miRNAMap 2.0: genomic maps of microRNAs in metazoan genomes

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ABSTRACT

MicroRNAs (miRNAs) are small non-coding RNA molecules that can negatively regulate gene expression and thus control numerous cellular mechanisms. This work develops a resource, miRNAMap 2.0, for collecting experimentally verified microRNAs and experimentally verified miRNA target genes in human, mouse, rat and other metazoan genomes. Three computational tools, miRanda, RNAhybrid and TargetScan, were employed to identify miRNA targets in 3'-UTR of genes as well as known miRNA targets. Various criteria for filtering the putative miRNA targets are applied to reduce the false positive prediction rate of miRNA target sites. Additionally, miRNA expression profiles can provide valuable clues on the characteristics of miRNAs, including tissue specificity and differential expression in cancer/normal cell. Therefore, quantitative polymerase chain reaction experiments were performed to monitor the expression profiles of 224 human miRNAs in 18 major normal tissues in human. The negative correlation between the miRNA expression profile and the expression profiles of its target genes typically helps to elucidate the regulatory functions of the miRNA. The interface is also redesigned and enhanced. The miRNAMap 2.0 is now available at http://miRNAMap.mbc.nctu.edu.tw/

INTRODUCTION

MicroRNAs (miRNAs) are small non-coding RNA molecules that can negatively regulate gene expression by hybridizing to the 3'-untranslated regions (3'-UTR) of the target gene. miRNAs are important in cell development, cell death, cell proliferation, fat metabolism, hematopoiesis and nervous system patterning in animals, as well as stress responses, and leaf and flower development in plants (1–4).

Numerous miRNAs and miRNA targets have been discovered and experimentally confirmed over the last few years. miRBase (5), which is the most comprehensive database of experimentally validated miRNAs across many genomes, provides integrated interfaces for presenting information on miRNA and computationally predicted miRNA targets. DIANA TarBase (6) collects experimentally validated miRNA targets in eight species. It contains a total of 750 miRNA target sites in 550 target genes. miRGen (7) collects positional relationships between miRNAs and genomic sets and miRNA targets according to combinations of widely used target prediction programs. Recently, many biologists have been paying much more attention to the functions of miRNAs in biological systems. Several miRNA target prediction tools were have been developed, such as miRanda (8), TargetScan (9) and RNAhybrid (10), for determining the energetically favored hybridization sites of small RNA to large RNAs. Lu et al. (11) developed an miRNA microarray to measure the expression profiles of all known miRNA in various normal tissues and tumors.

This work develops a resource, miRNAMap 2.0, to collect experimentally verified microRNAs and miRNA target genes in human, mouse, rat and other metazoan genomes. Three computational tools—miRanda, RNAhybrid and TargetScan—were utilized to identify miRNA targets in 3'-UTR of genes as well as known miRNA targets. Three criteria are applied for filtering the putative miRNA target sites to retain the more probable

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miRNA target sites to reduce the rate of false positive predictions of miRNA target sites. In particular, the RNA accessibilities of the identified miRNA target site were examined, providing information on understanding the miRNA/target relationship.

The miRNA expression profiles offer valuable clues on the properties of the miRNAs, such as tissue specificity and differential expression in cancer/normal cell. Accordingly, (quantitative polymerase chain reaction) experiments were conducted to monitor the expression profiles of 224 human miRNAs in 18 major normal tissues in humans. The negative correlation between the miRNA expression profiles and the expression profiles of their target genes helps to elucidate the regulatory functions of the miRNA. Finally, both textual and graphical web interfaces were redesigned and enhanced to facilitate the retrieval of data from the miRNAMap.

The main contribution of this work is the extension of miRNAMap version 1.0. The new version supports more genomes of known miRNAs and known miRNA targets. Prediction of miRNA genes is eliminated to examine miRNA/target relationship. Three criteria were applied to reduce the rate of false positive prediction of miRNA targets. The analysis of the correlation between the miRNA expression profile and the expression profiles of the target genes is useful to evaluate the possibility of the miRNA/miRNA targets relationship.

**IMPROVEMENTS**

Table 1 presents the major differences between the previous version and miRNAMap 2.0. The major enhancements and new features of miRNAMap 2.0 are described subsequently. miRNAMap 2.0 collects the known miRNAs in metazoan genomes, including two insects, nine vertebrates and one worm. miRNAMap 2.0 supports, apart from human, mouse, rat and dog, other metazoan genomes, others such as chicken, fruit fly, worm, zebrafish, fugu, frog (Xenopus tropicalis), malarial mosquito and opossum. Twelve genomes contain 2241 known miRNAs, which were obtained from miRBase (release 9.2, May 2007). The experimentally verified miRNA targets were obtained from DIANA TarBase and by surveying the literature. The numbers of experimental miRNA targets extracted from the DIANA TarBase and by surveying the literature are 346 and 29, respectively. Table 2 presents statistics for each genome.

**Identification of miRNA target**

This version of miRNAMap incorporates three previously developed computational tools, such as miRanda (8), TargetScan (9) and RNAhybrid (10), to identify miRNA target sites within the conserved regions of 3'UTR of genes in 12 metazoan genomes. The conserved regions were extracted from the UCSC Genome Browser Most Conserved Regions (12). The MFE threshold of the miRNA and target duplex was $-12 \text{kcal/mol}$ and the miRanda score was specified as 120. Therefore, the miRNA targets whose MFES are smaller than $-12 \text{kcal/mol}$ and whose miRanda score exceeds 120 are identified and compiled in the miRNAMap database. The predictive parameters of TargetScan and RNAhybrid were set as default values. Each miRNA target prediction tool yields a set of candidate miRNA target sites. However, some of these candidates may be false positive predictions. This work presents three criteria for eliminating false positives and retaining better candidate miRNA target sites.

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**Table 1. Enhancements and new features of miRNAMap 2.0**

<table>
<thead>
<tr>
<th>Features</th>
<th>miRNAMap 1.0</th>
<th>miRNAMap 2.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Known miRNAs</td>
<td>MiRBase (version 6.0)</td>
<td>MiRBase (version 9.2)</td>
</tr>
<tr>
<td>Supported species</td>
<td>Human, mouse, rat and dog</td>
<td>Two insects, nine vertebrates and one worm</td>
</tr>
<tr>
<td>Experimental miRNA targets</td>
<td>Taken from the literature</td>
<td>Taken from TarBase (6) and the literature</td>
</tr>
<tr>
<td>miRNA expression profiling</td>
<td>Lu et al. (11): miRNA profiling in human</td>
<td>Lu et al. (11): miRNA profiling in human</td>
</tr>
<tr>
<td>Expression profiles of miRNA targets</td>
<td>–</td>
<td>Q-PCR miRNA profiling in human</td>
</tr>
<tr>
<td>miRNA target prediction tools</td>
<td>miRanda</td>
<td>NCBI-GEO-GDS96 (76 human tissues) (18)</td>
</tr>
<tr>
<td>Criteria for filtering predicted miRNA targets</td>
<td>–</td>
<td>Criteria 1: predicted by at least two tools</td>
</tr>
<tr>
<td>Accessible region of miRNA target sites</td>
<td>–</td>
<td>Criteria 2: target genes contain multiple sites</td>
</tr>
<tr>
<td>Tissue specificity of human miRNAs</td>
<td>–</td>
<td>Criteria 3: target site is accessible</td>
</tr>
</tbody>
</table>

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**Table 2. The data statistics**

<table>
<thead>
<tr>
<th>Species</th>
<th>Number of known miRNAs</th>
<th>Number of target sites</th>
<th>Number of miRNA target sites after filtering</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Criterion 1</td>
</tr>
<tr>
<td>C. elegans</td>
<td>132</td>
<td>5751</td>
<td>378</td>
</tr>
<tr>
<td>Mosquito</td>
<td>38</td>
<td>3168</td>
<td>122</td>
</tr>
<tr>
<td>Fruit fly</td>
<td>78</td>
<td>10180</td>
<td>0</td>
</tr>
<tr>
<td>Human</td>
<td>475</td>
<td>6750</td>
<td>1717</td>
</tr>
<tr>
<td>Mouse</td>
<td>377</td>
<td>6763</td>
<td>1266</td>
</tr>
<tr>
<td>Dog</td>
<td>6</td>
<td>7017</td>
<td>418</td>
</tr>
<tr>
<td>Rat</td>
<td>234</td>
<td>5087</td>
<td>865</td>
</tr>
<tr>
<td>Chicken</td>
<td>149</td>
<td>1021</td>
<td>140</td>
</tr>
<tr>
<td>Zebrafish</td>
<td>337</td>
<td>1393</td>
<td>155</td>
</tr>
<tr>
<td>Opossum</td>
<td>107</td>
<td>2616</td>
<td>204</td>
</tr>
<tr>
<td>Fugu</td>
<td>131</td>
<td>246</td>
<td>7</td>
</tr>
<tr>
<td>Frog</td>
<td>177</td>
<td>2646</td>
<td>658</td>
</tr>
<tr>
<td>Total</td>
<td>2241</td>
<td>52638</td>
<td>5930</td>
</tr>
</tbody>
</table>
Criterion 1: target sites must be predicted by at least two tools. The three tools, miRanda, RNAhybrid and TargetScan, were applied separately to identify miRNA targets within the conserved regions of 3'-UTR in all metazoan genomes. This criterion retains putative miRNA targets that have been predicted by at least two tools for a miRNA, as displayed in Figure 1(a).

Criterion 2: target gene contains multiple target sites. Previous investigations have suggested that one gene can contain several miRNA target sites, bound by multiple distinct miRNAs or single miRNA. For example, six let-7 miRNA target sites were discovered in lin-4 (13–15), and miR-33, miR-124, miR-277 and miR-312 target the eye development gene seven-up (svp) in Drosophila (14). Hence, this criterion retains the miRNA target sites and the corresponding gene, which contains multiple target sites, as shown in Figure 1(a).

Criterion 3: target site must be located in accessible regions. The conventional target prediction tools exploit the complementarity between the miRNA and its target sequence, the conservation of the target sites, and the kinetics and thermodynamics of the miRNA/target duplex. Although these properties importantly determine the miRNA target sites, the sequence context that surrounds the miRNA target sites influences the binding affinities of the miRNA/target duplex. Robins et al. (16) hypothesized that single-strand miRNAs can only bind to stretches of free mRNA for potential target sites. Long et al. (15) posited the accessible model of miRNA target sites for predicting miRNA targets and successfully interpreted the published data on the in vivo activity of C. elegans reporter genes that contain modified lin-41 3'-UTR sequences. This work exploits the aforementioned concept to filter out the false positive predictions, such that miRNAs hybridize to the target sites that are located in the accessible regions, are more likely to be real, as presented in Figure 1(b). The accessibility of RNA sequences is determined by Sfold (17).

Expression profiling of microRNAs and target genes

As stated above, the computational tools for identifying miRNA target sites are developed based on the complementarities of miRNA and target sites, and of the kinetics and the thermodynamics of miRNA/target duplex, the combinatorial properties of miRNA target sites, and the accessibility of the target sites. Additionally, the expression profiles of miRNAs are useful in elucidating the roles of miRNAs in complex complicated biological systems.
In this work, two data sets of miRNA expression profiles, which were obtained by different experimental methods, Q-PCR and miRNA-bead array (11), are integrated.

The expression level of 224 human miRNAs in 18 major normal tissues in humans was detected by using a real-time PCR-based 220-plex miRNA expression profiling method to determine the tissue-specificity of human miRNAs. The detailed experimental protocol is described in Supplementary Material available online. The expression levels of miRNAs are currently provided in the miRNAMap to examine the tissue-specificity of human miRNAs. Another data set was generated by Lu et al. (11), who employed a bead-based flow cytometric miRNA expression profiling method to present a systematic expression analysis of 217 mammalian miRNAs from 334 human samples. GDS596 (GEO accession), which is the gene expression profiles of coding genes in 79 human tissues (18), was obtained from NCBI GEO (19).

Figure 2. The miRNAMap 2.0 enhanced web interface.
The expression profile of miRNA and the expression profiles of its target genes are typically negatively correlated since the miRNA downregulates its target gene. For each miRNA target site that is associated with a miRNA and a target gene (coding gene), the Pearson correlation coefficient is computed from the miRNA expression profile and the target gene expression profile, to elucidate the described phenomenon in experimental expression data in humans. Thirteen overlapping human tissues exist between the Q-PCR data set of the miRNA expression profiles and the GDS596 data set of the expression profiles of the target genes.

ACKNOWLEDGEMENTS
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CONCLUSION
miRNAmap 2.0 is an integrated resource for explicated regulatory functions of miRNAs. Various criteria were developed herein for filter out predicted miRNA target sites and elucidating the negative correlation between miRNA expression and its target gene expression, especially in humans, helping to elucidate the miRNA/target relationship.

Future works should evaluate the effectiveness of the filtering criteria using experimental miRNA targets. The disease/cancer-related expression profiles in humans will be considered and integrated into the database. The miRNA expression profiles and the expression profiles of the miRNA target genes in other genomes, such mouse and rat, will be incorporated into the resource.

REFERENCES

AVAILABILITY
The miRNAmap database will be continuously maintained and updated. The database is now freely available at http://miRNAmap.mbc.nctu.edu.tw/.

REFERENCES