LncRNA2Target v2.0: a comprehensive database for target genes of IncRNAs in human and mouse

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ABSTRACT

Long non-coding RNAs (lncRNAs) play crucial roles in regulating gene expression, and a growing number of researchers have focused on the identification of target genes of lncRNAs. However, no online repository is available to collect the information on target genes regulated by lncRNAs. To make it convenient for researchers to know what genes are regulated by a lncRNA of interest, we developed a database named LncRNA2Target to provide a comprehensive resource of lncRNA target genes in 2015. To update the database this year, we retrieved all new lncRNA–target relationships from papers published from 1 August 2014 to 30 April 2018 and RNA-seq datasets before and after knockdown or overexpression of a specific lncRNA. LncRNA2Target database v2.0 provides a web interface through which its users can search for the targets of a particular lncRNA or for the lncRNAs that target a particular gene, and is freely accessible at http://123.59.132.21/Incrna2target.

INTRODUCTION

Development of RNA sequencing technology leads to recognition of large amount of novel transcripts (1,2). The corresponding large-scale genomic and transcriptomic analysis has shown that over 90% of the human genome is actively transcribed (2), while only ∼2% of the genome can encode proteins (3). In comparison, a large portion of the transcripts are non-coding RNAs (ncRNAs) (4), such as long ncRNAs (lncRNAs)

lncRNA plays a crucial role in biological processes (5,6) and even in diseases (7–13). In 2014, Sun et al. presented that BANCR could be a biomarker for poor prognosis of non-small cell lung cancer (NSCLC) by investigating its expression in multiple NSCLC tissues (14). In 2016, Zhu et al. observed that lncRNA MEG3 may be a potential target and therapeutic strategy for diabetes since that the upregulation of MEG3 enhances hepatic insulin resistance (15). Many important databases such as lncRNA-Disease (16), lncRNAsNP2 (17), LncRNA-Wiki (18), MNDR (19), EVLncRNAs (20), NONCODE (21), LncRNA2Function (22) and TF2LncRNA (23) were developed for collecting the sequence information and functional characteristics of lncRNAs (16–23). For example, LncRNA2Function (22) mapped lncRNAs to Gene Ontology (GO) terms and biological process, lncRNADisease (16) documented associations between lncRNAs and diseases.

Accumulating evidence has shown that lncRNA exert its functions by regulating the expression of target genes. Researchers used to infer lncRNA–target relationships by examining whether a candidate gene is differentially expressed after knocking down or overexpressing a specific lncRNA (24). For example, in 2014, Bell et al. found the decreased expression of Myocardin and numerous smooth muscle contractile genes after knocking down the lncRNA SENCR (25). In 2015, LncRNA2Target v1.0 was initially released with these scattered lncRNA–target relationships (24), which was widely used for predicting potential lncRNA–target relationships, lncRNA disease associations, and so on (26–28). Whereas, the differentially expressed genes after knocking down or overexpressing a lncRNA are potential target genes with the lack of direct lncRNA–target interaction evidence. Recent years, some binding experiments such as immunoprecipitation assays, RNA pull-down assays and luciferase reporter assays was used to identify target genes of lncRNAs (29,30). Therefore, LncRNA2Target 2.0 not only collects all differentially expressed genes after knocking down or overexpressing a

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lncRNA, but also collects all lncRNA–target relationships confirmed by binding experimental technologies such as luciferase reporter assays, immunoprecipitation assays and pull down arrays.

DATA COLLECTION

To update LncRNA2Target, we collected all lncRNA papers and datasets published from 1 August 2014 to 30 April 2018 by searching the PubMed literature database and Gene Expression Omnibus (GEO) with keywords ‘lncRNA’, ‘lincRNA’ and ‘long non-coding RNA’, respectively. Then, over 1500 papers and 140 new available high-throughput microarray or RNA-seq datasets were retrieved. Subsequently, we downloaded all the published papers and manually extracted information about associations between lncRNAs and their target genes. To ensure data quality, lncRNAs were annotated by NCBI GenBank (31), Ensembl (32) and GENCODE (33), and each of these associations was double checked. Besides, we also downloaded all the high-throughput microarray and RNA-seq datasets before and after knocking down or overexpressing a lncRNA of interest and identified all differentially expressed genes.

A unified analysis process was developed to reanalyze public microarray and RNA-sequencing datasets before and after knocking down or overexpressing a lncRNA. First, microarray raw data was preprocessed by oligo package (34). For RNA-seq data, NGS QC Toolkit (35) was used for quality filtering and trimming, TopHat (36) was used to map reads to reference genome, and HTSeq (37) was used to count how many reads map to each gene. Then, limma (38) was selected for gene expression normalization and differential expression analysis, due to it can be applied to both microarray and RNAseq data with very similar pipelines. At last, genes with significant expression change (adjusted \( P \leq 0.05 \)) were considered as targets of a specific lncRNA.

DATABASE CONTENT

LncRNA2Target v2.0 contains 152 137 lncRNA–target associations from 1047 papers and 224 datasets. The detailed statistics on lncRNA–target associations is shown in Ta-
The total number of lncRNA–target associations increased significantly compared to the previous version. For example, LncRNA2Target v1.0 contains 278 human lncRNA–target associations between 68 lncRNAs and 216 target genes from low-throughput methods such as RT-qPCR and western blot. While LncRNA2Target v2.0 contains 1465 human lncRNA–target associations between 356 lncRNAs and 689 target genes from low-throughput methods such as RT-qPCR, western blot, luciferase reporter assays, immunoprecipitation assays, and RNA pull-down assays.

LncRNA2Target v2.0 contains 35 lncRNA–target associations from low-throughput experiments before 2010, and 33, 59, 59, 84, 133, 129, 296, 579, and 268 associations per year from 2010 to 2018, respectively. The number of lncRNA–target associations from low-throughput experiments has been annually increasing since 2010. Especially in 2017, up to 579 lncRNA–target associations were reported. Figure 1 shows that lncRNA knockdown and overexpression were the most common methods to infer lncRNA–target relationships, and the number of lncRNA–target associations validated from luciferase reporter assays, immunoprecipitation assays, and RNA pull-down assays is also increasing rapidly.

DATABASE ACCESS

LncRNA2Target v2.0 is publicly available at http://123.59.132.21/Lncrna2Target. Users can browse, search, and download all lncRNA–target relationship data through our web interface. Figure 2 shows the schematic workflow of browsing lncRNA–target associations by lncRNA symbol. LncRNA2Target contains information and target genes. Furthermore, the description of association between lncRNA and gene from the literature could be shown by clicking the hyperlink of the gene. Figure 3 shows the schematic workflow of searching lncRNA–target associations by lncRNA Entrez ID/symbol, lncRNA Ensembl ID, and target Entrez ID/symbol. Here, fuzzy search function was provided for retrieving lncRNAs and target genes. The figure gives the searching results of two examples CDKN1A and HOTAIR. In the download page, details of all the lncRNA–target associations could be accessed. In addition, LncRNA2Target web server also provides a submission page, which allows researchers to submit new experimentally verified lncRNA–target associations to the database.
CONCLUSION

LncRNA2Target v2.0 provides a comprehensive resource of lncRNA–target relationships in human and mouse, which aims to facilitate users to browse, search and download all literature-based lncRNA–target associations. The associations based on low-throughput and low-throughput experiments were manually extracted from literature and reanalyzed using a unified analysis process, respectively. Now LncRNA2Target v2.0 contains 152,137 lncRNA–target associations from 1047 papers and 224 datasets. With the development of experimental technologies, more and more relationships between lncRNAs and target genes will be identified. Therefore, to make researchers convenient access the new lncRNA–target associations, we will update LncRNA2Target regularly. We believe that LncRNA2Target v2.0 will be of particular interest to lncRNA community.

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