miRecords: an integrated resource for microRNA–target interactions

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ABSTRACT

MicroRNAs (miRNAs) are an important class of small noncoding RNAs capable of regulating other genes’ expression. Much progress has been made in computational target prediction of miRNAs in recent years. More than 10 miRNA target prediction programs have been established, yet, the prediction of animal miRNA targets remains a challenging task. We have developed miRecords, an integrated resource for animal miRNA–target interactions. The Validated Targets component of this resource hosts a large, high-quality manually curated database of experimentally validated miRNA–target interactions with systematic documentation of experimental support for each interaction. The current release of this database includes 1135 records of validated miRNA–target interactions between 301 miRNAs and 902 target genes in seven animal species. The Predicted Targets component of miRecords stores predicted miRNA targets produced by 11 established miRNA target prediction programs. miRecords is expected to serve as a useful resource not only for experimental miRNA researchers, but also for informatics scientists developing the next-generation miRNA target prediction programs. The miRecords is available at http://miRecords.umn.edu/miRecords.

INTRODUCTION

MicroRNAs (miRNAs) are a class of small (19–27 nt) noncoding RNAs capable of base-pairing to the transcripts of protein-coding genes (which are termed the targets of the miRNAs), leading to downregulation or repression of the targeted genes(1–3). The miRNA gene family is one of the largest in higher eukaryotes: more than 700 mature miRNAs have been identified in the human genome, according to the current release of miRBase (4), and these miRNAs account for >2.5% of all human genes. The exact mechanisms by which miRNAs regulate their target genes’ expression remain obscure, although several models have been proposed [see recent reviews, e.g. (5,6)], suggesting that miRNAs could induce translation repression at both the initiation phase and the elongation phase of translation. Alternatively, the translation may not be directly affected, but miRNAs could lead to rapid proteolysis of nascent polypeptides; or they may lead to accumulation of target miRNAs in the P-bodies, isolating them from the translation machinery (5,6). The miRNAs could also lead to degradation of the target transcripts. Recent evidence suggests that miRNA-induced target transcript degradation through a complex process that includes the deadenylation and decapping of mRNAs, distinct from the siRNA-induced RNA silencing mechanism (6,7).

Computational prediction of miRNA targets is much more challenging in animals than in plants, because animal miRNAs often form imperfect base-pairing with their target sites (3,8–11), unlike plant miRNAs which almost always bind their targets with near perfect complementarity (12). In the past several years, a large number of target prediction programs have been developed for animal miRNAs [see recent reviews, e.g. (8,10,11,13)]. Several of the earliest target prediction programs [including TargetScan (14)/TargetScanS (15), PicTar (16), miRanda (17,18), DIANA-microT (19) and MicroInspector (20)] adopted hand-derived rules based on a number of principles summarized from known miRNA–target interactions. These principles emphasize: (i) near-perfect complementarity in the 6–8 nt region close to the 5’ end of the miRNA (the so-called ‘seed’ region) with the 3’UTR region of the target sequence; (ii) evolutionary conservation of the target sequences between species; (iii) strong thermodynamic stability of miRNA–mRNA duplex; (iv) cooperativity between multiple sites in close proximity.
proximity; and (v) existence of a central nonmatched region (forming a loop or bulge). RNAhybrid (21), one of the earliest developed miRNA target prediction programs, implemented an efficient algorithm that finds energetically most favorable hybridizations between the miRNA and mRNA molecules, avoiding intramolecular hybridizations. A newer miRNA target prediction program, Rna22 (22), applied a procedure that first finds significant sequence motifs (or ‘patterns’) among all known miRNA sequences, then defines ‘target islands’, or regions in mRNAs where reverse complements of the miRNA patterns aggregate and focuses on these target islands when searching for miRNA target sites. Most recently developed miRNA target prediction programs [miTarget (23), MirTarget2 (24) and NBmiRTar (25)] employed machine learning techniques to construct predictors directly from validated miRNA target datasets. Furthermore, several recent studies suggested that target site accessibility is an important factor for effective targeting of miRNAs (26–28). One program, PITA, was developed to make predictions based on target site accessibility features.

Comparative studies conducted with the earlier miRNA target prediction programs (including TargetScanS, PicTar, miRanda, DIANA-microT, RNAhybrid, MicroInspector and Rna22) suggested that no one program was consistently superior to all others (13,29). Indeed, it has become a common practice for experimental researchers to look at predictions made by several target prediction programs and focus on their intersections (30,31). Machine learning methods have good potential to produce more accurate target predictors. Yet, recently developed machine-learning-based target prediction tools (miTarget, MirTarget2 and NBmiRTar) have not been through rigorous independent assessments. The performance of machine learning-based methods is known to be heavily dependent on the quantity and quality of the dataset used in the training. The lack of large, high-quality datasets of experimentally validated miRNAs will likely be the bottleneck for developing more accurate miRNA target prediction programs.

Here, we present miRecords, a new resource for animal miRNA–target interactions. miRecords consists of two components. The Validated Targets component is a large, high-quality database of experimentally validated miRNA targets resulting from meticulous manual literature curation. As the largest known collection of experimental validated miRNA targets, it emphasizes systematic and structured documentation of experimental support of miRNA–target interactions. This database not only serves the experimental researchers by providing the lists of confirmed targets of the miRNAs of their interest, but also provides a large and high-quality dataset that will facilitate the development of the next-generation miRNA target prediction programs. The Predicted Targets component of miRecords is an integration of predicted miRNA targets produced by 11 established miRNA target prediction programs. As the most complete integration of predicted miRNA targets, it is expected to provide considerable help to researchers investigating new miRNA targets.
product (mRNA or protein) of a putative target has reduced following over- or misexpression of a miRNA, or that the full-length gene product has accumulated following underexpression of the miRNA, it is considered as target gene level evidence. The target gene level evidence also includes endogenous miRNA experiments leading to the finding of inverse correlations between the endogenous miRNA levels and the full-length protein products of the putative target genes. The target gene level experiments are often regarded as indirect support of the miRNA–target interactions, because the level of the gene product may change due to other reasons, e.g. a change in expression of another protein (which is a true target of the miRNA) that it interacts with.

When the experimental evidence indicates that a region of the mRNA of the putative target (shorter than the full-length transcript) is responsible for the miRNA–target interaction, it is documented as target region level evidence. Most target region level experiments were conducted with fusion of the 3′UTR of the putative target gene (or a section of the 3′UTR) to a reporter construct (e.g. luciferase or GFP), followed by observations that the reporter expression is downregulated (or upregulated) in response to overexpression/misexpression (or underexpression) of the miRNA.

When an experiment points to a very short section of the mRNA (whose length is comparable with that of the miRNA) as being responsible for the miRNA–target interaction, it is classified as target site level evidence. The target site level experiments include reporter assays with fusion constructs made with short target sites, and target site mutation experiments (discussed below).

Over- or misexpression and underexpression of miRNAs. The exogenous miRNA experiments can be broadly classified into two categories based on the methods by which the miRNA levels are manipulated: miRNA overexpression or misexpression experiments, and miRNA underexpression experiments. The methods commonly applied to over- or misexpress miRNAs include mature miRNA transfection (38), miRNA precursor transfection (39) and indirectly induced miRNA overexpression [using the DNA demethylating agent 5-Aza-Deoxycytidine (40), or the histone deacetylase inhibitor phenylbutyrate (PBA) (41)]. The techniques applied to underexpress miRNAs include miRNA knockdown by siRNAs (42), miRNA knockdown by antisense modified oligonucleotides, e.g. morpholinos (43), locked nucleic acids (44), or 2′-O-Me oligonucleotides (45) and knockout of the miRNA gene (46).

Reporter assays, mRNA- and protein-level measurements. The means by which putative target expression levels are examined can be classified into four categories: reporter assays, mRNA-level measurements, protein-level measurements and ‘others’. In a reporter assay, the putative target region or target site is fused with a reporter vector, and the expression level of the putative target region or target site is quantified by measuring the reporter’s activity. Commonly used reporters include luciferase (16), GFP (47), YFP (43) and the lacZ/β-galactosidase reporter (48). Several methods of measuring mRNA levels of putative targets can be applied. They include RT–PCR (28), northern blot (49), 5′RACE (50), DNA microarrays (51), ribonuclease protection assay (52) and branched DNA probe assay (53). Commonly applied protein-level measuring methods include western blot (54), ELISA (55) and immunocytochemistry (56). The ‘others’ category includes rare target expression analyses that do not belong to other categories, e.g. phenotype analysis (43).

Target site mutations. In a target site mutation experiment, point mutations are introduced to the putative target site. If the introduced point mutations lead to abolishment of the miRNA-mediated downregulation, the site is convincingly verified as a true target site. Besides validating miRNA target sites, target site mutation experiments are frequently conducted in studies investigating general features that influence the miRNA targeting, e.g. it was applied to study the importance of the 5′ seed region for miRNA–target interactions (9), and of target accessibility features in assisting miRNA target discovery (26).

Web interface

The miRecords resource can be accessed through the URL http://miRecords.umn.edu/miRecords. In the Validated Targets section, the user can first select a species, then choose from a list of miRNAs for which experimentally validated targets have been documented. Optionally, the user can provide the RefSeq accession, the Entrez Gene ID or the gene name of the target gene and initiate a search. The result of the search is presented as a list of miRNA–target interactions. For each miRNA–target interaction, the information about the miRNA and about the target gene is displayed together with the prediction results of 11 established miRNA target prediction programs. Positive predictions are indicated by lit up symbols. The user can click a lit up symbol to obtain more information about the prediction.

When the user clicks the ‘Click for detail’ link displayed in the ‘Target Interaction’ column, detailed information about a validated miRNA–target interaction is presented. The detailed information about the miRNA includes the Stemloop ID and accession number, mature miRNA ID and accession number and the sequence of the miRNA. The detailed information about the target gene includes the RefSeq accession number, name, synonyms and the description of the gene.

Underneath the general information about the miRNA and the target gene, all records, or literature accounts of the miRNA–target interaction are presented. On the top of each record is the citation information, underneath which is the experimental support of the miRNA–target interaction, listed in the order of the target gene level evidence, followed by the target region level evidence, then by the target site level evidence. In each of the three levels of evidence, endogenous miRNA experiments (when available) are described first, followed by summaries of exogenous miRNA experiments. The summary of a target gene level, exogenous miRNA experiment includes...
the method of manipulating the miRNA level (e.g. ‘over-expression by mature miRNA transfection’, or ‘under-expression by siRNA knock-down’), reporter assay performed (e.g. luciferase assay), mRNA-level measurement performed (e.g. RT–PCR or 5′RACE), mRNA-level measurement result (e.g. ‘down-regulated’), protein-level measurement performed (e.g. western blot) and protein-level measurement result. The user can click the ‘Detail’ button for each individual experiment, and the description of the experiment from the original publication is displayed.

The summary of target gene level or target site level evidence also includes the section of the mRNA that was used in the fusion reporter assay, and about whether point mutation experiments were performed. When available, a drawing of the alignment between the miRNA and the putative target site is also presented.

The user can download the complete dataset of validated targets at the Download Validated Targets section accessible from the top of the web site.

The Predicted Targets section is organized similarly to the Validated Targets section. When the user submits a query, all putative targets (including unvalidated ones) predicted by one or more of the established miRNA target prediction programs are presented. The user can choose to display the result according to the predictions made by a particular target prediction program, and/or apply a filter to display putative targets predicted by at least a user-specified number of prediction programs. Moreover, an option is provided for the user to download the predicted targets of interest to his or her local computer for further examination.

Data access

The miRecords web site is publically accessible through the URL http://miRecords.umn.edu/miRecords. Additional requests can be made by emailing to miRecords@biocompute.umn.edu.

| Tool            | URL                                      | DIANA-microT | Micro-Inspector | miRanda       | mi-Target | Mir-Target2 | NBmir-Tar | Pic-Tar | PITA | RNA 22 | RNA Hybrid | TargetScan/TargetScanS |
|-----------------|------------------------------------------|--------------|----------------|---------------|-----------|-------------|-----------|---------|------|--------|------------|------------------------|
| miRGator (58)   | http://genome.ewha.ac.kr/miRGator.html  | ✓            |                |               |           |             |           |         |      |        |            |                        |
| miRGen (31)     | http://www.diana.pcbi.upenn.edu/miRGen.html | ✓            |                |               |           |             |           |         |      |        |            | ✓                      |
| MiRNAMap (59)   | http://mirnamap.mbc.ntcu.edu.tw/         | ✓            |                |               |           |             |           |         |      |        |            | ✓                      |
| miRecords       | http://miRecords.umn.edu/miRecords      | ✓            | ✓              | ✓             | ✓         | ✓           | ✓         | ✓       | ✓    | ✓      | ✓          | ✓                      |
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REFERENCES

1. Bartel,D.P. (2004) MicroRNAs: genomics, biogenesis, mechanism, and function. Cell, 116, 281–297.
2. Kim,V.N. (2005) MicroRNA biogenesis: coordinated cropping and dicing. Nat. Rev. Mol. Cell Biol., 6, 376–385.
3. Tochio,A., Stanley,S., Tuschl,T., Sander,C. and Enright,A.J. (2008) miRBase: tools for microRNA genomics. Nucleic Acids Res., 36, D154–D158.
4. Jackson,R.J. and Standart,N. (2007) How do microRNAs regulate gene expression? Sci. STKE, re1.
5. Pillai,R.S., Bhattacharyya,S.N. and Filipowicz,W. (2007) Repression of protein synthesis by miRNAs: how many mechanisms? Trends Cell Biol., 17, 118–126.
6. Reh wiklenk,J., Behm-Ansmnt,J., Gathfield,D. and Izzurralde,E. (2005) A crucial role for GW182 and the DCP1/DCP2 decapping complex in microRNA-mediated gene silencing. RNA, 11, 1640–1647.
7. Audhuri,K. and Chatterjee,R. (2007) MicroRNA detection and target prediction: integration of computational and experimental approaches. DNA Cell Biol., 26, 321–337.
8. Doencht,J.G. and Sharp,P.A. (2004) Specificity of microRNA target selection in translational repression. Genes Dev., 18, 504–511.
9. Doran,J. and Strauss,W.M. (2007) Bio-informatic trends for the identification of microRNA targets. DNA Cell Biol., 26, 353–360.
10. Lindow,M. and Gorodkin,J. (2007) Principles and limitations of computational microRNA gene and target finding. DNA Cell Biol., 26, 339–351.
11. Jones-Rhoades,M.W. and Bartel,D.P. (2004) Computational identification of plant microRNAs and their targets, including a stress-induced miRNA. Mol. Cell, 17, 787–799.
12. Rajewsky,N. (2006) microRNA target predictions in animals. Nat. Genet., 38 (Suppl.), S8–S13.
13. Lewis,B.P., Shih,I.H., Jones-Rhoades,M.W., Bartel,D.P. and Burge,C.B. (2003) Prediction of mammalian microRNA targets. Cell, 115, 787–798.
14. Lewis,B.P., Burge,C.B. and Bartel,D.P. (2005) Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Cell, 120, 15–20.
15. Krek,A., Grun,D., Poy,M.N., Wolf,R., Rosenberg,L., Epstein,E.J., MacMenamin,P., da Piedade,I., Gunsalus,K.C., Stoffel,M. et al. (2005) Combinatorial microRNA target predictions. Nat. Genet., 37, 495–500.
16. Enright,A.J., John,B., Gaul,U., Tuschl,T., Sander,C. and Marks,D.S. (2003) MicroRNA targets in Drosophila. Genome Biol., 5, R1.
17. John,B., Enright,A.J., Aravin,A., Tuschl,T., Sander,C. and Marks,D.S. (2004) Human MicroRNA targets. PLoS Biol., 2, e363.
18. Kiriakidou,M., Nsoso,P.T., Kouranov,A., Fitziev,P., Bouyioukos,C., Mouloumatou,Z. and Hatzigeorgiou,A. (2004) A combined computational-experimental approach predicts human microRNA targets. Genes Dev., 18, 1165–1178.
19. Rusanov,V., Baev,V., Minkov,I.N. and Tabler,M. (2005) MicroInspector: a web tool for detection of miRNA binding sites in an RNA sequence. Nucleic Acids Res., 33, W969–W700.
20. Rehmsmeier,M., Steffen,P., Hochsmann,M. and Giegerich,R. (2004) Fast and effective prediction of microRNA/target duplexes. RNA, 10, 1507–1517.
21. Miranda,K.C., Huynh,T., Tay,Y., Ang,Y.S., Tam,W.L., Thomson,A.M., Lim,B. and Rigoutsos,I. (2006) A pattern-based method for the identification of MicroRNA binding sites and their corresponding heteroduplexes. Cell, 126, 1203–1217.
22. Kim,K.C., Nam,J.W., Rhee,J.K., Lee,W.J. and Zhang,B.T. (2006) miTarget: microRNA target gene prediction using a support vector machine. BMC Bioinformatics, 7, 411.
23. Wang,X. and El Naqa,I.M. (2008) Prediction of both conserved and nonconserved microRNA targets in animals. Bioinformatics, 24, 325–332.
24. Yousef,J., Jung,S., Kossenkov,A.V., Shoewa,L.C. and Shoewa,M.K. (2007) Naive Bayes for microRNA target predictions–machine learning for microRNA targets. Bioinformatics, 23, 2987–2992.
25. Kertesz,Z., Iovino,N., U nerstall,U., Gaul,U. and Segal,E. (2007) The role of site accessibility in microRNA target recognition. Nat. Genet., 39, 1278–1284.
26. Long,D., Lee,R., Williams,P., Chan,C.Y., Ambros,V. and Ding,Y. (2007) Potent effect of target structure on microRNA function. Nat. Struct. Mol. Biol., 14, 287–294.
27. Zhao,Y., Samal,E. and Srivastava,D. (2005) Serum response factor regulates a muscle-specific microRNA that targets Hand2 during cardiogenesis. Nature, 436, 214–220.
28. Sethupathy,P., Megraw,M. and Hatzigeorgiou,A.G. (2006) A guide through present computational approaches for the identification of mammalian microRNA targets. Nat. Methods, 3, 881–886.
29. Sonkoly,E., Wei,T., Janson,P.C., Saaf,A., Lundeberg,L., Bengvall-Linder,M., Norstedt,G., Alenius,H., Homey,B., Lines,R. and Scheynius,A. (2007) MicroRNAs: novel regulators involved in the pathogenesis of Psoriasis? PLoS ONE, 2, e610.
30. Megraw,M., Sethupathy,P., Corda,B. and Hatzigeorgiou,A.G. (2007) miRGen: a database for the study of animal microRNA genomic organization and function. Nucleic Acids Res., 35, D149–D155.
31. Rajewsky,N. (2006) L(ou)sy microRNA targets? Nat. Struct. Mol. Biol., 13, 754–755.
32. Didiano,D. and Hobert,O. (2006) Perfect seed pairing is not a generally reliable predictor for miRNA-target interactions. Nat. Struct. Mol. Biol., 13, 849–851.
33. Johnson,S.M., Grosshans,H., Shingara,J., Byrom,M., Jarvis,R., Cheng,A., Labourier,E., Renert,K.L., Brown,D. and Slack,F.J. (2005) rAS is regulated by the let-7 microRNA family. Cell, 120, 635–647.
34. Wang,W.X., Rajeev,B.W., Stromberg,A.J., Ren,N., Tang,G., Huang,Q., Rigoutsos,I. and Nelson,P.T. (2008) The expression of microRNA miR-107 decreases early in Alzheimer’s disease and may accelerate disease progression through regulation of beta-site amyloid precursor protein-cleaving enzyme 1. J. Neurosci., 28, 1213–1222.
35. Asangani,I.A., Rasheed,S.A., Nikolova,D.A., Leupold,J.H., MacMenamin,P., da Piedade,I., Gunsalus,K.C., Stoffel,M. et al. (2007) Genetic unmasking of an epigenetically silenced microRNA in human cancer cells. Cancer Res., 67, 635–647.
36. Lujambio,A., Ropero,S., Ballestar,E., Fraga,M.F., Cerrato,C., Setien,F., Casado,S., Suarez-Gauthier,A., Sanchez-Cespedes,M., Cortina,E. et al. (2007) Genetic unmasking of an epigenetically silenced microRNA in human cancer cells. Cancer Res., 67, 1424–1429.
41. Saito,Y., Liang,G., Egger,G., Friedman,J.M., Chuang,J.C., Coetzee,G.A. and Jones,P.A. (2006) Specific activation of microRNA-127 with downregulation of the proto-oncogene BCL6 by chromatin-modifying drugs in human cancer cells. Cancer Cell, 9, 435–443.

42. Nakamoto,M., Jin,P., O’Donnell,W.T. and Warren,S.T. (2005) Physiological identification of human transcripts translationally regulated by a specific microRNA. Hum. Mol. Genet., 14, 3813–3821.

43. Woltering,J.M. and Durston,A.J. (2008) MiR-10 represses HoxB1a and HoxB3a in zebrafish. PLoS ONE, 3, e1396.

44. Elmen,J., Lindow,M., Silahtaroglu,A., Bak,M., Christensen,M., Lind-Thomsen,A., Hedtjarn,M., Hansen,J.B., Hansen,H.F., Straarup,E.M. et al. (2008) Antagonism of microRNA-122 in mice by systemically administered LNA-antimiR leads to up-regulation of a large set of predicted target mRNAs in the liver. Nucleic Acids Res., 36, 1153–1162.

45. Leaman,D., Chen,P.Y., Fak,J., Yalcin,A., Pearce,M., Unnerstall,U., Marks,D.S., Sander,C., Tuschl,T. and Gaul,U. (2005) Antisense-mediated depletion reveals essential and specific functions of microRNAs in Drosophila development. Cell, 121, 1097–1108.

46. Vigorito,E., Perks,K.L., Abreu-Goodger,C., Bunting,S., Xiang,Z., Kohlhaas,S., Das,P.P., Miska,E.A., Rodriguez,A., Bradley,A. et al. (2007) microRNA-155 regulates the generation of immunoglobulin class-switched plasma cells. Immunity, 27, 847–859.

47. Visvanathan,J., Lee,S., Lee,B., Lee,J.W. and Lee,S.K. (2007) The microRNA miR-124 antagonizes the anti-neural REST/SCP1 pathway during embryonic CNS development. Genes Dev., 21, 744–749.

48. Grosshans,H., Johnson,T., Reinert,K.L., Gerstein,M. and Slack,F.J. (2005) The temporal patterning microRNA let-7 regulates several transcription factors at the larval to adult transition in C. elegans. Dev. Cell., 8, 321–330.

49. Hossain,A., Kuo,M.T. and Saunders,G.F. (2006) Mir-17-5p regulates breast cancer cell proliferation by inhibiting translation of AIB1 mRNA. Mol. Cell Biol., 26, 8191–8201.

50. Davis,E., Caiment,F., Tordoir,X., Cavaille,J., Ferguson-Smith,A., Cockett,N., Geoges and Charlier,C. (2005) RNAi-mediated allelic trans-interaction at the imprinted Rtl1/Peg11 locus. Curr. Biol., 15, 743–749.

51. Lim,L.P., Lau,N.C., Garrett-Engele,P., Grimson,A., Schelter,J.M., Castle,J., Bartel,D.P., Linsley,P.S. and Johnson,J.M. (2005) Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs. Nature, 433, 769–773.

52. Yekta,S., Shih,I.H. and Bartel,D.P. (2004) MicroRNA-directed cleavage of HOXB8 mRNA. Science, 304, 594–596.

53. Sethupathy,P., Corda,B. and Hatzigeorgiou,A.G. (2006) TarBase: a comprehensive database of experimentally supported animal microRNA targets. RNA, 12, 192–197.

54. Nam,S., Kim,B., Shin,S. and Lee,S. (2008) miRGator: an integrated system for functional annotation of microRNAs. Nucleic Acids Res., 36, D159–D164.

55. Hu,H., Chu,C.H., Tsou,A.P., Chen,S.J., Chen,H.C., Hsu,P.W., Wong,Y.H., Chen,Y.H., Chen,G.H. and Huang,H.D. (2008) miRNAMap 2.0: genomic maps of microRNAs in metazoan genomes. Nucleic Acids Res., 36, D165–D169.