Published online 18 October 2018 *Nucleic Acids Research, 2019, Vol. 47, Database issue* ***D89–D93***
doi: 10.1093/nar/gky985

EVmiRNA: a database of miRNA profiling in extracellular vesicles

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Received August 14, 2018; Revised September 19, 2018; Editorial Decision October 05, 2018; Accepted October 09, 2018

ABSTRACT

Extracellular vesicles (EVs), such as exosomes and microvesicles, acted as cell-to-cell communication vectors and potential biomarkers for diseases. microRNAs (miRNAs) are the most well studied molecules in EVs, thus a comprehensive investigation of miRNA expression profiles in EVs will be helpful to explore their functions and biomarkers. We curated 462 small RNA sequencing samples of EVs from 17 sources/diseases and constructed the EVmiRNA database (http://bioinfo.life.hust.edu.cn/EVmiRNA) to show the miRNA expression profiles. We found >1000 miRNAs expressed in these EVs and detected specific miRNAs for EVs of each source/disease. EVmiRNA provides three functional modules: (i) the miRNA expression profiles and the sample information of EVs from different sources (such as blood, breast milk etc.); (ii) the specifically expressed miRNAs in different EVs that would be helpful for biomarker identification; (iii) the miRNA annotations including the miRNA expression in EVs and TCGA cancer types, miRNA pathway regulations as well as miRNA function and publications. EVmiRNA has a user-friendly web interface with powerful browse and search functions, as well as data downloading. It is the first database focusing on miRNA expression profiles in EVs and will be useful for the research and application community of EV biomarker, miRNA function and liquid biopsy.

INTRODUCTION

Extracellular vesicles (EVs) are cell-derived membranous surrounded vesicles originated from the endosomal system (exosomes) or shed from the plasma membrane (microvesicles: MVs) (1). Most cells including normal and cancer cells, can release EVs into the peripheral circulation (2). Both exosomes and MVs carry proteins, nucleic acids (mRNAs, non-coding RNAs and DNA sequences) and lipids etc. from host cells. These contents in EVs play important roles for intercellular communication and could server as potential biomarkers for special physiological/disease status (3). Tumor-derived exosomes and MVs shed light on novel microenvironment modulators, prospective cancer biomarkers for diagnosis, disease staging and assessing therapeutic responsiveness, as well as cancer vaccines and carriers of biological therapeutics (4–7). Thus, a comprehensive resource and study for the expression of important molecules in EVs will be useful for the community.

MicroRNAs (miRNAs) as important small non-coding RNAs play important roles in post-transcriptional regulation of biological processes. The expression profiles of miRNAs vary widely in normal and disease tissues, and exhibit condition-specific characteristics (8–10). Increasing evidence demonstrated that miRNAs could server as valuable pathological and therapeutic biomarkers in both cells and EVs (11–14). For example, miR-21 in the cerebrospinal fluid EVs has been proved as a platform for glioblastoma biomarker development (15). It has been reported that exosomal miR-1290 and miR-375 ware prognostic markers in castration-resistant prostate cancer (16).

High-throughput small RNA-sequencing technology brings conveniences to explore miRNAs in EVs and has generated many data for EVs from various conditions. A comprehensive survey of these data would be helpful for

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miRNA biomarker research of EVs and knowledge of regulatory roles mediated by EVs. Up to date, several resources have deposited the contents in EVs, such as Vesiclepedia (17), ExoCarta (18), EVpedia (19) and exoRBase (20). However, most of them only provide the lists of protein/RNA/lipid in exosomes and/or MVs, which were collected from publications. None of them provides the detailed miRNA expression profiles in EVs. Thus, the integration and visualization of miRNA expression profiles in exosomes and MVs is necessary and urgent for the related biomarker discovery and liquid biopsy technique development.

Here, we conducted a database named EVmiRNA, which curated and analyzed 462 miRNA expression profile datasets of EVs in 17 tissues/diseases. EVmiRNA offered valuable and comprehensive resources including EV samples classification (source/cancer and exosome/MV), miRNA expression profile for each sample, top expressed miRNAs, specifically expressed miRNAs for each EV type, as well as miRNA functions and regulations.

DATA GENERATION

Data collection and analyses

Firstly, more than 550 EV small RNA sequencing datasets were downloaded from NCBI SRA database. We classified these datasets into exosomes and MVs based on their sample introductions and isolated methods that adapted to the International Society for Extracellular Vesicles (ISEV) standards (21,22). Secondly, datasets with ambiguous description of experiment design and less raw reads were discarded. Subsequently, for each dataset, reads with the multiple N bases (>5) or unexpected length after adapter remove (>45 nt or <15 nt) or with low quality (mean quality $\leq$ 20) were filtered, and dataset with less reads (< 1M) after the procedure were discarded. Finally, 462 EV datasets were kept for further analysis, which included 225 exosome and 236 MV samples from 17 tissues and 10 cancer types (Table 1).

Clean reads were mapped to hg38 reference, and the mapped reads were re-aligned to rRNA, tRNA, snRNA, snoRNA and piRNA references to identify their prior belongings. Subsequently, the un-annotated reads above were aligned to canonical pre-miRNA sequences of homo sapiens from miRBase V21 (23) to identify known miRNAs by Bowtie (24) with 1 mismatch allowed. To better evaluate the expression profiling of mature miRNAs, the expression value of a given mature miRNA was constituted by the summation of reads from its all isoforms as RPM (expressed Reads Per Million reads). As a result, nearly 1000 miRNAs expressed in EVs, which occupied about 30% of all clean reads. About 50% of miRNAs were expressed at a very low level (RPM $\leq$ 1), whereas 14.3% of miRNAs were considered as high expression (RPM $\geq$ 100).

Identification of specifically expressed miRNAs

To support the identification of potential miRNA biomarkers in EVs, we applied the SEGtool (25) to detect specifically expressed miRNAs in 13 cancer exosome/MV categories and 21 normal tissue exosome/MV categories, and identified 755 (317 unique) specific miRNAs. Some of them have been reported as biomarkers. For example, the expression of circulating miR-21 in EVs was consistently upregulated in diffuse large B-cell lymphoma patients by a systematic review and was proposed as a biomarker for diagnosis (26). We also identified miR-21-3p as one of the specifically expressed miRNAs in lymphoma and miR-21-3p was highly specifically expressed in lymphoma.

Integration of miRNA profiling and regulatory annotation

Basic information of miRNAs were referred to miRbase (23), while the function information of miRNAs, publications and the small molecular drug's effects on miRNAs were obtained from NCBI GeneRIF, PubMed and SM2miR (27), respectively. The miRNA-Targets information was integrated experimental validated and predicted miRNA targets as our previous studies (28,29) and the procedure miRNA-pathway regulations detection was described in miR_path database (30). The expression profiling of miRNAs in each kind of EV and The Cancer Genome Atlas (TCGA) (31) cancer types was represented by the mean value of samples.

WEB INTERFACE AND CASE STUDY

Free and open-source document-oriented database MongoDB was employed for data deposition. Python FLASK-REST API frameworks were integrated with AngularJS to provide interfaces for EVmiRNA database. The EVmiRNA is free accessible for academic users via http://bioinfo.life.hust.edu.cn/EVmiRNA.

Data browse and search

Users can browse EVmiRNA by samples (Figure 1A), miRNAs and specific miRNAs. On the sample browse page, we divided samples into Exosome and MV groups. When an exosome or MV source was clicked, it will open a page to show the miRNA expression heatmap, the average expression value of each miRNA (Figure 1B), the sample summary and detailed sample information in the selected EV source. Users can search the database by miRNA ID by fuzzy match or miRNA expression level and sample sources. On the miRNA information page, we annotated the miRNA by miRNA basic information, miRNA expression (Figure 1C), miRNA function (Figure 1D), miRNA pathway regulations, miRNA target regulations, publications and the small molecular drug's effect on miRNA.

Case study

Here, we utilized an example to show the usage of EVmiRNA. B cell is an important component of the immune system. To investigate the miRNA expression profile of B-cell exosomes, we can go to the sample page by clicking the 'Sample' menu on the top of the homepage and then choose the 'B cell' icon under the exosomes section (Figure 1A). Thus, we will open the 'Exosomes from B-lymphoblastoid cell' page, which shows a heatmap for the top 40 highly expressed miRNAs in B-cell exosomes, sample summary, reads distribution, average expression of all B-cell exosome samples (Figure 1B) and sample information.

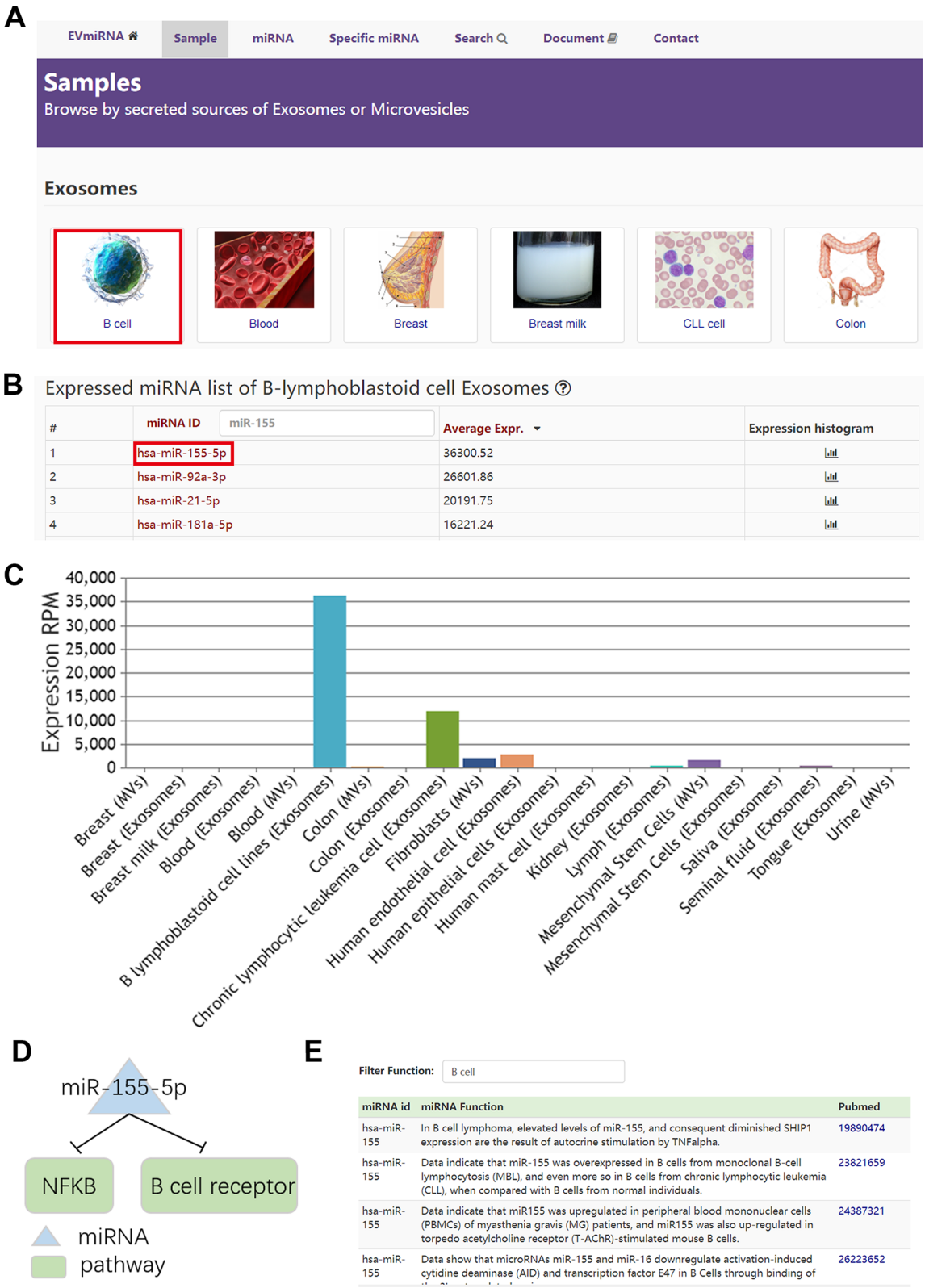

Figure 1. A case study of EVmiRNA for B-cell exosome miRNAs. (**A**) Sample browse for exosomes. (**B**) The top four highest expressed miRNA in B-cell exosomes. (**C**) The expression profile of miR-155-5p in each EV. (**D**) miRNA pathway regulation results figure out that miR-155-5p regulate NFKB signaling pathway and B cell receptor signaling pathway. (**E**) miR-155 function filtering with 'B cell

Table 1. Data summary of EV samples in EVmiRNA database

EV type	Source	Health/cancer type	No. of samples
Exosomes	Blood	Healthy control	57
	Blood	Prostate cancer	23
	B-lymphoblastoid cell lines	Healthy control	37
	Breast	Breast adenocarcinoma	3
	Breast milk	Healthy control	56
	CLL cell line	Chronic lymphocytic leukemia	2
	Colon	Colon carcinoma	11
	Human mammary epithelial cells	Breast adenocarcinoma	3
	Human mast cells	Mast cell leukemia	4
	Kidney	Healthy control	2
	Lymph	Lymphoma	7
	Mesenchymal stem sells	Healthy control	2
	Saliva	Healthy control	1
	Seminal fluid	Healthy control	6
	Tongue	Oral cancer	2
	Tongue	Squamous cell carcinoma	6
MVs	Blood	Prostate cancer	36
	Blood	Pancreatic cancer	6
	Blood	Healthy control	50
	Blood	Colon carcinoma	100
	Breast	Breast adenocarcinoma	1
	Colon	Colon carcinoma	1
	Fibroblasts	Healthy control	1
	Mesenchymal stem cells	Healthy control	3
	Mesenchymal stem cells	Chronic myelocytic leukemia	1
	Urine	Healthy control	38

Users can also download the whole miRNA expression profiles of all B-cell samples by clicking the 'Download' button on the top right of the page. Hsa-miR-155-5p is the highest expressed miRNA in B-cell exosomes (Figure 1B). By clicking the hsa-miR-155-5p, it will open the miRNA information page to show its expression in all EVs (Figure 1C) and TCGA cancer types. We can see that it is highly expressed in B-cell exosomes and two hematologic malignancies diffuse large B-cell lymphoma (DLBC) and acute myeloid leukemia (LAML)) in TCGA. Annotation in the 'miRNA pathway' section showed that hsa-miR-155-5p may regulate NFKB signaling pathway and B cell receptor signaling pathway (Figure 1D). Through filtering the miRNA function (Figure 1E) by 'B cell', the miRNA function in B cell and their publications will be shown. This example indicates that the miR-155 in B-cell exosomes may be a potential B-cell biomarker.

DISCUSSION AND FUTURE PERSPECTIVES

EVs, which carry selective cargos from host cells, are considered as a mode of intercellular communication. Interactions between recipient and host cells mediated by EVs mainly depend on the contents of EVs and uptakes of target cells. miRNAs have been considered as key and most well studied molecules in EVs (16,32–34). However, although several databases deposited inclusions of EVs, most of them only focus on the collection of the molecules in EVs without expression values. Although exoRBase (20) provides expression profiling of mRNA, lncRNA and cirRNAs in 92 exosome samples sourced from blood, the EV miRNAs profiling is still lacking. Our EVmiRNA is the first comprehensive database focusing on miRNAs profiling and the potential regulatory information in EVs. EVmiRNA offers comprehensive miRNA information of EVs, including miRNA average expression in each source type, miRNA expression abundance in each sample, top expressed miRNAs and specifically expressed miRNAs for each EV type, miRNA regulated pathways, as well as miRNA functions and publications. The experimental isolated methods and references were also included.

Finally, EVmiRNA is easy to use and is freely accessible without registration. As the expanding of EV sequencing data, we will continue to update our database by collecting more datasets about EV samples to cover more EV types and collecting the host cell samples of EVs for comparative analysis. By keep maintaining and updating, we believe the EVmiRNA will be a useful resource in the research and application community for biomarker discovery and exploration of regulatory mechanisms in EVs.

FUNDING

National Key Research and Development Program of China [2017YFA0700403]; National Natural Science Foundation of China (NSFC) [31822030, 31771458, 31801154, 31801113]; China Postdoctoral Science Foundation [2017M622455 and 2018M632830]. Funding for open access charge: NSFC [31771458].
Conflict of interest statement. None declared.

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