

1 **dbDEMC 3.0: Functional Exploration of Differentially** 2 **Expressed miRNAs in Cancers of Human and Model Organisms**

3

4 Feng Xu^{1#}, Yifan Wang^{2#}, Yunchao Ling², Chenfen Zhou², Haizhou Wang¹, Andrew
5 E. Teschendorff³, Yi Zhao⁴, Haitao Zhao⁵, Yungang He^{6*}, Guoqing Zhang^{2*}, Zhen
6 Yang^{1*}

7

8 ¹ *Center for Medical Research and Innovation of Pudong Hospital, Fudan University,*
9 *and the Shanghai Key Laboratory of Medical Epigenetics, the International*
10 *Co-laboratory of Medical Epigenetics and Metabolism, Ministry of Science and*
11 *Technology, Institutes of Biomedical Sciences, Fudan University, Shanghai 200032,*
12 *China*

13 ² *Bio-Med Big Data Center, CAS Key Laboratory of Computational Biology,*
14 *Shanghai Institute of Nutrition and Health, University of Chinese Academy of*
15 *Sciences, Chinese Academy of Sciences, Shanghai 200031, China*

16 ³ *CAS Key Lab of Computational Biology, Shanghai Institute for Nutrition and*
17 *Health, University of Chinese Academy of Sciences, Chinese Academy of Sciences,*
18 *Shanghai 200031, China*

19 ⁴ *Key Laboratory of Intelligent Information Processing, Advanced Computer*
20 *Research Center, Institute of Computing Technology, Chinese Academy of Sciences,*
21 *Beijing 100190, China*

22 ⁵ *Department of Liver Surgery, Peking Union Medical College Hospital, Chinese*
23 *Academy of Medical Sciences and Peking Union Medical College, Beijing, 100730,*
24 *China*

25 ⁶ *Shanghai Fifth People's Hospital, and the Shanghai Key Laboratory of Medical*
26 *Epigenetics, International Co-laboratory of Medical Epigenetics and Metabolism,*
27 *Ministry of Science and Technology, Institutes of Biomedical Sciences, Fudan*
28 *University, Shanghai 200032, China*

29

30 # Equal contribution.

31 * Corresponding authors.

32 E-mail: zhenyang@fudan.edu.cn (Yang Z), gqzhang@picb.ac.cn (Zhang GQ),

33 heyungang@fudan.edu.cn (He YG)

34

35 **Running title:** *Xu F et al. / a database for cancer miRNAs*

36

37 Total word counts (from “Introduction” to “Conclusions” or “Materials and
38 methods”): 3084

39 Total figures: 4

40 Total tables: 1

41 Total supplementary figures: 2

42 Total supplementary tables: 5

43 Total number of characters in the article title: 109

44 Total number of characters in the running title: 42

45 Total number of words of keywords: 5

46 Total number of words in Abstract: 191

47

48 **Abstract**

49 microRNAs (miRNAs) are important regulators in gene expression. The deregulation
50 of miRNA expression is widely reported in the transformation from physiological to
51 pathological state of cells. A large amount of differentially expressed miRNAs
52 (DEMs) have been identified in various human cancers by using high-throughput
53 technologies, such as microarray and miRNA-seq. Through mining of published
54 researches with high-throughput experiment information, the database of differentially
55 expressed miRNAs in human cancers (dbDEMC) was constructed with the aim of
56 providing a systematic resource for the storage and query of the DEMs. Here we
57 report an update of the dbDEMC to version 3.0, containing two-fold more data entries
58 than the previous version, now including also data from mouse and rat. The dbDEMC
59 3.0 contains 3,268 unique DEMs in 40 different cancer types. The current datasets for
60 differential expression analysis have expanded to 9 generalized categories. Moreover,
61 the current release integrates functional annotations of DEMs obtained from
62 experimentally validated targets. The annotations can greatly benefit integrative
63 analysis of DEMs. In summary, dbDEMC 3.0 provides a valuable resource for
64 characterizing molecular functions and regulatory mechanisms of DEMs in human
65 cancers. The dbDEMC 3.0 is freely accessible at <https://www.biosino.org/dbDEMC>.

66

67 **KEYWORDS:** miRNA; Cancer; Differential expression; Model Organisms;
68 Database

69

70

71 **Introduction**

72 Since the first discovery of microRNAs (miRNAs) at the beginning of this century,
73 this class of small non-coding RNA has received extensive attention [1]. As an
74 important gene expression regulator acting at the post-transcriptional level, studies
75 have disclosed the critical role of miRNAs in targeting mRNAs for the degradation or
76 translational repression [2]. Till now, a total of 2,654 miRNAs have been identified in
77 the human genome according to the latest version of miRBase database [3].
78 Significant researches on miRNAs have dramatically expanded our understanding
79 about gene regulatory network and its roles in physiological and pathological
80 conditions, such as in broad spectrums of biological processes including cell cycle,
81 cell proliferation, differentiation, apoptosis and cellular signaling [4, 5]. Owing to the
82 biological significance of miRNAs, alterations of the miRNAs are linked to the
83 development of many diseases including the cancer [6]. Differentially expressed
84 miRNAs (DEMs) are widely reported to hold great value in the diagnosis or prognosis
85 as well as treatment targeting for cancer research [7]. The potential use of circulating
86 miRNAs in serum, plasma and other body fluids as non-invasive cancer biomarkers
87 have also been thoroughly investigated [8].

88 Given the important functions of miRNA in cancer development, several on-line
89 resources have been built for warehousing information of cancer-related miRNAs,
90 such as the HMDD [9], miRCancer [10], and OncomiRDB [11]. With the
91 development of high-throughput techniques such as microarray and miRNA-seq, large
92 amount of cancer DEMs were identified from miRNA profiling data each year.
93 However, these valuable data were scattered in the vast literatures and it is of great
94 necessity to catalogue them in a favorable way, thus to provide integrative tools for
95 the effective utilization and systematic investigation. With this aim, we developed the
96 initial database of differentially expressed miRNAs in human cancers (dbDEMC) in
97 2010 [12] and further updated in 2017 [13]. To our knowledge, dbDEMC is the only
98 working repository currently available for storing DEMs from de-novo analysis of
99 high-throughput profiling data in human cancers, characteristic with miRNANome

100 data in various types of cancer. It greatly facilitates the efforts to excavate cancer
101 associated miRNAs and investigate their roles in pathological processes of cancer.
102 While the database could have been much more useful had there been more
103 high-quality data included.

104 In recent years, cancer quantitative miRNA profiling data has been increasing at
105 an unprecedented rate, and given the success of dbDEMC 2.0, this motivates an
106 update of this database. Here we introduce dbDEMC 3.0, a significantly expanded
107 version of this database. This update incorporates a substantial amount of new data.
108 Besides the human data, we have also incorporated the miRNA expression profiling
109 data of mouse and rat. A total of 403 datasets of high-throughput miRNA expression
110 encompassing 40 cancer-types, with the results of 807 differential expression
111 analyses, have been included. The present update is nearly doubling the data amount
112 over the previous version. In addition to the expanded data volume, the content of the
113 database has also been enriched. The new database firstly recorded the experimentally
114 validated DEMs targets and also their enrichment analysis on Gene Ontology (GO)
115 and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. In this way, we
116 provided the functional annotation of DEMs for various cancers. Also, the web
117 interface of the database was refined for a better visualization of the aforementioned
118 data. Taken together, the dbDEMC 3.0 is a comprehensive resource to systematically
119 characterize the function of DEMs in human cancers as well as other model
120 organisms.

121

122 **Data collection and processing**

123 **Data collection**

124 To compile the datasets, we used the keywords ‘cancer’, ‘tumor’, ‘carcinoma’ and
125 ‘neoplasm’, in combination with ‘microRNA’ or ‘miRNA’ to conduct an exhaustive
126 search for the microarray based miRNA expression profiles in Gene Expression
127 Omnibus (GEO) [14] and ArrayExpress [15], and further for miRNA-seq based
128 miRNA expression profiles in Sequence Read Archive (SRA) [16]. While the miRNA

129 profiles of mouse and rat were rapidly accumulating, we also incorporated the miRNA
130 data of the two model organisms in the current update. In addition, we also appended
131 the new miRNA profiling data from TCGA that was released since the last update of
132 dbDEMC 2.0. All the involved data were published as of June 2021. The data records
133 were manually reviewed and evaluated rigorously to guarantee that only high-quality
134 data sets were included. To ensure analysis reliability, we required at least three
135 biological replicates of samples each condition (for both case and control) as usual.

136 **Data processing**

137 For miRNA profiling data sets based on microarray, we used the same protocol as that
138 of dbDEMC 2.0 to identify the DEMs [13]. Briefly, the expression values were
139 logarithmically transformed (base 2) and quantile normalized. Then the limma (Linear
140 Models for Microarray Data) package was applied to select miRNAs whose mean
141 expression level is significantly different between case and control samples with FDR
142 value < 0.05 .

143 For miRNA-seq based profiling data obtained from SRA database, we
144 downloaded the SRA files of raw sequence reads and converted them into FASTQ
145 format using the fastq-dump of SRA Toolkit. Here we included only the data
146 produced on Illumina systems (Genome Analyzer I, II, Iix, HiSeq 1000, HiSeq 2000,
147 HiSeq 2500, HiSeq 4000, NextSeq, MiSeq). The involving miRNA-seq data was
148 analyzed by using QuickMIRSeq toolkit [17]. This toolkit utilizes the Cutadapt to
149 remove sequence adapters and performs quality control [18]. We collected detailed
150 information of DNA adapters of different miRNA-seq libraries from public resources
151 to guarantee that the adapters can be properly trimmed from the raw reads (Table S1)
152 [19]. The clean reads were then aligned to the reference genome by using Bowtie
153 [20], and miRDeep2 was used to obtain count tables of aligned reads for miRNA
154 quantification [21]. The reads count table was further normalized by using
155 limma-voom [22], and DEMs were then identified. For the datasets obtained from
156 TCGA, we directly used the prepared reads count data for further analysis [23].

157 Experimental validation of DEMs in low-throughput methods (such as real-time
158 PCR and northern blot, etc.) were manually collected from the original papers. These
159 types of information were carefully formatted and integrated into our update.

160 **Functional annotation**

161 For each obtained DEMs set, we collected the experimentally validated targets by
162 using multiMiR [24], which integrate miRNA target data from TarBase [25] and
163 miRTarBase [26]. Then we performed the enrichment analysis of the DEMs targets on
164 GO terms and KEGG pathways by using clusterProfiler package to facilitate the study
165 of context-dependent miRNA functional mechanisms [27]. Enriched GO terms and
166 KEGG pathways were selected where adjusted P value < 0.05. The data collection
167 and curation procedure for dbDEMC 3.0 is shown in **Figure 1**.

168 **Database construction**

169 All the data in dbDEMC 3.0 was managed by using MongoDB. The dynamic web
170 interface was developed using JSP and JavaScript. Data visualization was achieved
171 through the tools of vue, jquery, and Echarts, Elasticsearch was used for search
172 engine. The database was developed by Spring Boot framework. Apache Tomcat web
173 server was used for the http server. All the information in dbDEMC 3.0 is freely
174 available to the public domain through <https://www.biosino.org/dbDEMC>.

175 **miRNA cluster annotation**

176 A miRNA cluster is defined as set of miRNAs which located adjacent genomic
177 regions in the same or opposite orientation and not separated by other transcriptional
178 units. miRNAs within a cluster are thought to be regulated by common factors and
179 involved in same signaling pathways. According to Kabekkodu SP et al., among 1881
180 precursor miRNAs of human origin annotated in miRBase, 468 of which can be
181 attributed to 153 clusters [28]. Here we obtained these data about miRNA clusters and
182 annotated mature miRNAs by using annotation file from miRBase. Finally, a total of
183 688 (22.8%) mature miRNAs from 143 clusters were annotated in the human genome.
184 Analysis of homogeneous dysregulation pattern of miRNA clusters in cancer

185 For systematic study of co-dysregulation pattern of miRNA clusters in human
186 cancers, we considered all miRNAs associated with specific cancer. miRNAs not

187 belonging to any cluster and clusters of which at least half the members are not
188 associated with any cancer were discarded. To avoid potential bias introduced by
189 different expression platforms, here we only use the results obtained from TCGA and
190 checked for experimental design of Cancer vs. Normal for 19 kinds of epithelial
191 cancers. We finally obtained 106 unique clusters for these cancer types. A cluster was
192 designated to be homogeneous if at least half of its members show the same direction
193 of expression pattern (either up- or down-regulated). For each cluster, we computed
194 the homogeneous fraction as that of co-dysregulation throughout all cancer types
195 analyzed. A significant P-value for this fraction was calculated as follow: for each
196 cancer type, the expression of all its associated miRNAs was distributed randomly
197 within these miRNAs for 10,000 times, keeping the distribution of up- and
198 down-regulated miRNAs constant for each step. The homogeneous fraction over all
199 cancers was computed, which yields the P-value as the number of sampled
200 homogeneous fractions exceeding the original homogeneous fractions divided by
201 10,000.

202 In order to check whether clustered miRNAs are more enriched in cancer
203 development compared to single miRNA, we calculated an enrichment score of
204 log-odds (LOD) score for each cancer type:

$$205 \quad \text{LOD} = \log_2\left(\frac{X_c}{X_c+Y_c}\right) / \left(\frac{X_{all}}{X_{all}+Y_{all}}\right)$$

206 Where X_c and Y_c denote the number of cluster miRNAs and non-cluster
207 miRNAs for each cancer type, and X_{all} denotes the number of the cluster miRNAs
208 and denotes the number of the miRNAs not contained in any cluster. Here we take
209 into account all known human miRNAs annotated in the human genome, thus
210 designate as the 688 clustered miRNAs and 900 non-clustered miRNAs. In this case, a
211 positive LOD score indicates enrichment for cluster miRNAs compared to non-cluster
212 miRNAs in a specific cancer.

213

214 **Implementation and results**

215 **Database content**

216 In the current release of dbDEMC, the data of miRNA transcriptome of total 46,388
217 samples from 403 studies of human, mouse or rat were collected from public
218 resources (Table S2). These profiles are derived from 149 subtypes of 40 different
219 cancers and many different cell lines (Table S3). We then performed a systematic
220 analysis on each dataset, and yielded a total of 807 experiments for differential
221 expression analysis. dbDEMC 3.0 now hosts a total of 3,268 differentially expressed
222 miRNAs, among them, 2,584 are specific to human. A total of 160,799 miRNA
223 variations related to cancers were deposited in our database. The detailed information
224 about number of miRNAs, cancer types, datasets, and experiments for different
225 species was presented in **Table 1**.

226 **Figure 2A** depicts the number of DEMs for each type of human cancer. The
227 breast cancer presents a large number of DEMs that 1,833 up-regulated and 1,988
228 down-regulated DEMs had been identified. The number of DEMs from mouse and rat
229 can be found in Figure S1. Whereas Figure 2B demonstrates the number of DEMs
230 validated by low-throughput methods across major cancers, the brain cancer,
231 colorectal cancer and breast cancer are top ranked cancer types. Figure 2C shows the
232 percentages of experiments for top ranked cancers. The breast cancer accounted 15%
233 of the total experiments and ranked the first of the list. It was followed by colorectal
234 cancer and lung cancer. Whereas the 9 different comparisons, cancer samples vs.
235 normal controls constitutes about half of the total experiments, and followed by
236 comparisons of high-grade versus low-grade cancer samples (Figure 2D). Overall, the
237 sizes of analysis experiments and related literatures in dbDEMC 3.0 have a two-fold
238 increment by comparing with the previous version (Figure S2).

239 **New features**

240 In the dbDEMC 2.0, we assigned the different experimental designs to 7 different
241 classes: Cancer vs. Normal; High grade vs. Low grade; Metastasis vs. Primary cancer;
242 Subtype1 vs. Subtype2; Poor outcome vs. Good outcome; Blood sample of patients
243 vs. Blood sample of normal controls, and also Treatment vs. Non-treatment. In recent
244 years, many studies disclosed exosomes and microvesicles act as cell communication

245 agents, where miRNA is the most important molecular in exosomes and microvesicles
246 for regulating cancer progression [29]. In addition, circulating miRNAs were also
247 widely found in body fluids and represent a gold mine of noninvasive biomarkers in
248 cancer [30]. In this update version, we thus added these two classes of experimental
249 design: Exosome sample from patients vs. Exosome sample from control, and Body
250 fluid from patients vs. Body fluid from control. Moreover, for each DEMs set, targets
251 of miRNAs and enrichment information of the target genes in the KEGG pathways
252 and GO terms were deposited in the dbDEMC 3.0, which makes it possible for
253 inspecting functional mechanisms behind a set of miRNAs.

254 **Newly designed web interface**

255 The web interface of dbDEMC 3.0 has been significantly refined and improved,
256 allowing better use of the data. The Search page permits users to perform a quick
257 search and extract summarized information of a DEM list across cancer types. Users
258 can also specify the cancer type, experimental design or platform to select the
259 interested experiments (**Figure 3A–C**). After filtering the experimental results, users
260 can select interested experiments. Detailed information of DEM related experiment,
261 which includes description with the up-regulated and down-regulated miRNAs, can be
262 accessed. In the functional chart section, heatmap of the DMEs expression,
263 miRNA-target regulatory network for top ranked DEMs, as well as the bubble chart
264 for miRNA targets enriched KEGG and GO terms were presented (Figure 3D). Using
265 a single miRNA query, summary information of the interested miRNA can be
266 retrieved, including the general description of the interested miRNA and differential
267 expression summary heatmap which decided by number of experiments shows up
268 expression or down expression. In addition, summary statistics tables for both
269 high-throughput data analysis and low-throughput validation data were also displayed
270 (Figure 3E).

271 **Analysis tools**

272 miRBase is the central reference database for miRNA annotation by assigning names
273 and unique gene IDs for novel miRNAs. During its development, some miRNA
274 definition and annotation may have been changed. This leads to the inconsistency of

275 the miRNA IDs from different data sets, which derived from different miRBase
276 versions and making it difficult for comparing research results for integrative analysis.
277 To solve this problem, we provide a “ID convertor” in our database, by which users
278 could convert miRBase old version IDs to the latest version (v22.0) for the three
279 species of human, mouse, and rat. In addition, other analyzing tools including BLAST
280 and meta-profiling, which are used for sequence similarity search of unknown
281 miRNAs and identify the confident cancer related miRNAs in pan-cancer wide, are
282 also available in dbDEMC v3.0. For the meta-profiling study, the vote-counting
283 approach was used to calculate the consistent score of differential expression for
284 meta-analysis [31]. Common miRNAs identified in multiple cancer types with similar
285 differential expression pattern may suggest they could have similar regulatory
286 mechanisms and play important roles in cancer development.

287 **miRNA clusters are significantly overrepresented in cancers**

288 A large proportion of miRNAs localized as conserved clusters in the genome and
289 present similar expression pattern across tissues. It is critical to understand whether
290 miRNA clusters present similar differential expression across cancers and correlate
291 with the similar pathobiology. Here we obtained human miRNA cluster annotation
292 from public resources, which includes 22.8% (688/2588) of mature miRNAs appear
293 as 143 clusters of at least two members within (Table S4). We systematically
294 analyzed the homogeneity of expression patterns within miRNA clusters. We
295 excluded those clusters having less than half of all miRNAs annotated from the results
296 of TCGA, which leads to 106 remaining clusters. The clusters are denoted as
297 exhibiting a homogeneous expression pattern if annotated miRNA members are either
298 up- or down-regulated (see Data processing). In total, cancer-associated clusters
299 revealed homogeneous expression patterns for 74% of all annotated cancer, which
300 confirms the hypothesis of co-regulation pattern of miRNA clusters in cancer. For
301 example, the cluster of miR-142-5p, miR-142-3p, miR-4736 presents a consistent
302 differential expression pattern in 91% (11/12) of the cancers analyzed (Table S5). A
303 null model by randomly linking miRNA expression patterns (permutation 10,000
304 times within each cancer) indicated 52 clusters (49%, P-value < 0.05) showed a

305 significantly higher homogeneity pattern in all 19 kinds of cancer compared to that
306 expected by chance (Table S5). These clusters exhibit a homogeneous expression
307 pattern in at least 78.5% of all these types of cancer.

308 To further investigate the association of miRNA clusters with different kinds of
309 cancer, we estimated the enrichment of miRNA clusters in cancer-associated miRNAs
310 by using a LOD score. We found enrichment for all 19 kinds of cancer (**Figure 4**).
311 Within these 19 kinds of cancer, miRNAs located in clusters are, on average, 1.56
312 times (LOD = 0.65) enriched compared to random. In summary, our analyses show a
313 significant enrichment of clustered miRNAs in cancers compare to the single miRNA
314 members, which demonstrated that the different miRNAs within cluster act
315 synergistically in cancer development.

316

317 **Discussion**

318 Over the last decade, a large amount of miRNA transcriptome profiles of various
319 cancers have been generated. Many studies have performed miRNA transcriptome
320 analysis to explore the underlying molecular mechanisms of miRNA genes in cancer
321 development [32, 33]. This progress motivated a novel release of dbDEMC to keep
322 track of the latest published data. Along with this, we curate these data and provide a
323 platform to facilitate the study of miRNA-cancer associations. For dbDEMC 3.0, it
324 not only contains more miRNA-cancer associations, we also extended our database to
325 the species of mouse and rat, which benefits those studies characterizing the miRNA
326 functional machinery in cancer using the model organisms. Beyond the rapid increase
327 of data amount, our database now offers many new features and powerful tools for the
328 downstream analysis of DEMs, such as the integrated target identification and
329 functional enrichment analysis for miRNA-regulated biological processes.

330 One of the key questions of differential expression analysis of miRNAs is which
331 cancer types are regulated by a particular miRNA (miRNA-centric view), or
332 conversely, which miRNAs may involve in a given type of cancer (cancer-centric
333 view). Our databases support both miRNA- and cancer-centric investigations, users

334 are able to search miRNAs to determine the spectrum of cancer types that it involved,
335 or to find candidate miRNA list based on reference of the miRBase which link to
336 individual type of cancer. Although studies have indicated false positive and negative
337 records may exist in miRBase, researchers need to be cautious about resources based
338 on references from miRBase [34, 35]. Overall, we expect that dbDEMC 3.0 could
339 serve as a valuable resource with comprehensive data amount and data analysis tools
340 to facilitate the study of DEMs in cancers. In the future, we will continue to make
341 improvements to the functional characterizations as well as integrate more
342 heterogeneous data for the flexible analysis of miRNA functions in various cancers.
343 We believe that the development of dbDEMC database can help accelerate the
344 integration between miRNANome and cancer studies.

345

346

347 **Data availability**

348 dbDEMC v3.0 is freely accessible at <https://www.biosino.org/dbDEMC/>.

349

350 **CRedit author statement**

351 **Feng Xu:** Data curation, Formal analysis. **Yifan Wang:** Methodology, Validation.

352 **Yunchao Ling:** Visualization. **Chenfen Zhou:** Data curation. **Haizhou Wang:** Data

353 curation. **Andrew E. Teschendorff:** Methodology, Software. **Yi Zhao:** Investigation,

354 Software. **Haitao Zhao:** Validation. **Yungang He:** Supervision, Investigation.

355 **Guoqing Zhang:** Methodology, Supervision. **Zhen Yang:** Conceptualization,

356 Supervision, Writing- Original draft. All authors read and approved the final

357 manuscript.

358

359 **Competing interest**

360 The authors declare that they have no competing interests.

361

362 **Acknowledgments**

363 This work is supported by National Natural Science Foundation of China [91959106,

364 31871255, 91731310, and 81827901]; Strategic Priority Research Program of the

365 Chinese Academy of Sciences [XDB38030100]; Shanghai Municipal Science and

366 Technology [2017SHZDZX01].

367

368 **ORCID**

369 0000-0001-5185-2164 (Feng Xu)

370 0000-0003-4256-2696 (Yifan Wang)

371 0000-0002-5438-0753 (Yunchao Ling)

372 0000-0002-0294-7308 (Chenfen Zhou)

373 0000-0002-3376-386X (Haizhou Wang)

374 0000-0001-7410-6527 (Andrew E. Teschendorff)

- 375 0000-0001-6046-8420 (Yi Zhao)
- 376 0000-0002-3444-8044 (Haitao Zhao)
- 377 0000-0002-2931-2871 (Yungang He)
- 378 0000-0001-8827-7546 (Guoqing Zhang)
- 379 0000-0002-5647-9976 (Zhen Yang)
- 380

381 **References**

- 382 [1] Ambros V. microRNAs: tiny regulators with great potential. *Cell* 2001;107:823-6.
- 383 [2] Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell*
384 2004;116:281-97.
- 385 [3] Kozomara A, Birgaoanu M, Griffiths-Jones S. miRBase: from microRNA
386 sequences to function. *Nucleic Acids Res* 2019;47:D155-D62.
- 387 [4] Shivdasani RA. MicroRNAs: regulators of gene expression and cell
388 differentiation. *Blood* 2006;108:3646-53.
- 389 [5] Hwang HW, Mendell JT. MicroRNAs in cell proliferation, cell death, and
390 tumorigenesis. *Br J Cancer* 2007;96 Suppl:R40-4.
- 391 [6] Esquela-Kerscher A, Slack FJ. Oncomirs - microRNAs with a role in cancer. *Nat*
392 *Rev Cancer* 2006;6:259-69.
- 393 [7] Wang J, Chen J, Sen S. MicroRNA as Biomarkers and Diagnostics. *J Cell Physiol*
394 2016;231:25-30.
- 395 [8] Wang H, Peng R, Wang J, Qin Z, Xue L. Circulating microRNAs as potential
396 cancer biomarkers: the advantage and disadvantage. *Clin Epigenetics* 2018;10:59.
- 397 [9] Huang Z, Shi J, Gao Y, Cui C, Zhang S, Li J, et al. HMDD v3.0: a database for
398 experimentally supported human microRNA-disease associations. *Nucleic Acids Res*
399 2019;47:D1013-D7.
- 400 [10] Xie B, Ding Q, Han H, Wu D. miRCancer: a microRNA-cancer association
401 database constructed by text mining on literature. *Bioinformatics* 2013;29:638-44.
- 402 [11] Wang D, Gu J, Wang T, Ding Z. OncomiRDB: a database for the experimentally
403 verified oncogenic and tumor-suppressive microRNAs. *Bioinformatics*
404 2014;30:2237-8.
- 405 [12] Yang Z, Ren F, Liu C, He S, Sun G, Gao Q, et al. dbDEMC: a database of
406 differentially expressed miRNAs in human cancers. *BMC Genomics* 2010;11 Suppl
407 4:S5.

- 408 [13] Yang Z, Wu L, Wang A, Tang W, Zhao Y, Zhao H, et al. dbDEMC 2.0: updated
409 database of differentially expressed miRNAs in human cancers. *Nucleic Acids Res*
410 2017;45:D812-D8.
- 411 [14] Barrett T, Wilhite SE, Ledoux P, Evangelista C, Kim IF, Tomashevsky M, et al.
412 NCBI GEO: archive for functional genomics data sets--update. *Nucleic Acids Res*
413 2013;41:D991-5.
- 414 [15] Athar A, Fullgrabe A, George N, Iqbal H, Huerta L, Ali A, et al. ArrayExpress
415 update - from bulk to single-cell expression data. *Nucleic Acids Res*
416 2019;47:D711-D5.
- 417 [16] Kodama Y, Shumway M, Leinonen R, International Nucleotide Sequence
418 Database C. The Sequence Read Archive: explosive growth of sequencing data.
419 *Nucleic Acids Res* 2012;40:D54-6.
- 420 [17] Zhao S, Gordon W, Du S, Zhang C, He W, Xi L, et al. QuickMIRSeq: a pipeline
421 for quick and accurate quantification of both known miRNAs and isomiRs by jointly
422 processing multiple samples from microRNA sequencing. *BMC Bioinformatics*
423 2017;18:180.
- 424 [18] Chen C, Khaleel SS, Huang H, Wu CH. Software for pre-processing Illumina
425 next-generation sequencing short read sequences. *Source Code Biol Med* 2014;9:8.
- 426 [19] Zhong X, Heinicke F, Lie BA, Rayner S. Accurate Adapter Information Is
427 Crucial for Reproducibility and Reusability in Small RNA Seq Studies. *Noncoding*
428 *RNA* 2019;5.
- 429 [20] Langmead B, Trapnell C, Pop M, Salzberg SL. Ultrafast and memory-efficient
430 alignment of short DNA sequences to the human genome. *Genome Biol* 2009;10:R25.
- 431 [21] Friedlander MR, Mackowiak SD, Li N, Chen W, Rajewsky N. miRDeep2
432 accurately identifies known and hundreds of novel microRNA genes in seven animal
433 clades. *Nucleic Acids Res* 2012;40:37-52.
- 434 [22] Law CW, Chen Y, Shi W, Smyth GK. voom: Precision weights unlock linear
435 model analysis tools for RNA-seq read counts. *Genome Biol* 2014;15:R29.

- 436 [23] Chu A, Robertson G, Brooks D, Mungall AJ, Birol I, Coope R, et al. Large-scale
437 profiling of microRNAs for The Cancer Genome Atlas. *Nucleic Acids Res*
438 2016;44:e3.
- 439 [24] Ru Y, Kechris KJ, Tabakoff B, Hoffman P, Radcliffe RA, Bowler R, et al. The
440 multiMiR R package and database: integration of microRNA-target interactions along
441 with their disease and drug associations. *Nucleic Acids Res* 2014;42:e133.
- 442 [25] Karagkouni D, Paraskevopoulou MD, Chatzopoulos S, Vlachos IS, Tastsoglou S,
443 Kanellos I, et al. DIANA-TarBase v8: a decade-long collection of experimentally
444 supported miRNA-gene interactions. *Nucleic Acids Res* 2018;46:D239-D45.
- 445 [26] Huang HY, Lin YC, Li J, Huang KY, Shrestha S, Hong HC, et al. miRTarBase
446 2020: updates to the experimentally validated microRNA-target interaction database.
447 *Nucleic Acids Res* 2020;48:D148-D54.
- 448 [27] Yu G, Wang LG, Han Y, He QY. clusterProfiler: an R package for comparing
449 biological themes among gene clusters. *OMICS* 2012;16:284-7.
- 450 [28] Kabekkodu SP, Shukla V, Varghese VK, J DS, Chakrabarty S, Satyamoorthy K.
451 Clustered miRNAs and their role in biological functions and diseases. *Biol Rev Camb*
452 *Philos Soc* 2018;93:1955-86.
- 453 [29] Sun Z, Shi K, Yang S, Liu J, Zhou Q, Wang G, et al. Effect of exosomal miRNA
454 on cancer biology and clinical applications. *Mol Cancer* 2018;17:147.
- 455 [30] Cortez MA, Bueso-Ramos C, Ferdin J, Lopez-Berestein G, Sood AK, Calin GA.
456 MicroRNAs in body fluids--the mix of hormones and biomarkers. *Nat Rev Clin*
457 *Oncol* 2011;8:467-77.
- 458 [31] Guan P, Yin Z, Li X, Wu W, Zhou B. Meta-analysis of human lung cancer
459 microRNA expression profiling studies comparing cancer tissues with normal tissues.
460 *J Exp Clin Cancer Res* 2012;31:54.
- 461 [32] Ueda T, Volinia S, Okumura H, Shimizu M, Taccioli C, Rossi S, et al. Relation
462 between microRNA expression and progression and prognosis of gastric cancer: a
463 microRNA expression analysis. *Lancet Oncol* 2010;11:136-46.

464 [33] Iorio MV, Croce CM. MicroRNA dysregulation in cancer: diagnostics,
465 monitoring and therapeutics. A comprehensive review. *EMBO Mol Med*
466 2012;4:143-59.

467 [34] Fromm B, Keller A, Yang X, Friedlander MR, Peterson KJ, Griffiths-Jones S.
468 Quo vadis microRNAs? *Trends Genet* 2020;36:461-3.

469 [35] Alles J, Fehlmann T, Fischer U, Backes C, Galata V, Minet M, et al. An estimate
470 of the total number of true human miRNAs. *Nucleic Acids Res* 2019;47:3353-64.

471

472

473 **Figure legends**

474 **Figure 1 Schematic illustration of the data collection and architecture of the**
475 **dbDEMC 3.0**

476

477 **Figure 2 Statistics of data content in dbDEMC 3.0 for humans**

478 **A.** Number of DEMs from each cancer types identified by high-throughput methods.

479 **B.** Number of DEMs from major cancer types identified by low-throughput methods.

480 **C.** The percentage of experiments for major cancer types. **D.** The percentage of
481 experiments in seven types of experimental design.

482

483 **Figure 3 Web interface of dbDEMC 3.0**

484 **A.** Search page. miRNAs can be searched via miRBase IDs, or filter experiments with
485 interested conditions. **B.** Filtering result page of experiments. **C.** Search result page

486 with example miRNAs. **D.** Experiment page. The page summarizes the description of
487 the experiments and associated differentially expressed miRNA list, functional chart

488 for expression heatmap, regularly network and miRNA targets enriched KEGG
489 pathways and GO terms are also depicted. **E.** miRNA page. This page mainly consists

490 of four sections: miRNA Summary, Expression Profile and Expression Detail and
491 Validation.

492

493 **Figure 4 miRNA cluster enrichment for 19 kinds of cancer**

494 For each cancer type, the log-odds (LOD) score is plotted. There is an enrichment of
495 miRNA cluster members for all 19 kinds of cancer (100%, P-value < 1e-4). Within
496 these 19 types of cancer, miRNAs located in clusters are, on average, 1.56 times
497 (LOD = 0.65) enriched compared to random.

498

499 **Tables**

500 **Table 1 Summary of the data content of the current release of dbDEMC**

501

502

503 **Supplementary material**

504 **Figure S1 Number of differentially expressed miRNAs identified by**
505 **high-throughput methods for each cancer type. A. Number of DEMs for mouse; B.**
506 **Number of DEMs for rat.**

507

508 **Figure S2 Increasing number of experiments for each cancer type.** The number
509 of experiments for each cancer type in dbDEMC v3.0 and v2.0 are depicted.

510

511 **Table S1 Adapters for miRNA-seq kits for the Illumina platform.**

512

513 **Table S2 The table below lists datasets collected from public resources,**
514 **including the GEO, ArrayExpress, SRA and TCGA.** The Source Data ID, PubMed
515 ID, Species, Cancer Type, platform ID and the total number of samples for each
516 dataset were listed.

517

518 **Table S3 Cancer types and associated subtypes and cell line names covered in**
519 **dbDEMC 3.0.**

520

521 **Table S4 All miRNA clusters and associated miRNA IDs identified.** A total of
522 143 miRNA clusters and associated 688 human mature miRNAs were obtained in the
523 human genome.

524

525 **Table S5 miRNA clusters showing a homogenous expression pattern in all 19**
526 **kinds of cancer. T:** number of cancers for which each cluster showing a
527 homogeneous expression pattern; **F:** number of diseases for which each cluster shows
528 no homogeneous expression pattern; **Homogeneous-fraction:** number of diseases for
529 which each cluster shows a homogeneous expression pattern for each miRNA cluster
530 as defined; **P-value:** estimate by randomly linking miRNA-expression patterns 10,000
531 times within each cancer.

532

Collecting miRNA Profiling Data From Public Resources

GEO, ArrayExpress, SRA, TCGA

Data Preprocessing

microarray

- Probe mapping
- Data imputation
- Quantile normalization

miRNA-seq

- Adapter trimming
- Reads mapping
- miRNA quantification

Data Analysis

- Experiment condition annotation
- Sample curation
- Differential expression analysis
- ID conversion
- Target identification
- Regulatory network
- Functional annotation

Low-throughput
validation data

dbDEMC
v3.0

presentation

miRBase NCBI Ensembl HUGO MGI RGD TarBase miRTarBase KEGG GO

Sample Info Clinical Info Tumor Stage Tumor Grade Metastasis
Treatment Vital Status Overall Survival

Search & Browse

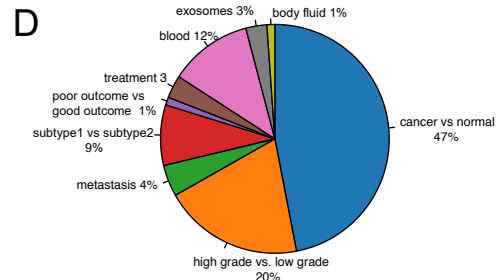
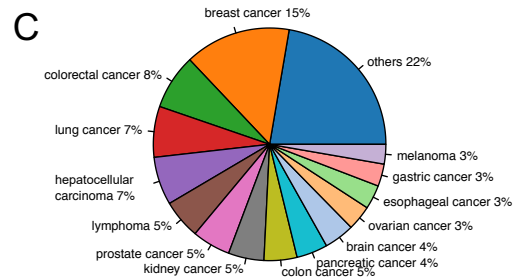
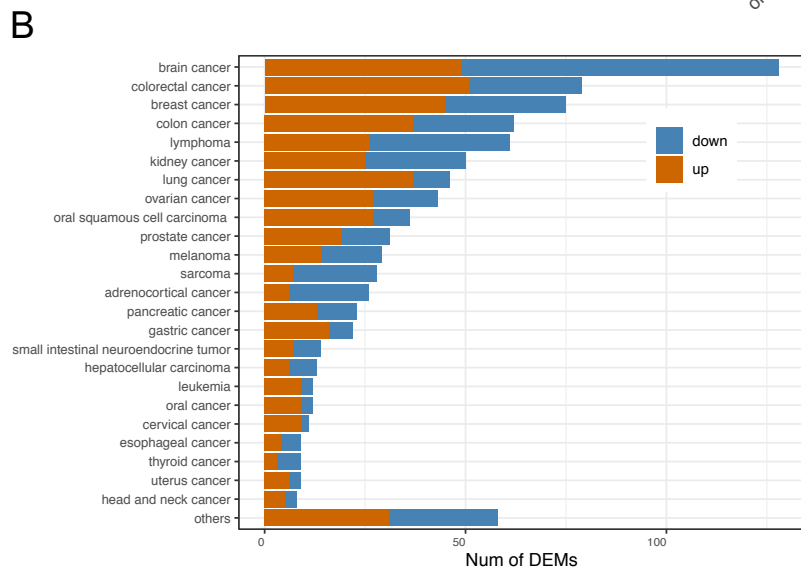
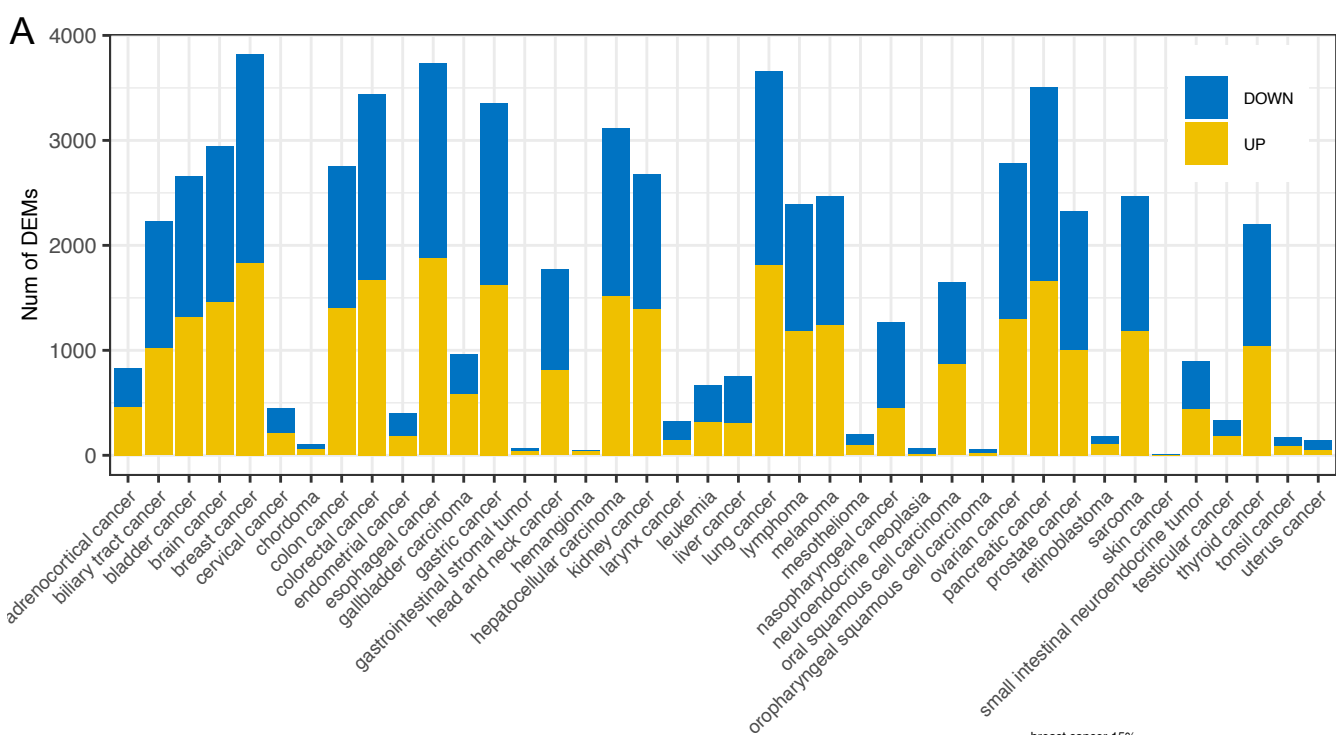
- miRNA identifier
- Cancer types
- Platforms
- Experimental design

Visualization

- Differential expression miRNAs
- Expression heatmap
- Regulatory network
- Gene Ontology terms
- KEGG terms
- miRNA expression summarization

Analysis Tools

- Blast
- Id convertor
- Meta-profiling



User Interface

A Search miRNA ID

Input: hsa-miR-122a, hsa-miR-133a

Buttons: Submit, Reset

Search experiment

Filters: Cancer Type, Platform, Experimental Design, Sample Type

Buttons: Submit, Reset

B Experiment List

Please select conditions to filter the experiment results.

Experiment ID	Cancer Type	Cancer Subtype	Design	Sample Case	Sample Control	Up	Down	Download
EXP00001	lymphoma	B cell lymphoma	cancer vs normal	OCI-L4	Normal B cell	55	53	▲
EXP00002	lymphoma	B cell lymphoma	cancer vs normal	OCI-L7	Normal B cell	48	51	▲
EXP00003	lymphoma	B cell lymphoma	cancer vs normal	OCI-L6	Normal B cell	36	35	▲
EXP00004	lymphoma	Burkitt's lymphoma	cancer vs normal	Namawa	Normal B cell	55	47	▲
EXP00005	lymphoma	Burkitt's lymphoma	cancer vs normal	Raj	Normal B cell	46	45	▲
EXP00006	lymphoma	Spleenic lymphoma	cancer vs normal	Karpas-1718	Normal B cell	51	45	▲
EXP00007	lymphoma	Gastric lymphoma	cancer vs normal	Mexico	Normal B cell	54	47	▲
EXP00008	lymphoma	Lymphoblastic	cancer vs normal	HG-1275	Normal B cell	47	43	▲
EXP00009	lymphoma	T cell leukemia	cancer vs normal	Jurkat	Normal B cell	42	35	▲
EXP00010	lymphoma	B cell lymphoma	subtype1 vs subtype2	OCI-L4	OCI-L7	20	14	▲
EXP00011	lymphoma	B cell lymphoma	subtype1 vs subtype2	OCI-L4	OCI-L6	18	20	▲
EXP00012	lymphoma	B cell lymphoma	subtype1 vs subtype2	OCI-L7	OCI-L6	8	16	▲
EXP00013	lymphoma	Spleenic lymphoma	subtype1 vs subtype2	Karpas-1718	OCI-L6	11	15	▲
EXP00014	lymphoma	Spleenic lymphoma	subtype1 vs subtype2	Karpas-1718	Mexico	14	16	▲
EXP00015	lymphoma	Gastric lymphoma	subtype1 vs subtype2	Mexico	OCI-L4	7	13	▲

Total rows: 807, page size: 15, skip to: page 54

C miRNA List

Differentially Expressed miRNAs List

miRNA ID	Source ID	Cancer Type	Cancer Subtype	Cell Line	Design	logFC	Expression Status	Experiment ID
hsa-miR-122a	GSE18188	esophageal cancer	esophageal cancer	NA	cancer vs normal	-2.36	DOWN	EXP00012
hsa-miR-133a	GSE5635	prostate cancer	prostate cancer	NA	cancer vs normal	-4.09	DOWN	EXP00012
hsa-miR-133a	GSE30259	colorectal cancer	colorectal cancer	NA	cancer vs normal	-2.41	DOWN	EXP00047
hsa-miR-133a	GSE30259	colorectal cancer	colorectal cancer	NA	cancer vs normal	-3.18	DOWN	EXP00049
hsa-miR-133a	GSE30259	colorectal cancer	colorectal cancer	NA	cancer vs normal	-2.12	DOWN	EXP00050
hsa-miR-133a	GSE18192	ovarian cancer	ovarian cancer	NA	cancer vs normal	-1.22	DOWN	EXP00090
hsa-miR-133a	GSE22018	hepatocellular carcinoma	hepatocellular carcinoma	NA	cancer vs normal	-0.22	DOWN	EXP00010
hsa-miR-133a	GSE11316	prostate cancer	prostate cancer	NA	cancer vs normal	-1.55	DOWN	EXP00011
hsa-miR-133a	GSE11036	prostate cancer	prostate cancer	NA	metastasis	-3.12	DOWN	EXP00010
hsa-miR-133a	GSE16416	esophageal cancer	esophageal cancer	NA	cancer vs normal	1.15	UP	EXP00075
hsa-miR-133a	GSE2564	leukemia	leukemia	NA	subtype1 vs subtype2	0.37	UP	EXP00016
hsa-miR-133a	GSE4849	breast cancer	breast cancer	NA	cancer vs normal	-0.57	DOWN	EXP00020
hsa-miR-133a	GSE16025	lung cancer	lung cancer	NA	cancer vs normal	-0.54	DOWN	EXP00071
hsa-miR-133a	GSE16025	lung cancer	lung cancer	NA	cancer vs normal	-0.50	DOWN	EXP00072
hsa-miR-133a	GSE16025	lung cancer	lung cancer	NA	cancer vs normal	-0.62	DOWN	EXP00073

Total rows: 234, page size: 15, skip to: page 16

D Experiment Description

Reference: Wang S, Thompson JF, Hironaka M, ...

Differentially Expressed miRNA list

miRNA ID	Cancer Type	Design	logFC	AveExpr	T value	P value	adj P value	Status	Plot
hsa-miR-106a	lymphoma	cancer vs normal	2.89	5.20	14.56	4.35e-17	4.02e-15	UP	▲
hsa-miR-7g	lymphoma	cancer vs normal	-3.58	1.77	-14.49	4.92e-17	4.02e-15	DOWN	▲
hsa-miR-17-5p	lymphoma	cancer vs normal	3.12	4.54	14.38	6.33e-17	4.02e-15	UP	▲
hsa-miR-7f	lymphoma	cancer vs normal	-4.01	3.21	-13.61	3.66e-16	1.74e-14	DOWN	▲
hsa-miR-7a	lymphoma	cancer vs normal	-2.86	2.59	-13.45	5.27e-16	2.00e-14	DOWN	▲
hsa-miR-150	lymphoma	cancer vs normal	-5.20	0.20	-12.96	1.70e-15	5.37e-14	DOWN	▲
hsa-miR-9	lymphoma	cancer vs normal	3.70	-0.05	12.13	1.28e-14	3.48e-13	UP	▲
hsa-miR-20	lymphoma	cancer vs normal	2.75	4.40	11.82	2.82e-14	6.75e-13	UP	▲
hsa-miR-92	lymphoma	cancer vs normal	2.56	2.32	11.37	8.85e-14	1.87e-12	UP	▲
hsa-miR-150a	lymphoma	cancer vs normal	2.74	2.04	11.24	1.06e-13	1.66e-11	UP	▲
hsa-miR-7a	lymphoma	cancer vs normal	-3.09	2.40	-11.24	1.06e-13	1.66e-11	DOWN	▲
hsa-miR-7b	lymphoma	cancer vs normal	-2.09	2.02	-10.91	1.32e-13	1.96e-11	DOWN	▲
hsa-miR-10b	lymphoma	cancer vs normal	2.39	1.51	10.82	1.32e-13	1.96e-11	UP	▲
hsa-miR-30b	lymphoma	cancer vs normal	-1.12	-2.35	-10.72	1.32e-13	1.96e-11	DOWN	▲
hsa-miR-1	lymphoma	cancer vs normal	-2.72	-1.77	-10.72	1.32e-13	1.96e-11	DOWN	▲

Functional Charts

E miRNA Detail

miRNA ID: hsa-miR-106a

miRBase ID: MI00000203

miRBase Accession: MIMAT0000203

miRNA Sequence: AAAAGUCGUUACAGUGCCAGGUAG

Precursor miRNA ID: MIM000113

Precursor miRNA Accession: MIM000113

Precursor miRNA sequence: CCUGGCCUUGUUAUACAUUACCAUGG

Organism: Homo sapiens

Genomic Location: chrX:134370244-134

Other Database Links: HNGC, 31496, Ensembl, TargetScan, DIANA

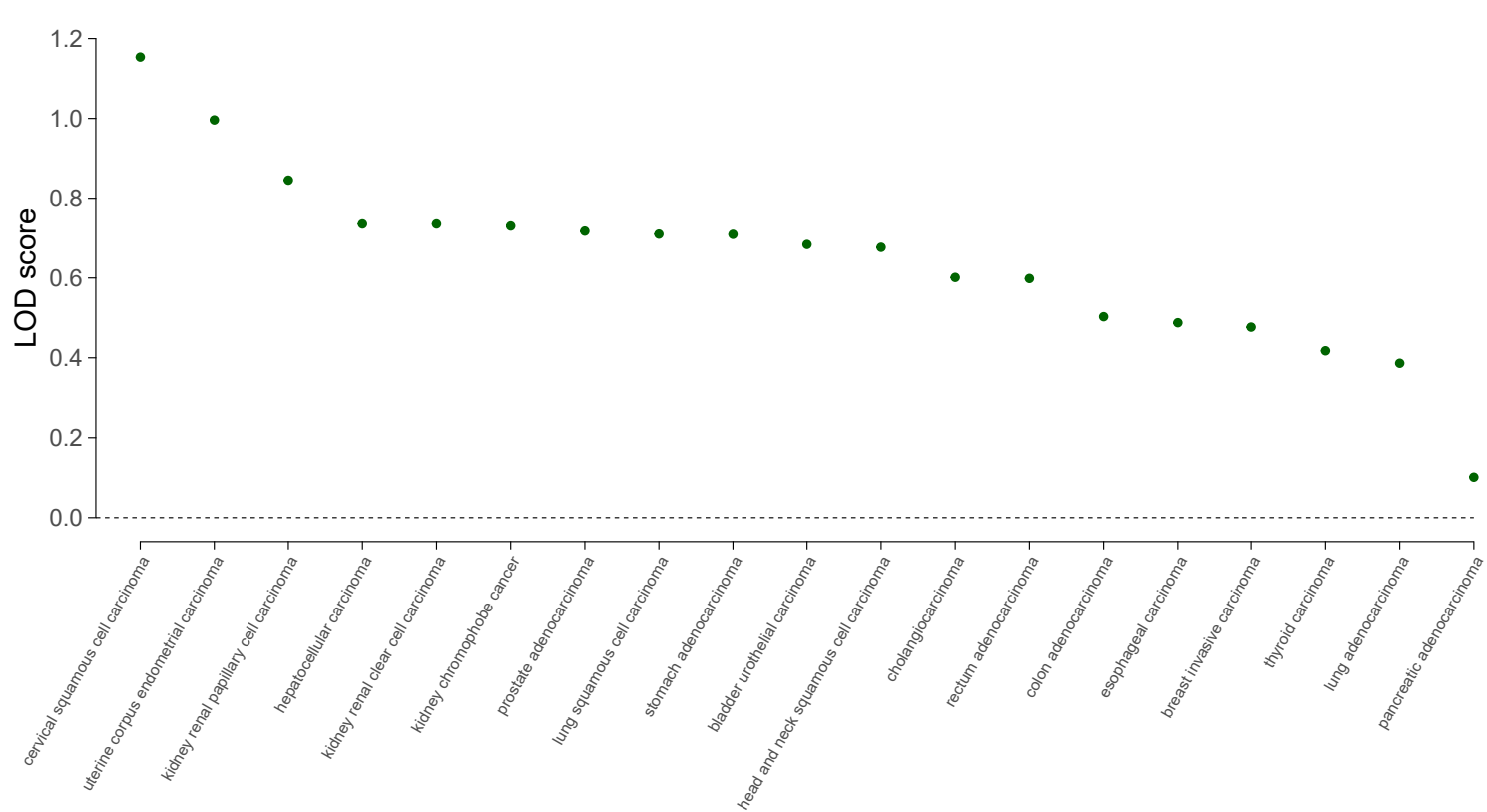
Expression Chart

Comparison: Cancer vs. Normal

High Grade vs. Low Grade, Metastasis, Subtype1 vs. Subtype2, Poor Outcome vs. Good Outcome, Blood

Validation Information

Cancer type	tumor subtype or cell line	Design	platform	status	PubMed ID
lymphoma	NA	cancer vs normal	QRT-PCR	up	18230780
brain cancer	meningioma	cancer vs normal	QRT-PCR	up	19703993
lung cancer	squamous cell carcinoma	cancer vs normal	QRT-PCR	up	19584273
colorectal cancer	colon adenocarcinoma/tumor/stolon adenomas	cancer vs normal	QRT-PCR	up	18230780



Status DOWN UP



DOWN



UP

