IRNdb: The database of immunologically relevant non-coding RNAs.

Elena Denisenko[1], Daniel Ho[1], Ousman Tamgue[2,3], Mumin Ozturk[2,3], Harukazu Suzuki[4], Frank Brombacher[2,3], Reto Guler[2,3], Sebastian Schmeier[1*]

[1]Massey University, Institute of Natural and Mathematical Sciences, Auckland 0745, New Zealand, [2]International Centre for Genetic Engineering and Biotechnology (ICGEB), Cape Town Component, Cape Town, South Africa, [3]University of Cape Town, Health Science Faculty, Institute of Infectious Diseases and Molecular Medicine (IDM), Division of Immunology, Observatory 7925, Cape Town, South Africa, [4]Division of Genomic Technologies, RIKEN Center for Life Science Technologies, 1-7-22 Suehiro-cho, Tsurumi-ku, Yokohama 230-0045, Japan

[*]Corresponding author: Tel: +64 9 2136538; Email: s.schmeier@massey.ac.nz

Abstract

MicroRNAs (miRNAs), long non-coding RNAs (lncRNAs) and other functional non-coding RNAs (ncRNAs) have emerged as pivotal regulators involved in multiple biological processes. Recently, ncRNA control of gene expression has been identified as a critical regulatory mechanism in the immune system. Despite the great efforts made to discover and characterize ncRNAs, the functional role for most remains unknown. To facilitate discoveries in ncRNA regulation of immune system related processes we developed the database of immunologically relevant ncRNAs (IRNdb), a database of ncRNAs, their immunologically relevant targets, associated biological pathways and experiments. We integrated mouse data on predicted and experimentally verified ncRNA-target interactions, ncRNA and gene annotations, biological pathways and processes, and experimental data in a uniform format with a user-friendly web interface. The current version of IRNdb documents 3,799 experimentally confirmed miRNA-target interactions between 265 miRNAs and 1,633 murine and human-derived immune-related targets. In addition, we recorded 22,453 lncRNA - immune target and 377 PIWI-interacting RNA - immune target interactions. IRNdb is a comprehensive searchable data repository which will be of help in uncovering the role of ncRNAs in immune system related processes.

Database URL: http://irndb.org

Introduction

Rapid development of high-throughput technologies enabled identification of non-coding RNAs (ncRNAs) as a highly abundant class of transcripts in the pervasively transcribed eukaryotic genome (1,2). Among ncRNAs, microRNAs (miRNAs), long non-coding RNAs (lncRNAs), and PIWI-interacting RNAs (piRNAs) have been attracting an increasing interest over the last decade as master regulators of numerous genes and diverse biological processes.

MiRNAs constitute a family of ~22 nucleotides small ncRNAs that bind to target mRNAs to mediate post-transcriptional repression or degradation of the mRNA (3,4). More than 20,000 miRNA loci are described to date in different species, including over 2,000 miRNAs in mammals (5). More than 50% of the mammalian genome is thought to be under miRNA control (6,7). Nevertheless, the functional and biological roles of many of miRNAs remain to be determined.

Complexity of miRNA action mechanisms poses challenges on deciphering their roles. Different possible models of miRNA action were proposed (8,9). MiRNA may control single or multiple mRNA targets involved in the establishment of a certain biological outcome or phenotype. Alternatively, miRNAs may regulate multiple mRNA targets associated with different biological outcomes (9-12). Finally, multiple miRNAs may be required to regulate multiple mRNA targets,

which contribute additively to the same specific biological outcome (9,10,13). Additional challenges in miRNA studies exist, e.g. multiple targets might be repressed by different miRNAs to different extent (8,14), miRNA levels might be reciprocally controlled by their targets (14-16), and miRNA regulation might differ between tissues and pathologies (7,9). Thus, characterization of miRNA targets alone fails to improve our understanding of miRNA biology. Instead, the knowledge of target gene interconnection and involvement in biological processes and pathways under different conditions is essential.

Recently, the role of miRNAs in controlling the immune system has begun to emerge (9,17). A number of miRNAs have been described as important regulators acting in both adaptive and innate immune cells (18). Cooperative action of multiple miRNAs in various immune cells contributes to a systemic regulation of development and homeostasis of the immune system and the host response to invading pathogens (9,17,19,20). Therefore, it is not surprising that dysregulation of miRNAs contributes to a number of inflammatory conditions (19) and autoimmune diseases (21-24). However, for the majority of miRNAs their roles in immune related processes still remain to be elucidated.

PiRNAs constitute another class of small ncRNAs whose well-established function is to silence transposable elements in complex with PIWI proteins in germline cells (25). Recent studies provided evidence that piRNA might possess a wider range of functions including protein-coding gene silencing (including immune system related genes) and epigenetic regulation (26,27).

LncRNAs are defined as ncRNAs longer than 200 nucleotides (28). Several classification systems and numerous subclasses of lncRNA genes have been described (29). LncRNAs are believed to possess a wide range of molecular functions including transcriptional regulation, regulation of mRNA processing, control of post-transcriptional events and regulation of protein activity (30,31). A number of lncRNAs were shown to regulate the expression of their adjacent immune genes and be functionally important in both innate and adaptive immunity (32,33). LncRNAs synthesized by both host and pathogen are involved in the regulation of host-pathogen interactions and might be crucial for the infection outcome (32-34). Despite the clear importance of certain lncRNAs for regulatory mechanisms, the functionality and biological role of the vast majority of lncRNAs remains unknown (30).

The numbers of experimental studies on ncRNA-target interactions as well as the numbers of computational prediction approaches and tools have increased drastically over the years (35-39). Accordingly, several databases have been published aiming at collecting miRNA, piRNA, and lncRNA data. Nevertheless, little overlap among database entries and results of prediction methods require researchers to employ multiple independent sources (40,41). In addition, lack of utilization of pathway information and integration with tissue specific processes broadens a list of public data sources that should be utilized in answering even simple biological questions.

In this study, we report the development of IRNdb, a specialized database for immune-related ncRNAs in mice, a model system for the study of the immune system. We aimed to integrate information on murine miRNAs, lncRNAs, piRNAs, and their immunologically relevant targets. We combined different sources of ncRNA-target interactions, ncRNA and gene annotations, signalling pathways, and experimental data to create one comprehensive, easily accessible knowledge base for immunologically relevant ncRNAs. Hence, IRNdb offers a new data repository to improve our understanding of the roles of ncRNAs in the immune system.

Materials and methods

Database construction / data sources and implementation

IRNdb is an open-access database implemented in the DJANGO web-framework (https://www.djangoproject.com/). In IRNdb we integrated public domain data from various sources (see Figure 1). Experimentally validated interactions between miRNAs and their targets were retrieved from two databases: miRTarBase (42) and miRecords (43). Predicted interactions were obtained from nine tools/databases: DIANA microT-CDS (44), ElMMo3 (45), MicroCosm (46), microRNA.org (47), miRDB (48), miRNAMap2 (42), PicTar2 (DoRiNA 2.0) (49), PITA (50), and TargetScanMouse (51). These tools employ different prediction algorithms including seed sequence match, free energy of microRNA:mRNA duplex, and evolutionary conservation. We also included lncRNA-target interaction from LncRNADisease (52), LncRNA2Target (38), and LncReg (39) and piRNA-target interactions from piRBase (53).

The set of genes involved in immunological processes was retrieved from six sources: InnateDB (54) and SepticShock (http://www.septicshock.org/) contain mouse-specific immunological gene information, whereas immunologically relevant human genes were extracted from ImmPort (55), the Immunome database (56,57), Immunogenetic Related Information Source (IRIS) (58), and the MAPK/NFKB network (http://www.innatedb.com/redirect.do?go=resourcesGeneLists) (54) and converted to murine genes via the NCBI Homologene database (http://www.ncbi.nlm.nih.gov/homologene).

General information on miRNAs and corresponding targets was extracted from miRBase (5) and NCBI gene (https://www.ncbi.nlm.nih.gov/gene). Biological pathway and process information were downloaded from KEGG (59), Wikipathways (60), and the Gene Ontology (GO) (61). In addition, lists of up- and down-regulated genes in immunological experiments were downloaded from Molecular Signature Database (MsigDB) (62).

Transcription factor binding site (TFBS) information was retrieved from ENCODE (63), hmChIP (64), and HT-ChIP (65). TFBSs locations were converted to the mm10 genome build using the UCSC liftOver tool (https://genome.ucsc.edu/cgi-bin/hgLiftOver) and mapped within 1000bp upstream of miRNA associated promoter regions (see "Tuberculosis dataset").

Tuberculosis dataset

Gene expression time-course data derived from murine macrophages infected with *Mycobacterium tuberculosis* (*M.tb*) were incorporated in the database (66,67). In the experiment, bone marrow-derived macrophages, important immune cells in tuberculosis development, were isolated from mice. Macrophages activated in four different ways (IFN-gamma, IL-13, IL-4 or IL-13/ IL-4) and non-activated macrophages were left alone or infected with the hypervirulent, clinical HN878 strain of *M.tb.* RNA samples were collected over time and subjected to CAGE-sequencing (68). Transcription start sites (TSSs) and expression of corresponding transcripts and genes were identified. In IRNdb we incorporated gene expression values averaged across replicates, normalized to tags per million (TPM), and log-transformed for representation purposes. Differences in gene expression between infected and non-infected macrophages were calculated as log2 fold-changes before log-transformation.

We inferred miRNA expression levels and corresponding TFBSs using the PROmiRNA algorithm (69) in conjunction with the CAGE tuberculosis dataset (see above). Briefly, PROmiRNA identified a subset of TSSs and transcripts, which are likely associated to particular miRNAs rather than coding

genes. The expression of miRNAs was calculated by summing up the expression of associated TSSs and averaging across replicates. TFBSs were identified by proximity to miRNA associated TSSs.

Results

Database overview

The content of IRNdb is divided into four main data sections 'microRNAs', 'piRNAs', 'lncRNAs', and 'Target genes'. Two more sections including 'Documentation' and 'Contact' provide instructions and examples for using the database and contact information. A statistical summary of the IRNdb interaction data is provided in Table 1 and the 'Statistic'-tab of the 'Documentation'-page, where users can also download all immunologically relevant ncRNA-target interactions in tabular form.

Browsing and searching ncRNAs

IRNdb provides an interface for the convenient retrieval of ncRNA and target information. Users can browse ncRNAs in tabular form that is searchable, e.g. by identifier or names. Clicking the ncRNA name in the table leads to an individual ncRNA entry (e.g. 'miRNA view' in case of miRNAs).

An individual ncRNA entry provides tabs with information regarding the ncRNA. The 'Targets'-tab enables users to browse available ncRNA targets extracted from the respective sources as detailed above (see Materials and methods). The targets are listed in searchable tables which include target symbols, gene names, and GeneIDs. Additional columns indicate if the immune relevance of the target was inferred from human or mouse data and show the respective sources. The 'Target source'-column lists the sources of the ncRNA-target interactions, which helps users to evaluate the reliability of the target. In case of piRNA and lncRNA a reference column shows the publication source of the interaction if available. In case of miRNAs the targets are split into two tables showing experimentally verified and predicted targets, respectively.

The 'Pathways'-tab lets users browse WikiPathways and KEGG pathways that contain targets of the ncRNA under investigation. Pathways are presented as a table for each pathway source. For miRNAs it is split into experimentally verified and predicted targets, respectively. For each pathway the table lists gene symbol, name and total number of associated genes.

The 'GO'-tab is structured similarly to the 'Pathways'-tab and provides users with a list of GO-process and GO-function terms associated with the ncRNA targets. Exploring the 'GO'- and 'Pathways'-tab, users can identify biological pathways and processes, which might be affected by the ncRNA of interest.

The 'M.tb'-tab contains expression data for the immunologically relevant ncRNA targets available in the experiment on *M.tb*-infected macrophages (66,67). For each gene log2 fold-changes were calculated comparing *M.tb*-infected to non-infected macrophages within each stimulation type. The lowest log2 fold-change value across all time points per stimulation is shown as we assume, e.g. the miRNA to down-regulate genes.

In addition, the 'M.tb'-tab for an individual miRNA might contain information on the expression of the miRNA under investigation. As described above (see Materials and methods) we tried to infer CAGE-promoter regions for miRNAs. If this was successful we also show the expression of the sum of all promoters associated to the miRNA in the *M.tb* infection experiment (66,67).

With help of the miRNA associated TSS regions (see Materials and methods) we tried to infer TFBSs located in their vicinity. These are associated to transcription factors that might take part in regulating the expression of the miRNA under investigation. We show for miRNAs a 'TFBS'-tab and therein a table with inferred TFBSs with associated significance values from the origianl source (if available).

Browsing and searching target genes

Users can search for ncRNA target genes in a search interface. A user needs to specify a target type (immunologically relevant mouse targets or immunologically relevant mouse and human targets) and can search using gene symbol, gene name, GeneID, or their parts. Search results are presented as a table in the 'Search results'-page section. The resulting table includes gene symbol, gene name, GeneID, the numbers of experimentally verified and predicted miRNAs, and the number of piRNAs and lncRNAs that target each found gene. By selecting the specific gene users can switch from the search results or the browsing page to an individual target view page which includes seven tabs.

There are three tabs that give information about targeting ncRNAs: the 'microRNAs'-, 'piRNAs'-, and 'lncRNAs'-tab. Each tab shows a table containing the ncRNAs that target the gene in question with additional information, like the source of the interaction.

The 'Pathways'- and 'GO'-tabs provide the pathways and processes associated with the target gene. Following the links users can switch to the IRNdb pathway (or GO) view or open the original pathway (or GO) source website.

The 'Experiments'-tab comprises MSigDB immunologically relevant experiments in which the target gene was found to be up- or down-regulated. Expression data for the experiment on *M.tb*-infected macrophages for the gene under investigation is presented (if measurable) in time-course graphs in the 'M.tb'-tab. Top panels show expression levels of the gene across different time points and stimulation types for infected and non-infected macrophages as log-transformed TPM values (see Materials and methods). Bottom panels show the differences between the gene expression in infected and non-infected macrophages for each time point as log2 fold-changes.

Browsing biological pathways

The IRNdb repository includes information on biological pathways with immunologically relevant ncRNA target genes. Users can reach the pathway data in three different ways by selecting the pathway from the ncRNA view's 'Pathways'-tab, from the target view's 'Pathways'-tab, or from the 'Browse Pathways'-pages in the navigation panel. On the 'Browse Pathways'-page for each pathway we provide a list of immunologically relevant experimentally verified ncRNA target genes and corresponding ncRNAs that target the genes. Following the link to the IRNdb pathway view, users can explore association maps of ncRNA-target interactions for immunologically relevant genes involved in the pathway of interest (see Figure 2). Furthermore, we show an image of the pathway with, in the case of KEGG pathways, highlighted ncRNA-targeted immunologically relevant genes.

Browsing *M.tb* experimental data

Following the 'Browse M.tb' link in the navigation panel under 'Target genes', users can browse immunologically relevant genes in the experiment on *M.tb*-infected macrophages (see above). We show six different tabs, one tab for each stimulation type and one 'Overview'-tab. For the 'Overview'-tab, we calculated for each gene log2 fold-change comparing *M.tb*-infected to non-infected macrophages for each time point within each stimulation type. The lowest log2 fold-change

value per stimulation across all time points is shown. For individual stimulation tabs, we show the log2 fold-changes for *M.tb*-infected to non-infected macrophages for each time-point separately.

Conclusion

Over the past years, research into ncRNA has increased significantly within the scientific community. For example, several miRNA databases with diverse scopes and goals have been recently constructed (70-73). Undoubtedly, these efforts lead to a better data accessibility and understanding of e.g. miRNA biology and function. One of the insights in miRNA control is that it is exerted in a tissue- and condition-specific manner. Hence, without a specialized resource, an investigation of miRNA roles under a certain condition might require time-consuming navigation between multiple data sources and gathering condition-specific literature information. The same is true for other types of ncRNAs.

To bridge the gap in the understanding of ncRNA control of the immune system we developed IRNdb. To our knowledge, this is the first resource to focus on immune-related murine ncRNAs and their target genes. IRNdb is an integrative, user-friendly and easy-to-use platform with a wide range of applications (see Figure 3). IRNdb brings together experimentally verified and predicted ncRNA-target interactions for genes relevant to immunological processes, enriched with pathway and experimental information, which will benefit ncRNA research and will help in the functional characterization of ncRNAs. In the future we aim at integrating information on additional types of ncRNAs and expand IRNdb to include more mammalian systems.

Figures

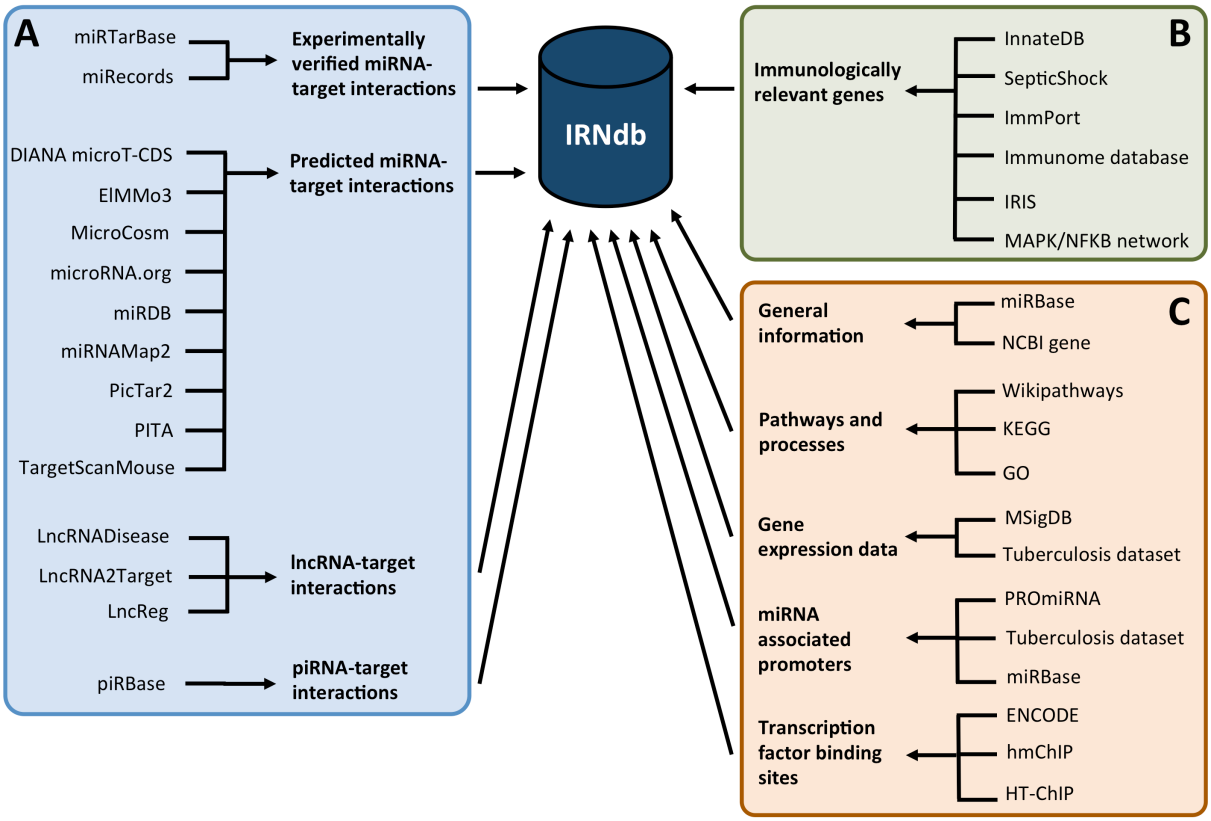

Figure 1. IRNdb construction: public domain data sources. (**A**) ncRNA-related information. (**B**) Information regarding immunologically relevant target genes. (**C**) Annotation data, e.g. general information regarding the biological entities, biological pathways, processes, gene expression data, transciption factor binding sites, etc.

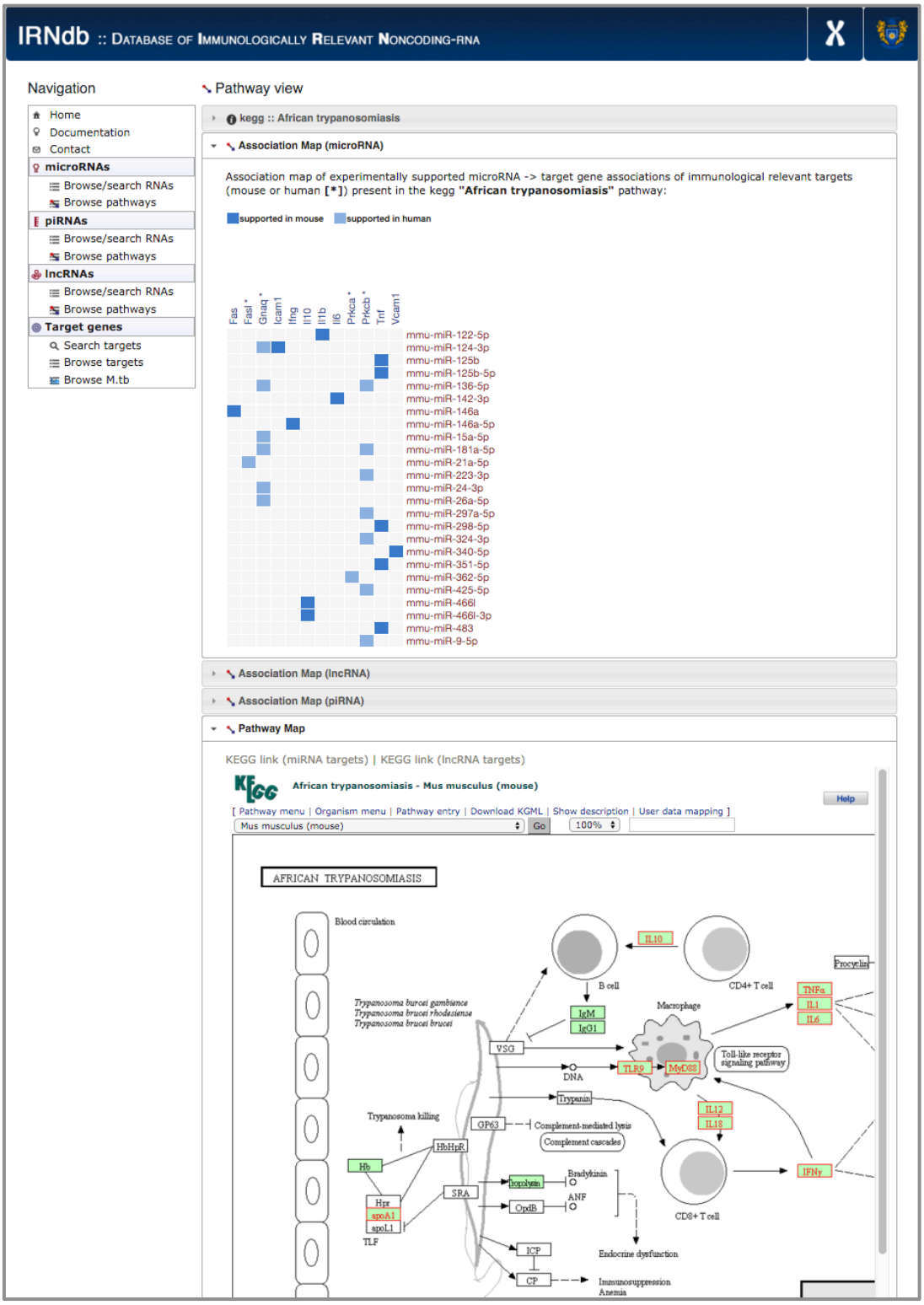

Figure 2. Example of a pathway-view (African trypanosomiasis). In the pathway view, ncRNAs associated to gene targets in the pathway are shown in form of a association map for each class of ncRNA seperately. Also shown is the KEGG pathway map with ncRNA-targeted genes highlighted.

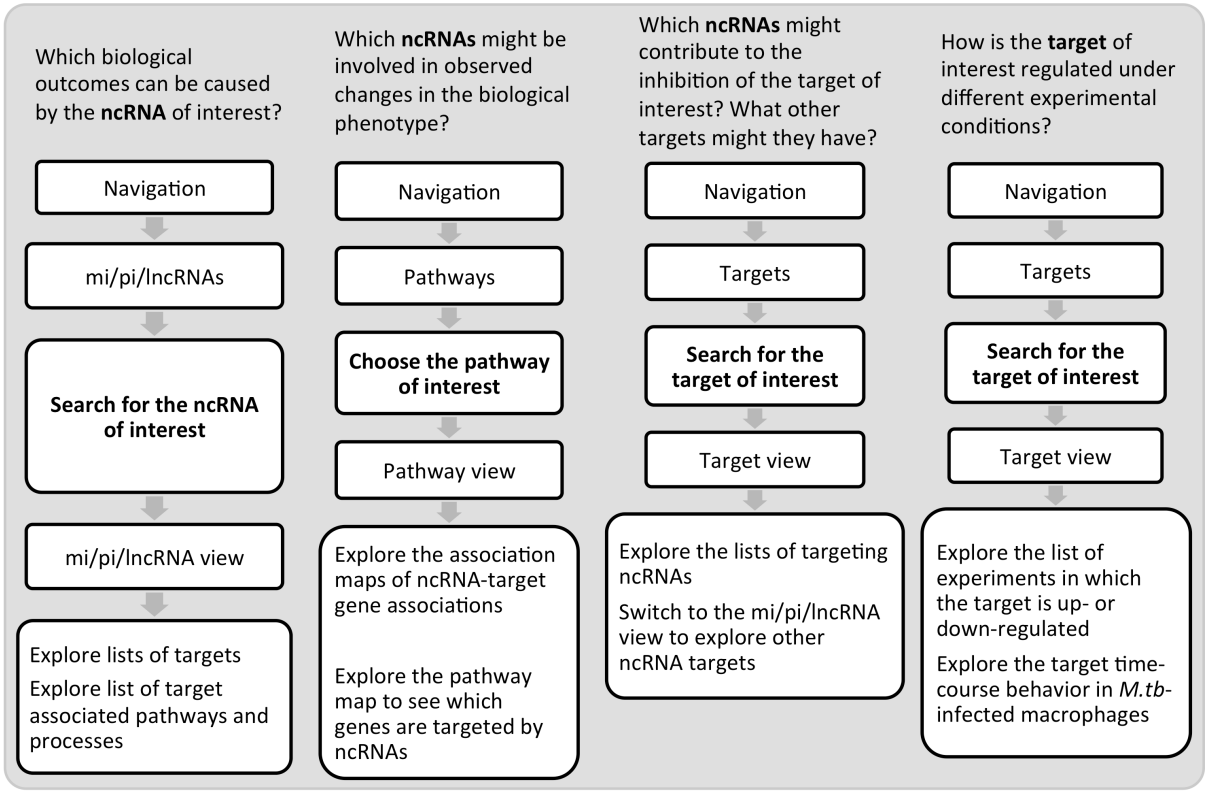

Figure 3. Examples of IRNdb applications in immunological research.

Tables

Table 1. IRNdb data repository statistics on ncRNA-target gene interactions.

	Immunological target information inferred from	
	Mouse	**Mouse+Human**
Experimentally verified *microRNA-target interactions*	1,350	3,799
MicroRNAs	170	265
Targets	576	1,633
Predicted *microRNA-target interactions*	386,409	1,188,681
MicroRNAs	1,798	1,802
Targets	1,752	5,487
Long non-coding-target interaction	7,771	22,453
lncRNAs	138	163
Targets	1,501	4,521
PIWI-interacting RNAs–target interactions	141	377
piRNAs	134	319
Targets	32	84

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