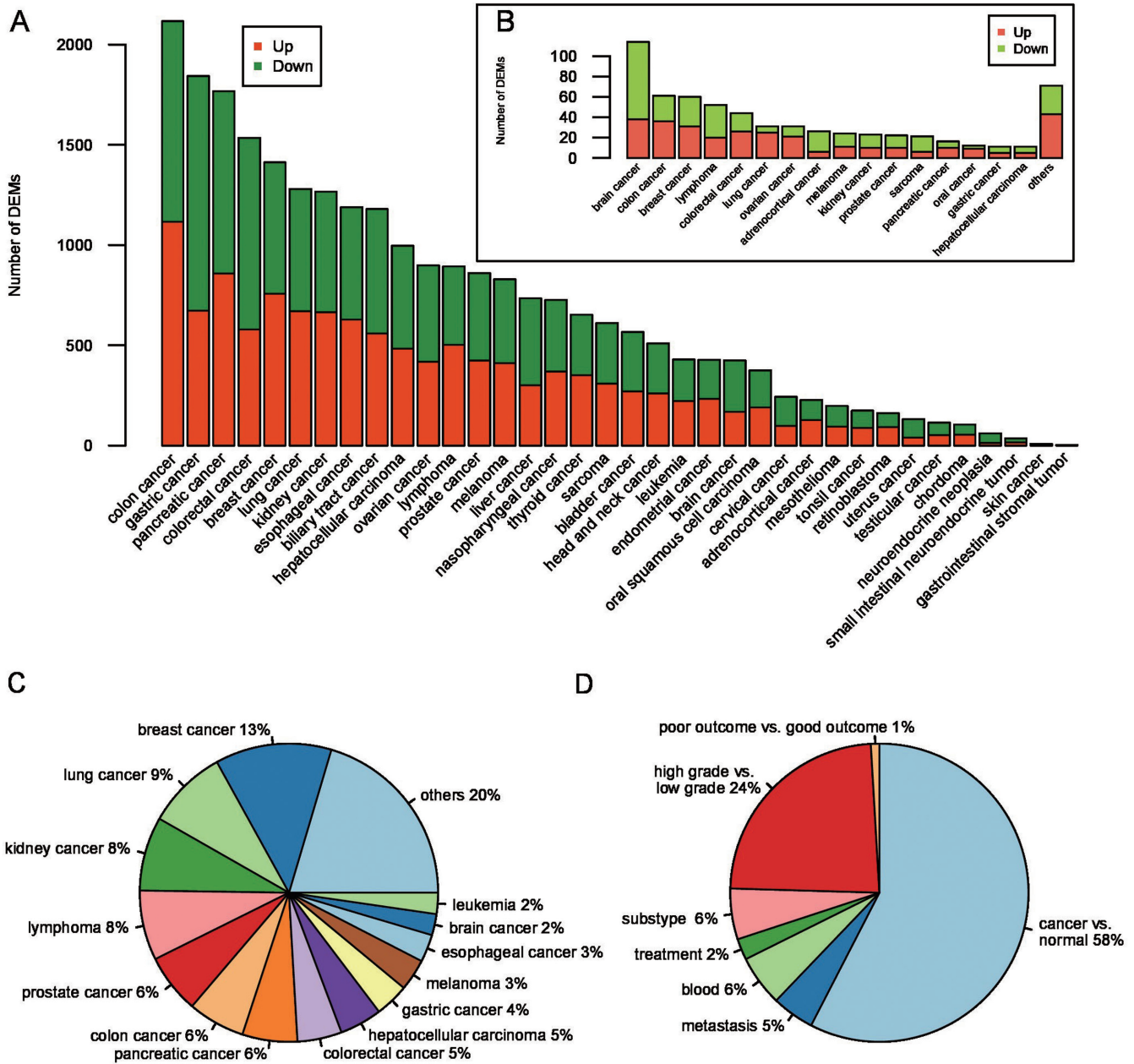
Figure 1. Data content in dbDEMC 2.0. (**A**) Number of DEMs in each cancer types identified by high-throughput methods. (**B**) Number of DEMs in major cancer types identified by low-throughput methods. (**C**) The percentage of experiment data sets in major cancer types. (**D**) The percentage of experiment data sets in seven types of experimental design.

This meta-profiling tool helps users to make a quick view of differential expression events of miRNAs from user defined cancer types.

COMPARISON TO RELATED DATABASES

Here we compared the content of dbDEMC 2.0 with cancer related databases including miR2Disease, TUMIR, HMDD, which are now available to download. We only selected the cancer associated miRNAs, then we unified the miRNA and cancer names for each database respectively. The Venn diagram for the cancer related miRNAs indicated that a great portion of the differentially expressed miR-

NAs in the dbDEMC 2.0 are newly identified, with ~27% of miRNAs shared with those of other external databases (Figure 3C). Whereas for the cancer types, 29 cancer types overlap with external databases, and seven cancers are not included by other databases (Figure 3D). This indicates db-DEMC 2.0 will be an important complement to other similar resources.

DISCUSSION

MiRNAs are widely involved in regulation of crucial signaling pathways by controlling the expression of important oncogenes in normal cells. Many studies have shown

Figure 2. A schematic workflow of dbDEMC 2.0. (**A**) Search page. miRNAs can be searched via miRBase ID, multiple miRNAs input is allowed. (**B**) miRNA list page. The page summarizes the search result that matched miRNAs in the database. (**C**) Browse page. Users can browse experiment list by selecting particular cancer type or subtype. (**D**) Experiment list page. The page summarizes the browse result that for cancers in the database. Users can select one or more experiment and click the 'view miRNAs' button to view the miRNA list. (**E**) miRNA page. This page mainly consists of four sections: miRNA Summary, Expression Profile and Expression Detail and Validation.

that aberrant expression of miRNAs plays a critical role in human cancers (30). The decreasing cost of the high-throughput methods has led to large amount of miRNA transcriptome data from cancer-related studies. This allows researchers to perform miRNA quantification analysis in cancer samples and identify cancer associated miRNAs. Here, we provide the dbDEMC 2.0 to utilize these resources and to provide a tool to facilitate the study of miRNA expression levels in cancer.

dbDEPC 2.0 now has documented much more datasets and differentially expressed miRNAs than the first version (Supplementary Figure S2). In addition to a greater number of miRNA–cancer associations included, dbDEMC 2.0 also has several advanced features that distinguish it from other sources. For instance, by studying the samples profiled in each of the collected data sets, we defined seven classes of differential expression analyses relevant to the processes of neoplastic transformation and progression. These included cancer versus respective normal tissue, high grade versus low grade samples, metastasis versus primary cancer, subtype1 versus subtype2, poor outcome versus good outcome, blood samples from cancer patient versus blood samples

from normal person, and also drug-treatment samples versus non-treatment samples. This approach provides a better way in application yet robust to the heterogeneous data formats and experimental designs for miRNA expression. For instance, circulating miRNAs have been one of the hot topics in cancer research, it has been suggested as an important class of potentially promising biomarkers in a variety of different cancers (31,32). Users can query the database by different cancer types and then filter the associated experimental design easily to check the differentially expressed miRNA list.

By analysing the data from database, we could find important miRNAs that may drive cancer development. Previous studies have shown many miRNAs present consistent differential expression pattern across cancer types. These miRNAs can cooperatively regulate oncogenic pathways and contribute to cancer hallmarks (33,34). We would like to re-assess this using the result in the database. Here, we only focus on the data from TCGA as it used high-quality miRNA-Seq method and avoid the heterogeneity from different miRNA expression platforms. We selected twelve cancer types with sufficient samples profiled, miR-

Figure 3. Meta-proofing tool and Venn-diagrams. (**A**) Meta-profiling page. Users can input a list of miRNAs, select particular cancer type and pick one type of experimental design to generate a differential expression heatmap. (**B**) An example heatmap visualize the miRNA differential expression profiles in multiple cancer types focus on cancer versus normal comparison. (**C**) Overlap of the miRNAs among dbDEMC 2.0 and other three external databases. (**D**) Overlap of the cancer types among dbDEMC 2.0 and other three external databases.

NAs present consistent up- or down-regulation across all or most cancer types are of particular interest, since these may represent candidate list of oncogenes or tumor suppressors. We identified a list of 42 miRNAs which were consistently deregulated across at least nine of the twelve cancer types, with 25 of these upregulated, and with the remaining 17 exhibiting downregulation (Supplementary Figure S3). Among the deregulated miRNAs, many have been previously demonstrated to be associated with cancers. For instance, miR-23b could function as a tumor suppressor that present downregulation in stomach cancer (35) and bladder cancer (36). Whereas the miR-130 family have been identified as upregulated in many cancer types that representing putative oncogene (37,38). This pan-cancer wide meta-profiling analysis indicated that the comprehensive investigation of the database may help to illuminate the complicated relationship between miRNAs and cancers and develop more effective treatment strategies for cancers.

In summary, dbDEMC 2.0 provides a comprehensive collection of cancer related miRNAs based on analysis of large scale expression profiling data. As the cancer related miRNA expression profiling data accumulate rapidly, this database will be updated periodically to incorporate new miRNA expression data. We hope to make dbDEMC 2.0 a useful resource that facilitate to cancer research, and contribute to the biomarker discovery or even cancer treatment related to miRNA.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

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