MicroRNAs – targeting and target prediction

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Abstract

MicroRNAs (miRNAs) are a class of small noncoding RNAs that can regulate many genes by base pairing to sites in mRNAs. The functionality of miRNAs overlaps that of short interfering RNAs (siRNAs), and many features of miRNA targeting have been revealed experimentally by studying miRNA-mimicking siRNAs. This review outlines the features associated with animal miRNA targeting and describes currently available prediction tools.

1. Introduction

MicroRNAs (miRNAs) were identified as a large sub-class of ncRNAs in 2001. Since then, an increasing number of studies have firmly established miRNAs’ importance in gene regulation in general and animal development and disease in particular [1–5]. miRNAs regulate protein-coding genes post-transcriptionally by guiding a protein complex known as the RNA-induced silencing complex (RISC) to messenger RNAs (mRNAs) with partial complementarity to the miRNA [6]. Through mechanisms not completely understood, RISC then inhibits protein translation and causes mRNA degradation [7, 8]. Current estimates indicate that miRNAs regulate at least 60% of the human protein-coding genes through this post-transcriptional gene silencing (PTGS) [9].

Incorporated into RISC, miRNAs are functionally equivalent to short interfering RNAs (siRNAs) [10, 11]. The main difference between these RNAs is that miRNAs are processed from imperfect hairpin structures, whereas siRNAs are processed from long double-stranded RNAs [12, 13]. Moreover, animal miRNAs typically target imperfect sites, whereas siRNAs target sites with near-perfect complementarity. siRNAs do target imperfect sites as well, however, and this miRNA-like targeting is the major source of siRNA off-target effects [14–16].

The list of known miRNAs is large and increasing. Currently, the official miRNA database miRBase lists 721 human miRNAs (http://www.mirbase.org; Release 14) [17], but estimates indicate that the human genome contains more than 1000 miRNAs. As only a few regulatory targets are known, predicting and validating miRNA targets is one of the major hurdles in understanding miRNA biology. Here, we review the features important for miRNA targeting and the bioinformatics tools available for predicting miRNA targets.

2. miRNA target features

Identifying miRNA targets in animals has been very challenging. This is mainly because the limited complementarity between miRNAs and their targets, which might lead to the finding of hundreds of potential miRNA targets per miRNA. Therefore, many studies have been conducted, both experimentally and computationally, to reveal more efficient approaches for miRNA target recognition. We have divided the miRNA target features reported in these studies into six categories, miRNA:mRNA pairing, site location, conservation, site accessibility, multiple sites and expression profile.

2.1. miRNA:mRNA pairing: ‘Seed site’ is the most important feature for target recognition

miRNA targets commonly have at least one region that has Watson-Crick pairing to the 5′ part of miRNA. This 5′ part, located at positions 2-7 from the 5′ end of miRNA, is known as the ‘seed’, as RISC uses these positions as a nucleation signal for recognizing target mRNAs [18–20]. The corresponding sites in mRNA are referred to as ‘seed sites’. A stringent-seed site has perfect Watson-Crick pairing and can be divided into four ‘seed’ types – 8mer, 7mer-m8, 7mer-A1 and 6mer – depending on the combination of the nucleotide of position 1 and pairing at position 8 (Fig. 1a). 8mer has both an adenine at position 1 of the target site and base pairing at position 8. 7mer-A1 has an adenine at position 1, while 7mer-m8 has base pairing at position 8. 6mer has neither an adenine at position 1 nor base pairing at position 8 [21]. An adenine on the target site corresponding to position 1 of miRNA is known to increase efficiency of target recognition [22].

In addition to this stringent-seed matching, moderate-stringent-seed matching is also functional because RISC can tolerate small mismatches or G:U wobble pairing within the seed region (Fig. 1b). This moderate-stringent-seed matching has five ‘seed’ types: GUM, GUT, BM, BT and LP, defined regarding to the mismatch type. GUM has one G:U wobble and the uracil on the seed site of miRNA, whereas GUT has the uracil on the target site of mRNA. BM has one bulge and...
2.2. Site location: most target sites reside within 3′ untranslated region (UTR) of target genes

Several studies have reported that most target sites can be found in the 3′ UTR segment of the target genes, even though miRNA-loaded RISC in theory can bind any segment of mRNA. Target genes tend to have longer 3′ UTR, whereas ubiquitously expressed genes, such as house-keeping genes, have shorter 3′ UTR – potentially to avoid being regulated by miRNAs [26]. Target sites are not evenly distributed within 3′ UTR, but are located near both ends when the length of 3′ UTR is ≥2000 nucleotides. For shorter 3′ UTRs, sites tend to be near the stop codon [23]. Sites are not located too close to the stop codon, however, but 15-20 nucleotides away from the stop codon [21]. In addition, some genes have alternative splicing in their 3′ UTR segments, especially genes with long 3′ UTRs. These genes might therefore have different potential target sites for alternatively spliced 3′ UTRs [27]. Finally, alternative polyadenylation sites can shorten 3′ UTRs and affect miRNA regulation [28].

Although functional miRNA sites are preferentially located in 3′ UTR, seed sites in the coding sequence (CDS) and 5′ UTR regions can also give downregulation [29, 30]. Why does RISC then appear to prefer the 3′ UTR? The most probable explanation is that RISC competes with other protein complexes, such as ribosomes in CDS and translation initiation complexes in 5′ UTR; see discussion in the following section ‘Multiple sites: cooperativity enhances site efficacy’. The 3′ UTR might simply be more accessible for long-term binding than the two other mRNA regions [5].

Despite this general trend for 3′ UTR targeting, there are some notable exceptions. One recent study reported that many miRNAs preferentially target 5′ UTR sites with high complementarity to the miRNAs’ 3′ end in a species-specific manner [31]. The targets also showed signs of weaker interactions between the miRNA seed sequence and the 3′ UTR. The authors proposed that these sites represented a new miRNA target class called ‘miBridge’, in which one miRNA simultaneously interacts with a seed pairing site in 3′ UTR and a 3′ pairing site in 5′ UTR. The molecular mechanisms behind and the biological extent of these miBridge targets are still unknown, however.

Most miRNA target prediction studies only focus on the 3′ UTR, which results in that all the available data are biased toward 3′ UTR. Moreover, few studies consider alternative splicing or polyadenylation because of shortcomings in current annotations. As transcript usage often depends on cellular context – for example, whether the cell is proliferating or terminally differentiated – future tools for miRNA target analyses should probably use available information about cellular state to increase prediction performance.

2.3. Conservation: miRNAs and their targets are conserved among related species

miRNA families are comprised of miRNAs that have the same seed site, and are well conserved among related species. In addition, miRNA families have targets that are conserved among related species [9]. There are also species-specific miRNAs and targets, and one study showed that about 30% of the
experimentally validated target genes might not be well con-
erved [32].

siRNA off-target effects occur no matter whether the site is 
conserved or not [33], therefore searching for all potential tar-
get sequences without considering evolutionally conservational 
might increase siRNA off-target detection efficacy.

Applying a filter that requires predicted target sites to be con-
served can decrease the false-positive rate, but such a filter is ef-
fective only for conserved miRNAs. It is important to identify 
targets both with and without conservation – especially when 
species-specific miRNAs or siRNA off-targets is of interest.

2.4. Site accessibility: mRNA secondary structure affects site 
accessibility

The mRNA secondary structure is very important for miRNA 
targeting. An effective miRNA:mRNA interaction needs an 
open structure on the target site to begin the hybridization rea-
tion. After binding, RISC can disrupt the secondary structure 
on the site to elongate hybridization [34, 35]. Minimum free 
ergy is usually used to estimate the secondary structure and 
RNA hybridization, but the amount of A:Us surrounding the 
site can also be used to estimate the site accessibility. Effective 
target sites often have A:U rich context in approximately 30 
nucleotides upstream and downstream from the seed matching 
region of the target site [21].

Calculating the minimum free energy of accessibility and hy-
bridization with the mRNA secondary structure requires ana-
lyzing different mRNA folding patterns. This requires enorm-
ous amounts of computing power, as finding the most stable 
RNA structure is a computational problem that scales with the 
cube of the length of the RNA sequence [36]. Hence, finding 
hybridization sites in long 3’ UTRs tends to be time consum-
ing. Moreover, the current thermodynamic models used in RNA 
secondary structure prediction algorithms are only 90-95% ac-
curate, which results in that the algorithms tend to have only 
50-70% of the base pairs correct [36]. Thus, despite being the-
etically sound, calculating site accessibility has limited prac-
tical value when predicting miRNA target sites; heuristics that 
are easy to compute, such as local A:U context, have similar 
performance.

2.5. Multiple sites: cooperativity enhances site efficacy

Strong miRNA targets tend to have multiple target sites in-
stead of one single site [37]. Considering the number of pu-
tative miRNA sites per mRNA can therefore significantly en-
han ce target prediction.

Although the general effect of multiple sites appears to be 
additive, miRNA targeting can also be synergistic. Our pre-
vious study showed that two target sites within optimal distance 
enhance target site efficacy. The preferable optimal length is 
between 17 and 35 nucleotides, but the length between 14 and 
46 nucleotides also enhances the efficacy (Fig. 1d). This co-
operability is functional between the same miRNAs as well as 
two different miRNAs [25]. Multiple sites involving more than 
two sites can also contribute to the enhancement [38].

The exact mechanism underlying the synergism remains un-
known. As translational suppression is a relatively slow process 
compared with RISC’s catalytic cleavage [39], however, multi-
ple RISC complexes bound at closely spaced target sites might 
cooperatively stabilize each other at the sites or possibly accel-
erate the regulatory process. This could explain why miRNAs 
prefer targets in 3’ UTRs, as ribosomes would displace RISC 
from sites in CDS before RISC could effect translational sup-
pression. Indeed, a cluster of rare codons that stall the ribo-
some can, when placed in front of a nonfunctional miRNA site 
in CDS, change the site to a functional site [40]. Moreover, the 
genes that currently have verified miRNA target sites in CDS 
tend to have either one very strong target site [41, 42] or multi-
ple, closely spaced sites [43, 44].

2.6. Expression profile: miRNA:mRNA pairs are negatively 
correlated in expression profiles

One miRNA can potentially regulate many genes; therefore, 
expression profiles of miRNAs might vary substantially depend-
ing on the miRNA expression levels. Many miRNAs are also 
expressed differently in different tissues. Consequently, if nega-
tively correlated expression levels of a miRNA:mRNA pair are 
detected across different tissue profiles, the mRNA of the pair 
is probably targeted by the miRNA [45, 46]. Filtering putative 
targets based on expression profile correlations is an effective 
approach to reduce the false-positive rate. Although the major-
ity of miRNA targets appear to be regulated both at the mRNA 
and protein level, some targets only show an effect at the pro-
tein level, however [47, 48]. Researchers should therefore be 
aware that such filtering will exclude potential targets.

3. Target prediction tools

Many target prediction tools have been developed (Table 1), 
but the types of methods applied, the miRNA and mRNA se-
quenc es used and the output prediction data and performance 
evaluation vary widely between tools. Direct comparison of 
prediction performance among tools is not straightforward be-
cause the set of predicted target genes from different tools do 
not overlap well. What is clear, however, is that conventional 
tools with simple stringent-seed search are prone to high false-
positive rates. Therefore, most tools are designed to reduce 
the false-positive rate and maximize the accuracy at the sam-
time. We have compared the currently available tools based on 
the target features the tools use in their predictions (Table 1), 
and the tools’ availability (Table 2). Availability is especially 
important for researchers that are using their own miRNA or 
mRNA annotations, or are working in a nonstandard species. 
In these cases, only tools that can be downloaded or allows the 
user to input own miRNAs and mRNAs can be used.

Most tools rely on either one or a combination of seed match-
ing, site accessibility and evolutionary conservation features, 
although some recently developed tools use expression profiles. 
No tools have successfully incorporated some of the important 
features, such as optimal distances of multiple miRNA sites or 
supplementary sites in CDS and 5’ UTR.

TargetScan [9, 21, 22], PicTar [49–52], miRanda [37, 53], 
RNAhybrid [56, 57] and PITA [35] have been frequently used
Table 1: List of miRNA target prediction tools

| Tool               | Pair\(^a\) | Site\(^b\) | Consv\(^c\) | Access\(^d\) | Multi\(^e\) | Expri\(^f\) | Refs    |
|--------------------|------------|------------|-------------|--------------|-------------|------------|---------|
| TargetScan         | o          | •          | •           | o            | o           | [9, 21, 22]|
| PicTar             | •          | o          | •           | •            | o           | [49–52]   |
| miRanda            | •          | o          | •           | o            | o           | [37, 53]  |
| MicroCosm Targets  | •          | o          | •           | o            | o           | [17, 54, 55]|
| RNAhybrid          | •          | o          | •           | o            | o           | [56, 57]  |
| PITA               | •          | •          | o           | o            | o           | [35]      |
| STarMir            | •          | o          | •           | o            | o           | [34]      |
| Rajewsky & Socci   | •          | o          | •           | o            | o           | [19]      |
| Robins             | •          | •          | o           | o            | o           | [58]      |
| mirWIP             | •          | o          | •           | o            | o           | [24]      |
| MicroInspector     | •          | o          | •           | o            | o           | [59]      |
| MicroTar           | •          | o          | •           | o            | o           | [60]      |
| MirTarget2         | o          | •          | •           | o            | o           | [61]      |
| miTarget           | •          | o          | •           | o            | o           | [62]      |
| TargetMiner        | •          | o          | •           | o            | o           | [63]      |
| EIMMo              | •          | o          | •           | o            | o           | [23]      |
| NbmiRTar           | •          | o          | •           | o            | o           | [64]      |
| TargetBoost        | •          | o          | •           | o            | o           | [65]      |
| RNA22              | •          | o          | •           | o            | o           | [66]      |
| TargetRank         | o          | o          | •           | o            | o           | [67]      |
| EMBL               | •          | o          | •           | o            | o           | [18][26][68]|
| MovingTarget       | •          | o          | •           | o            | o           | [69]      |
| DIANA-microT       | •          | o          | •           | o            | o           | [70]      |
| HOCTAR             | •          | o          | •           | o            | o           | [71]      |
| Stanhope           | •          | o          | •           | o            | o           | [72]      |
| GenMiR++           | o          | o          | •           | o            | o           | [73]      |
| HuMiTar            | •          | o          | •           | o            | o           | [74]      |
| MirTif             | •          | o          | •           | o            | o           | [75]      |
| Yan et al.         | •          | o          | •           | o            | o           | [76]      |
| Xie et al.         | o          | o          | •           | o            | o           | [77]      |

\(^a\)miRNA:mRNA pairing. •: stringent seeds, ◦: moderately stringent seeds, Blank: seed sites not considered.
\(^b\)Site location. •: target positions considered, Blank: target positions not considered.
\(^c\)Conservation. •: with/without conservation filter, ◦: with conservation filter, Blank: conservation not considered.
\(^d\)Site accessibility. •: site accessibility with minimum free energy considered, ◦: A:U rich flanking considered, Blank: site accessibility not considered.
\(^e\)Multiple sites. •: multiple sites considered, ◦: the number of putative sites consided, Blank: multiple co-operability not considered.
\(^f\)Expression profile. •: expression profiles used, Blank: expression profiles not used.

Finding true functional miRNA targets is still challenging even though many biological features of miRNA targeting have been revealed experimentally and computationally. Building a model with more features might achieve higher accuracy and enhance site recognition efficacy, but its implementation might also become more complex. None of the existing prediction tools has been able to incorporate all currently known features. We expect that a new approach that can combine the features from the six categories we have shown will significantly improve computational miRNA target prediction.

Another important problem that has hardly been addressed is predicting target interactions between different miRNAs. Different miRNAs can cooperatively regulate individual targets, but miRNA expression signatures differ between cell types and cellular conditions. Determining how varying miRNA expression affects target regulation in cancerous versus normal cells, for example, will therefore be a major problem in the coming years.
| Tool                | Predicted species | Web access | Online tool | Own miRNA | Own mRNA | SW<sup>a</sup> | URL                                      |
|---------------------|-------------------|------------|-------------|-----------|----------|--------------|------------------------------------------|
| TargetScan          | 23 vertebrates, f, w | Yes        | Yes         | Yes       | Yes      | Yes          | http://www.targetscan.org                  |
| PicTar              | v, m, f, w        | Yes        | No          | No        | No       | No           | http://pictar.mdc-berlin.de               |
| miRanda             | h, m, r           | Yes        | No          | No        | Yes      | No           | http://www.microrna.org                   |
| MicroCosm           | 44 species        | Yes        | No          | No        | Yes      | No           | http://www.ebi.ac.uk/enright-srv/microcosm/htdocs/targets/v5 |
| RNAhybrid           |                   | No         | No          | No        | Yes      | No           | http://bibiserv.techfak.uni-bielefeld.de/rnahybrid |
| PITA                | h, m, f, w        | Yes        | Yes         | Yes       | Yes      | Yes          | http://genie.weizmann.ac.il/pubs/mir07    |
| STarMir             | f                 | Yes        | Yes         | Yes       | No       | No           | http://sfold.wadsworth.org/starmir.pl     |
| Rajewsky & Socci    |                   | f          | No          | No        | No       | No           |                                          |
| Robins              | f, w              | No         | No          | No        | No       | No           |                                          |
| mirWIP              | w                 | Yes        | No          | No        | Yes      | No           | http://146.189.76.171/query.php           |
| MicroInspector      |                   | Yes        | Yes         | Yes       | No       | No           | http://mirna.imbb.forth.gr/microinspector  |
| MicroTar            |                   | No         | No          | No        | Yes      | No           | http://tiger.dbs.nus.edu.sg/microtar      |
| MirTarget2          | h, m, r, d, c     | Yes        | No          | No        | No       | No           | http://mirdb.org                          |
| miTarget            |                   | Yes        | Yes         | Yes       | No       | No           | http://cbit.snu.ac.kr/~miTarget            |
| TargetMiner         | h                 | Yes        | No          | No        | No       | No           | http://www.isical.ac.in/~bioinfo_niu     |
| EIMMo               | h, m, f, z        | Yes        | No          | Yes       | No       | No           | http://www.mirz.unibas.ch/EIMMo2          |
| NBmiRTar            |                   | Yes        | Yes         | Yes       | No       | No           | http://wotan.wistar.upenn.edu/NBmiRTar    |
| TargetBoost         | w                 | Yes        | Yes         | No        | Yes      | No           | https://demo1.interagon.com/targetboost   |
| RNA22               |                   | Yes        | Yes         | No        | Yes      | No           | http://cbserv.watson.ibm.com/ra22.html    |
| TargetRank          | h, m              | Yes        | No          | No        | No       | No           | http://hollywood.mit.edu/targetrank       |
| EMBL                | f                 | Yes        | No          | No        | No       | No           | http://www.russell.embl-heidelberg.de/miRNAs |
| MovingTarget        | f                 | No         | No          | No        | No       | No           |                                          |
| DIANA-microT        |                   | Yes        | Yes         | Yes       | No       | No           | http://diana.pcbi.upenn.edu/cgi-bin/microT.cgi|
| HOCTAR              | h                 | Yes        | No          | No        | No       | No           | http://hoctar.tigem.it                     |
| Stanhope            |                   | No         | No          | No        | No       | No           |                                          |
| GenMiR++            | h                 | No         | No          | No        | Yes      | No           | http://www.psi.toronto.edu/genmir/        |
| HuMiTar             | h                 | No         | No          | No        | No       | No           |                                          |
| MirTif              |                   | Yes        | Yes         | Yes       | No       | No           | http://bsal.ym.edu.tw/mirtif               |
| Yan et al.          | h                 | No         | No          | No        | No       | No           |                                          |
| Xie et al.          | h, m, r, d        | No         | No          | No        | No       | No           |                                          |

<sup>a</sup>Both species of pre-computed prediction and the species available on the web tool are listed. Letters indicate the species: fly (f), worm (w), human (h), mouse (m), rat (r), chicken (c), zebra fish (z), and dog (d). Cells are left empty when no information is available.

<sup>b</sup>Yes/No indicate whether own miRNA sequences can be used on the web interface or not.

<sup>c</sup>Yes/No indicate whether own mRNA sequences can be used on the web interface or not.

<sup>d</sup>SW: Software availability (executable or source code).

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