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CircNet 2.0: an updated database for exploring circular RNA regulatory networks in cancers

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ABSTRACT

Circular RNAs (circRNAs), which are single-stranded RNA molecules that have individually formed into a covalently closed continuous loop, act as sponges of microRNAs to regulate transcription and translation. CircRNAs are important molecules in the field of cancer diagnosis, as growing evidence suggests that they are closely related to pathological cancer features. Therefore, they have high potential for clinical use as novel cancer biomarkers. In this article, we present our updates to CircNet (version 2.0), into which circRNAs from circAtlas and MiOncoCirc, and novel circRNAs from The Cancer Genome Atlas database have been integrated. In total, 2732 samples from 37 types of cancers were integrated into CircNet 2.0 and analyzed using several of the most reliable circRNA detection algorithms. Furthermore, target miRNAs were predicted from the full-length circRNA sequence using three reliable tools (PITA, miRanda and TargetScan). Additionally, 384 897 experimentally verified miRNA–target interactions from miRTarBase were integrated into our database to facilitate the construction of high-quality circRNA–miRNA–gene regulatory networks. These improvements, along with the user-friendly interactive web interface for data presentation, search, and visualization, showcase the updated CircNet database as a powerful, experimentally validated resource, for providing strong data support in the biomedical fields. CircNet 2.0 is currently accessible at https://awi.cuhk.edu.cn/~CircNet.

INTRODUCTION

Circular RNAs (circRNAs) are single-stranded RNA molecules that have individually formed into a covalently closed continuous loop. Compared with linear RNAs, circRNAs do not have 5′ and 3′ ends and are therefore resistant to exonuclease-mediated degradation, making them highly stable molecules (1). Studies have indicated that circRNAs act as regulators of gene expression by 'sponging' microRNAs (miRNAs) and RNA-binding proteins, and thereby influencing transcription and translation processes (2). CircRNAs have been shown to play a significant role in the diagnosis of various diseases such as Alzheimer's disease, osteoporosis, osteoarthritis, and cardiovascular diseases (3–6). Several studies have focused on the association of circRNAs with the pathological and clinical aspects of malignant diseases and highlighted their roles in oncogenic pathways, demonstrating their potential application as diagnostic biomarkers of cancer (7–9). The high abundance and stability of circRNAs and their tissue-specific expression frameworks and wide distribution in body vessels render them as promising targets for drug development (5,7,10,11).

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Owing to the significance of circRNAs in eukaryotic cells in various biological and clinical aspects, the first version of CircNet database was published in January 2016, focusing on tissue-specific expression profiles and regulatory networks among circRNAs, miRNAs and genes (12). Since then, the database has accumulated data from over 200 research studies that have referred to CircNet annotations, especially those on regulatory networks of circRNAs in a wide array of cancers. For the updated version 2.0, we processed raw RNA-Seq data of six cancer types from The Cancer Genome Atlas (TCGA) database to detect novel circRNAs. To the best of our knowledge, CircNet 2.0 is the first database that includes circRNAs detected from TCGA samples. CircNet 2.0 also contains circRNA data from several online resources, such as circAtlas, a database that contains a collection of expression patterns and comprehensive annotations of highly reliable circRNAs derived from >1000 RNA-Seq samples (13). Another resource, MiOncoCirc, provides circRNA profiles that have been directly detected and captured in over 1000 human cancer tissue samples, using the exome capture RNA-Seq protocol (14). Through this update, CircNet 2.0 now encompasses over 2732 samples from 37 types of human cancers, providing a rich resource for cancer exploration and biological research.

To ensure the accuracy of the circRNAs obtained and the precise construction of the circRNA–miRNA–gene networks, the circRNA data were analyzed using a series of reliable bioinformatics algorithms for their identification and annotation. CIRI2 uses an adapted maximum-likelihood estimation based on multiple seed matching to accurately identify back-spliced circRNA reads and reduce false-positive estimations, achieving high sensitivity and reliability (15,16). The CIRCexplorer2 pipeline, upgraded from the original CIRCexplorer, enables the annotation of alternative back-splicing and splicing events in circRNAs and can reveal novel back-spliced or spliced exons with the *de novo* transcript assembly (17). We also applied circRNA detection tools such as Find_circ and DCC, which integrate a series of filters and data across replicate sets to harvest a list of precise circRNA candidates (18,19). By combining these algorithms in CircNet 2.0, we have enhanced this circRNA processing and data-incorporating pipeline. To build a reliable circRNA-miRNA-gene regulatory network, we annotated the circRNA data with full-length sequences by CIRI-full and then used PITA, miRanda and TargetScan to predict the miRNA interactions (20–23). Additionally, we integrated 384,897 experimentally verified miRNA–target interactions (MTIs) from miRTarBase, one of the most comprehensively annotated and experimentally validated MTI databases. miRTarBase provides comprehensive information on upstream and downstream regulatory targets of miRNAs, and its latest version contains more than 13,404 validated MTIs from 11 021 manually curated articles (24).

In the years since the publication of CircNet 1.0, the field of circRNA research has flourished. Many researchers have focused on circRNA-miRNA-gene regulatory networks in cancer research, and circRNAs are now considered as potential biomarkers of malignant diseases. The updated CircNet 2.0 aims to provide a more comprehensive resource of circRNA data derived from thousands of cancer samples and to allow the detection of novel circRNA from raw cancer data using reliable state-of-the-art algorithms. Furthermore, we have developed a novel pipeline for the construction of circRNA–miRNA-gene networks of high quality. We also built a more user-friendly web interface with comprehensive functionalities, to enhance user experience of the database.

DATA COLLECTION AND PROCESSING

Compared with CircNet 1.0, CircNet 2.0 has both novel and a larger number of integrated and processed data as well as a brand new network construction pipeline, as shown in Figure 1. In CircNet 2.0, we have integrated high-quality cancer-related circRNAs of the human species with their basic annotations and circRNA–miRNA interaction networks from circAtlas. We also integrated all the circRNAs registered on MiOncoCirc along with their basic annotation and expression levels. To ensure data integrity, we reprocessed a number of circRNAs that were missing parts of their annotation information and filtered out all irreparable data. All integrated samples can be accessed through the Gene Expression Omnibus (GEO) database (25). Basic information on all the integrated circRNAs was obtained using one of the following four bioinformatics algorithms: CIRI2, CIRCexplorer2, find_circ, and DCC. All circRNA–miRNA interactions were predicted using three prediction tools: PITA, miRanda and TargetScan (20–22). To enrich the circRNA information in the cancer area, we also collected the controlled raw RNA-Seq data from TCGA. Although we had accumulated over 100 TB of sequencing data from 37 cancer types registered on TGCA, the limitation of computing resources at our institute allowed us to process only the top six most important cancer types for finding novel related circRNAs; namely, breast cancer (breast invasive carcinoma), lung cancers (lung adenocarcinoma, lung squamous cell carcinoma), colon and rectal cancers (colon adenocarcinoma, rectum adenocarcinoma), and leukemia (acute myeloid leukemia). After obtaining basic information along with the full-length sequences of circRNAs in each sample, we predicted the circRNA–miRNA interactions using the three tools mentioned above. Suitable energy cut-off was set for each tool to filter the high quality circRNA–miRNA interactions prediction results. The circRNA–miRNA interactions were clustered into three groups, respectively correspond to the number of tools successfully predicted such interaction. Overall, we integrated 289 303 circRNAs from 2732 cancer samples covering 37 types of cancers into our database. For each circRNA, details on its genomic position, strand, host gene, full-length sequence, expression counts and interactions with miRNAs are provided. The circRNA nomenclature in CircNet 2.0 follows a similar rule to that of MiOncoCirc and Cancer-Specific CircRNA Database 2.0 (26), where the human genome assembly GRCh38 (hg38) position of the circRNA is used directly as its name and identity (ID).

To facilitate the construction of circRNA–miRNA–gene regulatory networks of high quality, we have integrated three databases of miRNAs and genes into CircNet 2.0. The latest release of miRBase (v22) contains miRNA sequences from 271 organisms, 38 589 hairpin precursors and 48 860 mature miRNAs (27). We incorporated all human mature

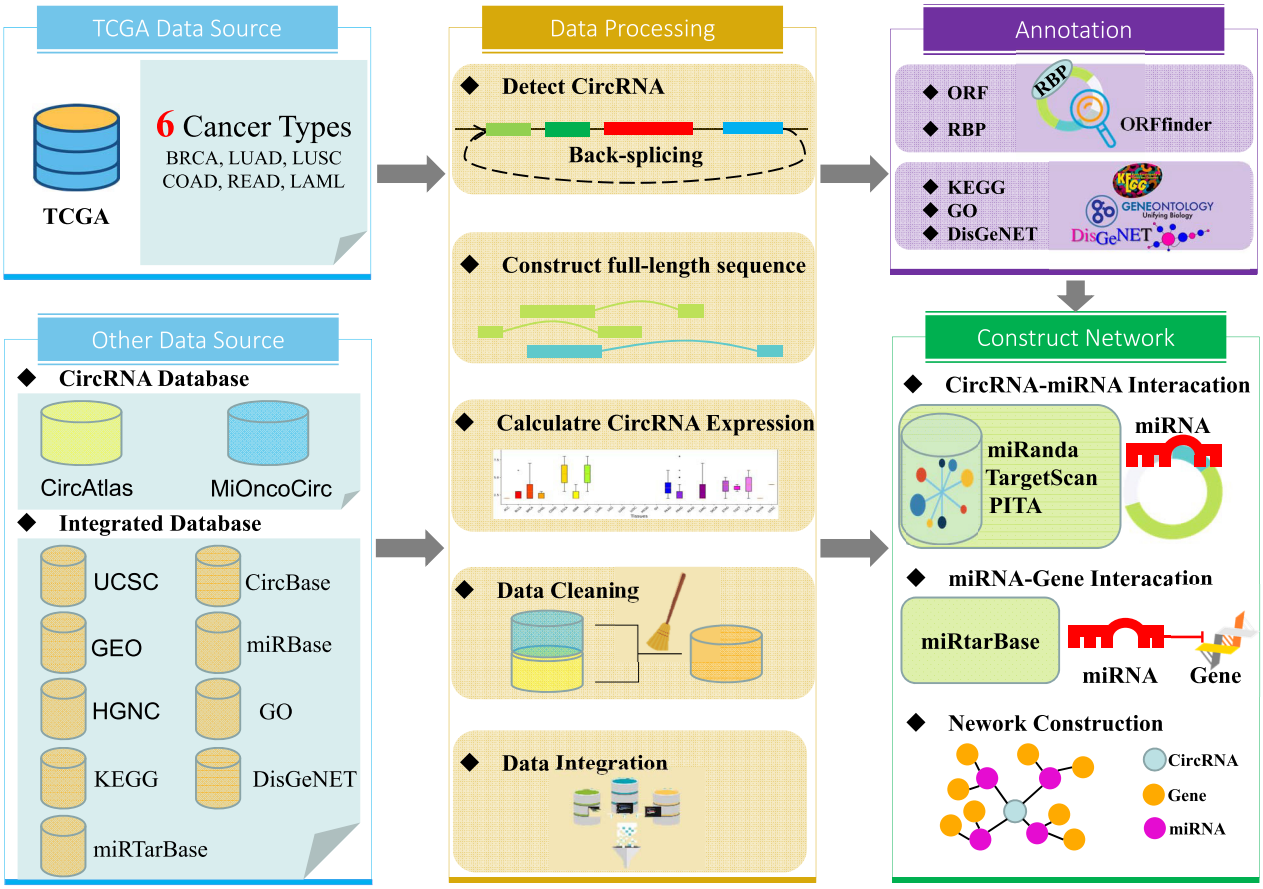

Figure 1. System workflow of CircNet 2.0.

miRNAs from miRBase (v22) into CircNet 2.0. Finally, from the HUGO Gene Nomenclature Committee (HGNC) guidelines for naming protein-coding genes and different classes of RNA genes and pseudogenes in humans (28), we integrated all HGNC gene symbols with annotations into our database. To facilitate the construction of the interaction networks between miRNAs and genes, 384 897 experimentally verified MTIs from miRTarBase were also integrated (24). All the tools included in the CircNet 2.0 data processing pipeline were listed in Supplementary Table S1.

To further enrich the functionalities of CircNet 2.0, we integrated other databases containing information about circRNAs, biological pathways, and diseases and developed several bioinformatics tools for user convenience. circBase is a database that merges and unifies datasets of circRNAs, from which the evidence supporting their expression can be accessed, downloaded, and browsed within the genomic context (29). As circBase ID is widely used in circRNA research, we integrated the database into CircNet 2.0 and developed a function that automatically converts the circBase ID into the CircNet ID, thereby allowing users to search for any circRNA on CircNet 2.0 by inputting its circBase ID. We also developed similar ID conversion function for circAtlas. Gene Ontology (GO) (30) and Kyoto Encyclopedia of Genes and Genomes (KEGG) (31) are well-known enrichment analysis databases that focus on providing functional interpretations of genes and genomes at both the molecular and higher levels. Additionally, DisGeNET is a discovery platform containing one of the largest publicly available collections of genes and variants associated with human diseases (32). On the basis of the collective data from GO, KEGG and DisGeNET that were also integrated into CircNet 2.0, we built a functional tool for the construction of circRNA-miRNA-gene interaction networks for disease analysis in our system. Lastly, we also integrated miRNA expression information of different TCGA cancers from UCSC Xena (33). The amalgamation of these circRNA expression and miRNA expression data allowed for the corroboration of circRNA–miRNA interactions in the network. Supplementary Table S2 summarized all the databases included in CircNet 2.0.

RESULTS

Data statistics and database content of CircNet 2.0

Of the 289 303 circRNAs recorded on CircNet 2.0 (Table 1), 126 491 are related to breast cancer, accounting for approximately half of the updated data. CircRNAs were detected in 2732 samples downloaded from TCGA, circAtlas, MiOncoCirc, and GEO. The detail information of sample id was available in Supplementary Table S3. We also integrated 2656 miRNAs from 8239 samples that covered 37 differ-

Table 1. Data statistics of CircNet 2.0

Abbrev cancer name	Cancer type	Sample sources	Number of circRNAs	TCGA included	Number of samples	Number of miRNAs	miRNA sample counts
BRCA	Breast cancer	circAtlas, MiOncoCirc, GEO, TCGA	126 491	yes	429	2238	832
PRAD	Prostate cancer	circAtlas, MiOncoCirc, GEO	80 744	yes	341	2111	544
LAML	Acute leukimia lyphoma	circAtlas, MiOncoCirc, GEO, TCGA	73 320	yes	194	1834	188
DLBC	Diffuse large B cell lymphoma	circAtlas, MiOncoCirc, GEO	70 255	yes	124	1883	47
MM	Multiple myeloma	circAtlas, MiOncoCirc, GEO	68 019	no	212	NA	NA
SARC	Sarcoma	circAtlas, MiOncoCirc, GEO	67 392	yes	167	2093	260
MISC	Miscellaneous	circAtlas, MiOncoCirc, GEO	65 707	no	126	NA	NA
MPN	Myeloproliferative neoplasms	circAtlas, MiOncoCirc, GEO	60 037	no	151	NA	NA
LUAD	Lung adenocarcinoma	circAtlas, MiOncoCirc, GEO, TCGA	46 502	yes	168	2228	495
CHOL	Bile duct cancer	circAtlas, MiOncoCirc, GEO	45 601	yes	63	1779	45
SECR	Adenoid cystic carcinoma	circAtlas, MiOncoCirc, GEO	44 390	no	44	NA	NA
PAAD	Pancreatic cancer	circAtlas, MiOncoCirc, GEO	40 841	yes	60	2050	182
BLCA	Bladder urothelial carcinoma	circAtlas, MiOncoCirc, GEO	40 698	yes	49	2210	429
HNSC	Head and neck squamous cell carcinoma	circAtlas, MiOncoCirc, GEO	36 504	yes	49	2246	529
LIHC	Liver cancer	circAtlas, MiOncoCirc, GEO	36 020	yes	21	2172	420
LUNG	Lung cancer	circAtlas, MiOncoCirc, GEO	33 303	no	42	NA	NA
LUSC	Lung squamous cell carcinoma	circAtlas, MiOncoCirc, GEO, TCGA	32 884	yes	125	2213	380
READ	Rectal cancer	circAtlas, MiOncoCirc, GEO, TCGA	32 431	yes	103	2003	92
ESCA	Esophageal cancer	circAtlas, MiOncoCirc, GEO	32 345	yes	19	2092	195
GBM	Glioblastoma	circAtlas, MiOncoCirc, GEO	32 158	yes	26	1388	5
KDNY	Kidney cancer	circAtlas, MiOncoCirc, GEO	29 474	no	23	NA	NA
STAD	Stomach cancer	circAtlas, MiOncoCirc, GEO	29 125	yes	27	2178	428
SKCM	Melanoma	circAtlas, MiOncoCirc, GEO	26 869	yes	31	2220	452
ACC	Adrenocortical cancer	circAtlas, MiOncoCirc, GEO	26 839	yes	24	1952	79
COLO	Squamous cell carcinoma	circAtlas, MiOncoCirc, GEO	25 983	no	29	NA	NA
NRBL	Neuroblastoma	circAtlas, MiOncoCirc, GEO	25 939	no	13	NA	NA
OV	Ovarian cancer	circAtlas, MiOncoCirc, GEO	22 922	yes	20	2165	485
MBL	Medulloblastoma	circAtlas, MiOncoCirc, GEO	22 298	no	7	NA	NA
THCA	Thryoid cancer	circAtlas, MiOncoCirc, GEO	20 298	yes	13	2,217	569
RHABDO	Rhabdomyosarcoma	circAtlas, MiOncoCirc, GEO	19 899	no	10	NA	NA
TGCT	Testicular cancer	circAtlas, MiOncoCirc, GEO	13 377	yes	4	2212	155
LYMP	Lymphoma	circAtlas, MiOncoCirc, GEO	11 765	no	3	NA	NA
MESO	Mesothelioma	circAtlas, MiOncoCirc, GEO	8140	yes	3	1964	87
THYM	Thymoma	circAtlas, MiOncoCirc, GEO	5918	yes	2	2128	126

Table 1. Continued

Abbrev cancer name	Cancer type	Sample sources	Number of circRNAs	TCGA included	Number of samples	Number of miRNAs	miRNA sample counts
UCEC	Uterine carcinosarcoma	circAtlas, MiOncoCirc, GEO	5532	yes	2	2238	430
LGG	Lower grade glioma	circAtlas, MiOncoCirc, GEO	5414	yes	2	2157	524
COAD	Colon cancer	circAtlas, MiOncoCirc, GEO, TCGA	1487	yes	6	2113	261
Total			**289 303**		**2732**	**2656**	**8239**

Table 2. Comparison of CircNet 2.0 with other circular RNA databases

	MiOncoCirc	circAtlas 2.0	CSCD2.0	CircNet 1.0	CircNet 2.0
Publication	Cell (2019)	Genome Biol. (2020)	NAR (2021)	NAR Database Issue (2016)	Submitted
Last update	2018	2019	2021	2015	2021
Support species	Homo sapiens	6 species	Homo sapiens	Homo sapiens	Homo sapiens
Number of cancer types	19	0	23	0	37
Number of samples	2000 + samples	1070 samples	1113 cancer samples	464 samples	2732 cancer samples
Data sources	GEO	SRA, NGDC, GeneBank	ENCODE, SRA	GEO	TCGA, GEO
Circular RNA detection methods	CIRCexplorer, CrossMap	CIRI2/CIRI-full, CIRCexplor2, Find_circ, DCC	CIRI2, circRNA_finder, find_circ, circexplorer2	Memczak's algorithm	CIRI2/CIRI-full, CIRCexplor2, Find_circ, DCC
circRNA-miRNA interaction	no	yes	yes	yes	yes
miRNA-gene interaction	no	no	no	yes	yes
circRNA expression	yes	yes	yes	yes	yes
microRNA expression	no	no	yes	no	yes
other characteristics	-	Conserved score, circRNA-miRNA network, circRNA annotation about full-length sequence and ORF.	CircRNA annotation about full-length sequence and ORF.	Expression profiles of circRNA isoforms	CircRNA annotation about full-length sequence and ORF, circRNA-miRNA-Gene network, GO analysis, KEGG analysis, Disease enrichment analysis.

ent types of cancer, 26 of which were included in TCGA projects. One in five samples for circRNA discovery and 1 in 10 samples for miRNA integration were of breast cancers. CircRNAs from prostate cancer, lymphoma, myeloma, sarcoma, and lung adenocarcinoma samples were also processed to enrich our database for wider applications and utility in cancer research. Furthermore, Supplementary Table S4 shown the distribution of circRNAs on chromosomes.

For CircNet 2.0, we focused on updating circRNAs in cancers and their interactions with miRNAs. Table 2 presents the improvements and updated content of CircNet 2.0 in comparison with CircNet 1.0 and other circRNA databases. As indicated in Table 2, CircNet 2.0 provides expression profiles and interaction information of circRNAs, miRNAs, and genes across 37 human cancers, including breast cancer, prostate adenocarcinoma, and lymphoma. The number of processed samples has been significantly increased from 464 to 2732. Full-length circRNA sequences were annotated, allowing for a more precise prediction of circRNA–miRNA interactions. As mentioned above, miRTarBase was included to provide high-quality miRNA–gene interactions. Therefore, this update can facilitate the construction of comprehensive circRNA–miRNA–gene regulatory networks of high quality. Furthermore, CircNet 2.0 not only provides the genomic annotation and expression profiles of circRNAs but also displays the genomic information and expression patterns of miRNAs in different TCGA cancer types.

Construction of circRNA–miRNA–gene regulatory networks

CircNet 2.0 facilitates the construction of novel circRNA–miRNA–gene regulatory networks. The interactions between circRNAs and miRNAs were initially predicted using miRanda, TargetScan, and PITA based on the atomic principles of miRNA interactions and popular bioinformatics algorithms. Then, 384 897 experimentally validated MTIs between miRNAs and mRNAs from miRTarBase were incorporated into CircNet 2.0, making circRNA–miRNA–gene regulatory networks of high quality available. With miRNAs as intermediates, circRNAs could be connected

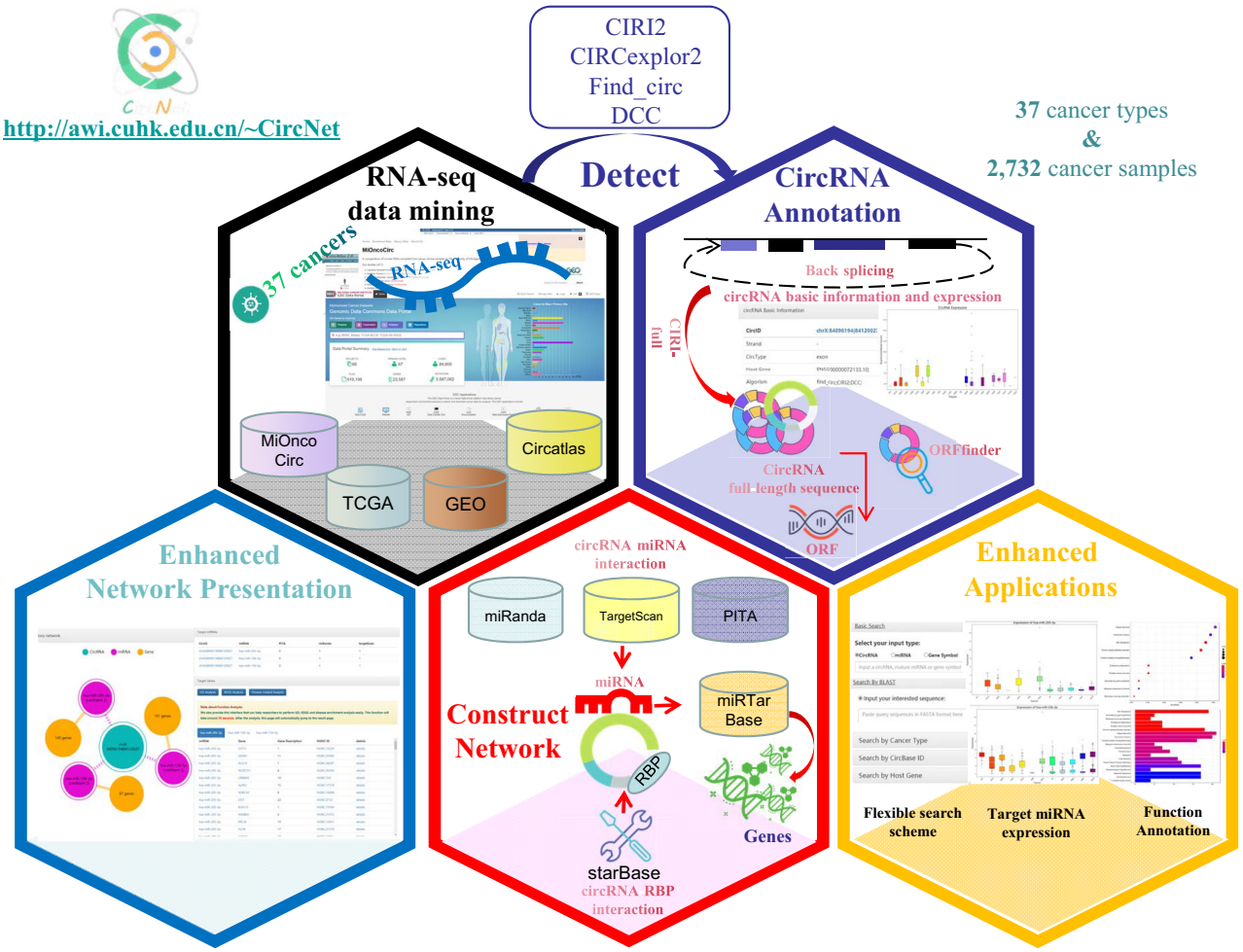

Figure 2. Highlighted improvements of CircNet 2.0.

with genes, which proved to be a powerful tool for the daily discovery of novel potential drug targets for replacing known drug targets with obvious side effects or for solving the problems of undruggable targets. The regulatory networks among circRNAs, miRNAs and genes may draw the attention of researchers to pathways that have been overlooked. For example, Zhang *et al.* (34) discovered that circTRIM33-12 upregulated ten-eleven translocation 1 (TET1) expression by sponging miR-191, leading to a significant decrease in 5-hydroxymethylcytosine and DNA methylation levels in hepatocellular carcinoma (HCC). Their findings provided new insight into the role of circRNAs in HCC progression.

Comprehensive web functionalities of CircNet 2.0

The highlights of the CircNet 2.0 functionalities are shown in Figure 2. Users can browse circRNA–miRNA–gene regulatory networks by clicking on any node of the visualized network. To further illustrate the circRNA–miRNA interactions, CircNet 2.0 now contains the expression profiles of both circRNAs and target miRNAs that act as indicators of their interactions in different cancer categories. For example, for this update, the potencies of circRNAs in encoding proteins were calculated through the identification of their open reading frames (ORFs). Additionally, this update also integrated the RNA-binding proteins of circRNAs predicted by circAtlas, which can enhance the regulatory network. Furthermore, CircNet 2.0 can provide a one-click functional enrichment analysis of the genes interacting with a specific circRNA, using GO, KEGG and related disease enrichment analysis tools. Such analyses of gene functions are important for the interpretation of biological data and have become an indispensable routine in clinical research. The data capacity in CircNet 2.0 for allowing the cancer-specific analysis of cancer-related circRNAs would greatly support disease enrichment analyses. Working as an indicator of disease pathology, it would help to shed light on the correlations between circRNA expression patterns and the oncology of various cancer types and facilitate the discovery of new cancer drug targets.

Enhanced user interface of CircNet 2.0

As shown in Figure 3, we updated the web interface to provide a user-friendly experience in data presentation, search, and visualization. CircNet 2.0 allows users to browse the network by simply searching for the circRNA, miRNA or

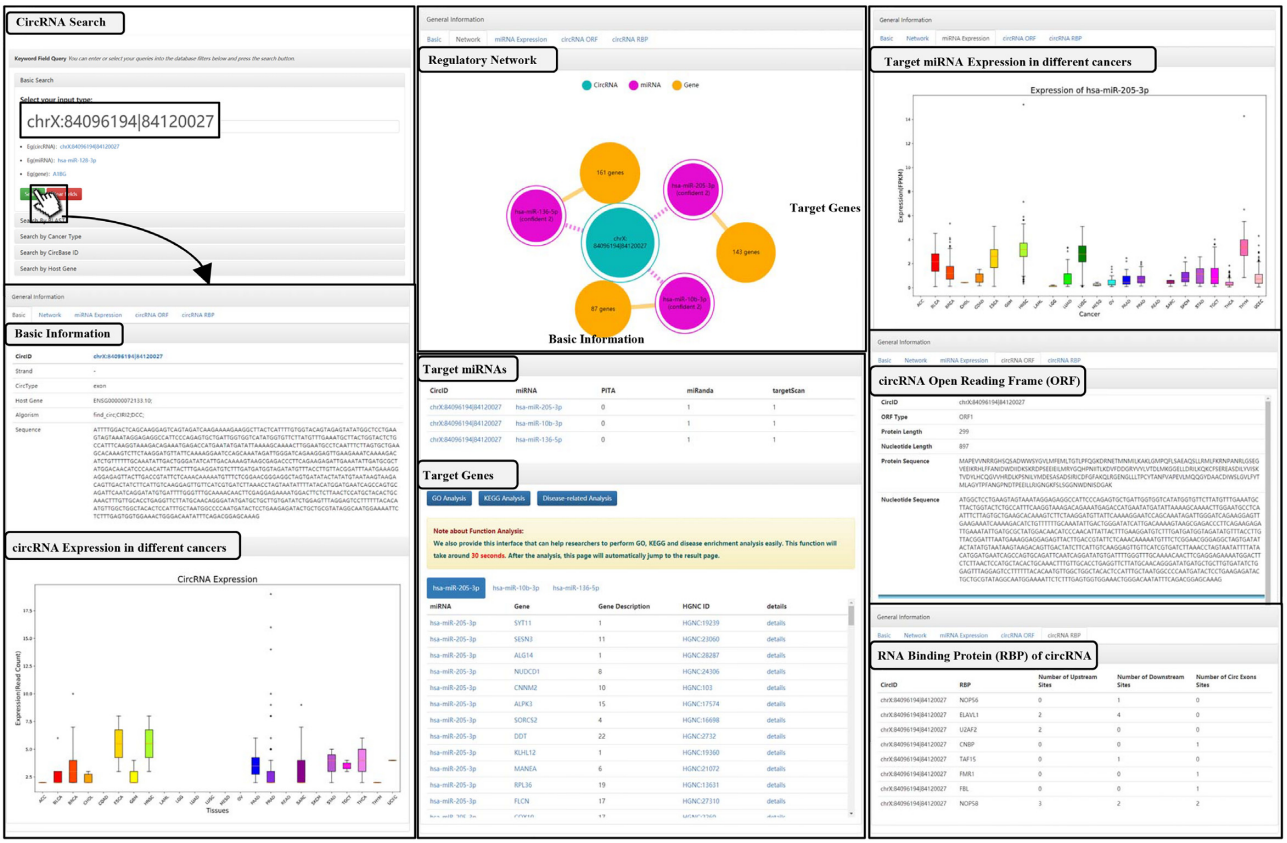

Figure 3. Demonstration of the web interface of CircNet 2.0. We use basic search as an example. After input of the circRNA ID, with one click, CircNet 2.0 will provide the annotation of the circRNA, its expression, its interactions with miRNA, and detailed information of the circRNA–miRNA–gene regulatory network. Users can also click on any node of the network to browse and zoom-in on it.

gene symbol of interest. Given the lack of a common nomination rule of circRNAs, CircNet 2.0 also provides a BLAST search to allow users to match potential circRNAs to the data in our database by sequence similarity (Supplementary Figure S1). Once successfully matched, users can obtain not only the sequence and expression profiles of circRNAs and related miRNAs in certain types of cancers but also their ORFs, RNA-binding protein information, and potential target miRNAs and affected mRNAs. Additionally, CircNet 2.0 allows users to search for significantly expressed cancer-related circRNAs for cancer-specific analysis, which would be of great convenience to researchers and supply them with a vast amount of reliable information for their target screening and exploration. The interface also allows users to search the database by circBase ID, which will be converted to the CircNet ID, thereby improving the efficiency of data browsing. Additionally, CircNet 2.0 facilitates the search of circRNAs via a search of their host gene (Supplementary Figure S2). By simply clicking on the details of a circRNA, CircNet 2.0 provides its functional annotation and expression pattern, its interactions with miRNAs, and comprehensive information of the circRNA–miRNA–gene regulatory network. Users can also click on any node of the visualized network to browse and zoom-in on it. Overall, CircNet 2.0 provides a practical means for the analysis of circRNA regulation processes.

DISCUSSION AND CONCLUSION

The discovery of circRNAs and their potential targets in diseases has become an important research direction, and published studies have provided promising strategies for the development of novel therapeutic methods. For example, Du *et al.* (35) reported that circ-Dnmt1 overexpression in breast cancer cells increased their proliferation and survival as mediated through the activation of autophagy by the interaction of the circRNA with p53 and AUF1. The silencing of circ-Dnmt1 reversed these processes. According to Wang *et al.* (36), the sponging of miR-442a by overexpressed circNT5E promoted the formation of glioblastoma (GBM) in vivo, with the circRNA affecting multiple biological functions of the cancerous cells, including their proliferation, apoptosis, and metastasis. CircRNAs also play important roles in the tumor microenvironment (TME), where they are involved in the activities of various types of cells, such as cancer-associated epithelial cells, immune cells (e.g. tumor-associated macrophages, dendritic cells, and mast cells), and cancer stem cells (37). Zhan *et al.* (38) provided the first evidence for differentially expressed circRNA patterns in macrophages of various polarization states, which shed light on the role of these RNAs in the oncogenesis of cancers due to changes in the TME. Furthermore, circRNAs have been identified as novel targets for reversing drug resistance in various cancer types. For exam-

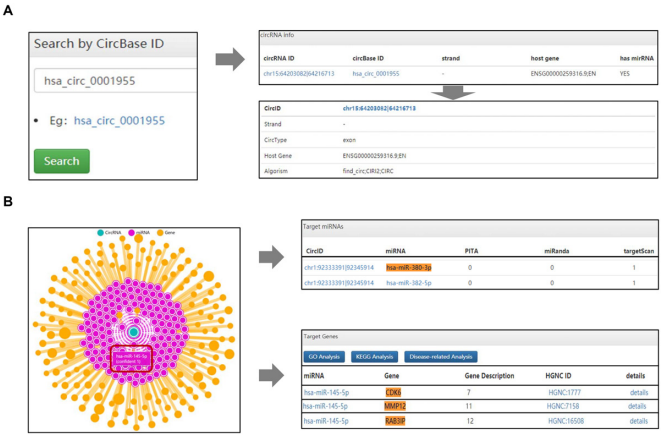

Figure 4. Case study of the hsa_circ_0001955 regulatory network. (**A**) Using 'Search by CircBase ID,' hsa_circ_0001955 is converted to the CircNet ID chr15:64203082|64216713. The circRNA annotation is shown after one click. (**B**) In the network page of chr15:64203082|64216713, we can see that TargetScan has predicted the target of the circRNA to be has-miR-145-5p, which corresponds to the result of previous studies. We can find that all three target genes are proven as the interaction targets of has-miR-145-5p in our network.

ple, it was confirmed that circPAN3 can be used as a target for reversing adriamycin resistance in patients with acute myeloid leukemia through the miR-153-5p/miR-183-5p-X-linked inhibitor of apoptosis protein (XIAP) axis (35,39). The CircNet 2.0 database will facilitate the acquisition of knowledge about such circRNA-associated biological processes and the discovery of potential biomarkers by helping researchers to explore circRNA annotations and circRNA–miRNA–gene regulatory networks.

To ensure the reliability and accuracy of the database contents provided to users, the analyzed data were confirmed with the findings detailed in existing research articles on circRNA–miRNA–gene regulatory networks in cancers. In 2020, Ding *et al.* (40) proposed that the circRNA hsa_circ_0001955 had the potential to regulate the miRNA hsa-miR-145-5p in colorectal cancer (CRC), where the genes encoding cell division protein kinase 6 (CDK6), matrix metalloproteinase-12 (MMP12), and Ras-related protein Rab-3A interacting protein (RAB3IP) acted as potential downstream targets. The circRNA dataset of 10 patients with CRC was extracted from the GEO database, and differential expression analysis was performed using the GEO2R tool provided by that database to identify differentially expressed circRNAs. Using data from the cancer-specific circRNA database, the differentially expressed circRNAs chosen were confirmed to have miRNA response elements, indicating that the circRNAs could act as miRNA sponges in CRC. Specific miRNA prediction and downstream gene identification were performed using the starBase database (41), whereupon it was found that there was a significant regulatory connection among hsa_circ_0001955, hsa-miR-145-5p, and CDK6/MMP12/RAB3IP. We conducted the same procedure in CircNet 2.0 and searched for hsa_circ_0001955 after converting its ID to chr15:64203082|64216713 in the database, and the circRNA was predicted to interact with dozens of miRNAs, including the TargetScan-predicted hsa-miR-145-5p. As presented in Figure 4, CircNet 2.0 can provide these experimentally verified circRNA regulatory networks, which are consistent with the findings of Ding *et al.* (42) and other studies, validating the reliability of the database content. An additional case study of the circ_0004463/miR-380-3p/FOXO1 axis is presented in Supplementary Figure S3.

In summary, we have not only integrated data from existing circRNA databases into CircNet 2.0 but also detected novel circRNAs from the sequencing data of the GEO and TCGA databases. The application of multiple circRNA prediction tools ensured the accuracy of the data contained in CircNet 2.0. We also designed a more user-friendly web interface to aid researchers in searching and browsing circRNAs of interest conveniently. In conclusion, CircNet 2.0 provides a practical and user-friendly platform on which researchers can explore novel cancer biomarkers and circRNAs related to the pathogenesis, diagnosis, and therapy of malignant and other diseases.

DATA AVAILABILITY

The CircNet 2.0 database will be continuously maintained and updated. The database is now publicly accessible at https://awi.cuhk.edu.cn/~CircNet.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

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