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Research Article

In silico miRNA Target Identification within the Human Peroxisome Proliferator -Activated Receptor Gamma (PPARG) Gene

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Abstract

MicroRNAs (miRNAs), an abundant class of 21-25 nucleotides long non-coding RNAs, regulate eukaryotic gene expression and therefore implicated in a wide range of biological processes. The miRNA- related genetic alterations are possibly more implicated in human diseases than currently appreciated. miRNA target prediction using bioinformatics tools is often the first line approach in studying gene regulation. Such predictions will help in setting search priorities for experimental validation of gene controlling mechanisms. But finding a functional miRNA target in the human genome yet remains a challenging task. In the present study, miRNA target sites within the complete sequences (5′ UTR, CDS and 3′ UTR) of human *PPARG* gene were investigated using miRwalk database. We found 26, 52 and 85 different miRNA target sites within the 5′ UTR, CDS and 3′ UTR regions of the gene, respectively. This computational approach will subsequently allow better *in vitro* confirmation of the miRNA regulatory networks in cellular systems.

Keywords: microRNA, *In silico*, target site, PPARG, miRWalk

INTRODUCTION

MicroRNAs (miRNAs) are a broad class of naturally occurring small non-coding RNAs of about 21-25 nucleotides in length and found in plants, animals and some viruses. The main functions of miRNAs are to down-regulate gene expression in translational repression, cleavage of messenger RNA (mRNA) and in a variety of other biological processes. Each miRNA is partially or completely complementary to one or more mRNAs (Friedman et al. 2009, Landgraf et al. 2007).

Transcription of miRNAs occurs through RNA polymerase II9 and subsequent processing is mediated by the nuclear ribonuclease III (RNase III) enzyme Drosha to form precursor miRNAs (70–100 nucleotides). Following transportation to the cytoplasm by exportin 5, a further cleavage occurs via another RNase III enzyme, Dicer, to form the mature miRNA (He and Hannon 2004, Zeng and Cullen 2006).

miRNAs modulate both physiological and pathological pathways by post-transcriptionally inhibiting the expression of a plethora of target genes. miRNAs deregulate gene expression mostly by imperfect binding to complementary sites within transcript sequences and suppresses their translation, stimulate their de-adenylation and degradation or induce their cleavage (Bartel 2004, Perron and Provost 2008).

The decisive regulatory functions exhibited by the miRNA are found to be associated with a wide variety of human diseases such as cancer, heart diseases, metabolic disorders, neurodegenerative disorders etc. as reviewed by Srinivasan et al. (Srinivasan et al 2013). Therefore, microRNAs displaying deregulated expression in the context of specific diseases are of particular interest as therapeutic targets especially if they can be shown to coordinate such disease networks.

Peroxisome proliferators-activated receptor gamma (PPARγ or PPARG) encoded by the *PPARG* gene in humans belongs to the nuclear hormone receptor superfamily of ligand-activated transcription factors and originally has been characterized to be important for adipogenesis and glucose metabolism. There are two isoforms described (PPARG 1 and -2) (Vidal-Puig A. J. et al. 1997). PPARG has been associated with various diseases including obesity, diabetes mellitus, atherosclerosis, and cancer. PPARG agonists have been used in the treatment of hyperlipidaemia and hyperglycemia (Li et al. 2008). PPARG is important to shape an anti-inflammatory macrophage phenotype and appears crucial for dampening inflammation (Rosen et al. 1999). miRNAs have been reported to destabilize PPARG mRNA which can lead to impaired PPARG abundance (Schoonjans et al. 1996, Vidal-Puig A. et al. 1996). Therefore, miRNA target site identification within the PPARG gene is quite important in studying PPARG gene regulation.

There are a number of miRNA target prediction algorithms exploiting different approaches have been recently developed, and many methods of experimental validation have been premeditated. However, it is difficult to predict miRNA targets within the animal genomes due to its partial complementation to their target mRNA (Martin et al. 2007). For this shortcoming, the interactions of miRNA with their mRNA counterparts are complex and poorly understood. In the study *in silico* based miRNA targets identification within the human *PPARG* gene was performed.

METHODS

The miRWalk, a comprehensive database of miRNA from human, mouse and rat was used to identify miRNA target sites within the human PPARG gene based on a comparison of identified miRNA binding sites with the 8 established miRNA-target prediction programs i.e. RNA22, miRanda, miRDB, TargetScan, RNA- hybrid, PITA, PICTAR, and Diana-microT (Dweep et al. 2011). The miRWalk algorithm identifies the longest consecutive complementary between miRNA and gene sequences. miRWalk was used for investigating predicted targets of microRNAs in the complete sequences (5′ UTR, CDS and 3′ UTR) of *PPARG* gene in the human genome. Default parameters were used regarding minimum seed length (7) and *p* value (0.05).

RESULTS AND DISCUSSION

Because of the several limitations associated with genetic screening and experimental approaches for discovering founding members of miRNAs such as low efficiency, time consuming and high cost, several web-based or non

web-based computer software programs for predicting miRNAs and their targets have been developed in order to predict targets for follow up experimental validation. Even though many computational methods for the identification of miRNA may have their own limitations, there is no other option now other than to use computational methods for miRNA predictions. The next step in miRNA research is to identify and experimentally validate their mRNA targets.

All computer-based miRNA target prediction programs are based on specific parameters where slight variation results for the same target input. Such weakness of single *in silico* studies can be partially compensated by predicting targets using multiple programs. Scoring methods using dynamic programming (John et al. 2004, Kiriakidou et al. 2004, Lewis et al. 2003) and a complementarily-based strategy (Lewis et al. 2003, Rajewsky and Socci 2004) are generally preferred to rank the prediction results. These approaches have been quite successful for a few top ranked results. miRNAs targets calculated from multiple prediction methods significantly improved target prediction accuracy. Therefore, 8 key programs were used in the present study to optimize our search and to unravel miRNA target sequences of the PPARG gene cluster with high accuracy.

Table 1: Predicted miRNA sequences within the 5′-untranslated region (5′-UTR) of human *PPARG* gene

miRNA	Stem Loop ID	Seed Length	Start	Position	End	P value
hsa-miR-181a-2*	hsa-mir-181a-2	10	120	1	111	0.0003
hsa-miR-345	hsa-mir-345	9	75	2	67	0.0010
hsa-miR-181a-2*	hsa-mir-181a-2	9	119	2	111	0.0010
hsa-miR-607	hsa-mir-607	8	205	2	198	0.0042
hsa-miR-423-3p	hsa-mir-423	8	95	1	88	0.0042
hsa-miR-922	hsa-mir-922	8	149	2	142	0.0042
hsa-miR-1226	hsa-mir-1226	8	153	1	146	0.0042
hsa-miR-345	hsa-mir-345	8	264	1	257	0.0042
hsa-miR-1226	hsa-mir-1226	7	152	2	146	0.0166
hsa-miR-1282	hsa-mir-1282	7	256	1	250	0.0166
hsa-miR-298	hsa-mir-298	7	181	1	175	0.0166
hsa-miR-192	hsa-mir-192	7	116	1	110	0.0166
hsa-miR-423-3p	hsa-mir-423	7	94	2	88	0.0166
hsa-miR-580	hsa-mir-580	7	252	1	246	0.0166
hsa-miR-377*	hsa-mir-377	7	145	1	139	0.0166
hsa-miR-624*	hsa-mir-624	7	32	2	26	0.0166
hsa-miR-329	hsa-mir-329-1	7	20	2	14	0.0166
hsa-miR-329	hsa-mir-329-2	7	20	2	14	0.0166
hsa-miR-299-5p	hsa-mir-299	7	224	1	218	0.0166
hsa-miR-634	hsa-mir-634	7	151	2	145	0.0166
hsa-miR-522	hsa-mir-522	7	247	1	241	0.0166
hsa-miR-548k	hsa-mir-548k	7	34	2	28	0.0166
hsa-miR-1224-3p	hsa-mir-1224	7	15	2	9	0.0166
hsa-miR-1300	hsa-mir-1300	7	252	1	246	0.0166
hsa-miR-559	hsa-mir-559	7	35	1	29	0.0166
hsa-miR-362-3p	hsa-mir-362	7	20	2	14	0.0166

miRNA: microRNA; hsa: Homo sapiens

Table 2: Predicted miRNA sequences within the coding sequence (CDS) of human *PPARG* gene

miRNA	Stem Loop ID	Seed Length	Start	Position	End	P value
hsa-miR-367	hsa-mir-367	10	507	2	498	0.0014
hsa-miR-1224-5p	hsa-mir-1224	10	1562	1	1553	0.0014
hsa-miR-101	hsa-mir-101-1	9	769	2	761	0.0055
hsa-miR-371-5p	hsa-mir-371	9	1382	1	1374	0.0055
hsa-miR-654-5p	hsa-mir-654	9	314	1	306	0.0055
hsa-miR-25	hsa-mir-25	9	507	2	499	0.0055
hsa-miR-101	hsa-mir-101-2	9	769	2	761	0.0055
hsa-miR-545	hsa-mir-545	9	1478	1	1470	0.0055
hsa-miR-1224-5p	hsa-mir-1224	9	1561	2	1553	0.0055
hsa-miR-923	hsa-mir-923	9	904	1	896	0.0055
hsa-miR-92a	hsa-mir-92a-1	9	507	2	499	0.0055
hsa-miR-92a	hsa-mir-92a-2	9	507	2	499	0.0055
hsa-let-7c*	hsa-let-7c	8	1224	2	1217	0.0216
hsa-miR-142-5p	hsa-mir-142	8	1366	1	1359	0.0216
hsa-miR-181c	hsa-mir-181c	8	607	2	600	0.0216
hsa-miR-1234	hsa-mir-1234	8	840	1	833	0.0216
hsa-miR-152	hsa-mir-152	8	1405	2	1398	0.0216
hsa-miR-513b	hsa-mir-513b	8	661	1	654	0.0216
hsa-miR-1243	hsa-mir-1243	8	456	2	449	0.0216
hsa-miR-199a-3p	hsa-mir-199a-2	8	393	1	386	0.0216
hsa-miR-578	hsa-mir-578	8	446	2	439	0.0216
hsa-miR-1205	hsa-mir-1205	8	1087	2	1080	0.0216
hsa-miR-206	hsa-mir-206	8	436	1	429	0.0216
hsa-miR-1825	hsa-mir-1825	8	1407	1	1400	0.0216
hsa-miR-199a-3p	hsa-mir-199a-1	8	393	1	386	0.0216
hsa-miR-371-5p	hsa-mir-371	8	1381	2	1374	0.0216
hsa-miR-541	hsa-mir-541	8	314	1	307	0.0216
hsa-miR-199b-3p	hsa-mir-199b	8	393	1	386	0.0216
hsa-miR-1207-3p	hsa-mir-1207	8	1538	1	1531	0.0216
hsa-miR-1	hsa-mir-1-1	8	436	1	429	0.0216
hsa-miR-1270	hsa-mir-1270	8	870	1	863	0.0216
hsa-miR-181a	hsa-mir-181a-1	8	607	2	600	0.0216
hsa-miR-1207-3p	hsa-mir-1207	8	887	1	880	0.0216
hsa-miR-654-5p	hsa-mir-654	8	313	2	306	0.0216
hsa-miR-885-5p	hsa-mir-885	8	351	1	344	0.0216
hsa-miR-1	hsa-mir-1-2	8	436	1	429	0.0216
hsa-miR-629*	hsa-mir-629	8	1051	2	1044	0.0216
hsa-miR-328	hsa-mir-328	8	1308	2	1301	0.0216
hsa-miR-33b	hsa-mir-33b	8	1403	1	1396	0.0216
hsa-miR-545	hsa-mir-545	8	1477	2	1470	0.0216
hsa-miR-148b	hsa-mir-148b	8	1405	2	1398	0.0216
hsa-miR-589	hsa-mir-589	8	1295	1	1288	0.0216
hsa-miR-545	hsa-mir-545	8	1388	2	1381	0.0216
hsa-miR-453	hsa-mir-453	8	1512	1	1505	0.0216

hsa-miR-33a	hsa-mir-33a	8	1403	1	1396	0.0216
hsa-miR-635	hsa-mir-635	8	1376	1	1369	0.0216
hsa-miR-181a	hsa-mir-181a-2	8	607	2	600	0.0216
hsa-miR-92b	hsa-mir-92b	8	507	2	500	0.0216
hsa-miR-923	hsa-mir-923	8	903	2	896	0.0216
hsa-miR-130a*	hsa-mir-130a	8	1485	1	1478	0.0216
hsa-miR-592	hsa-mir-592	8	292	2	285	0.0216
hsa-miR-485-3p	hsa-mir-485	8	934	1	927	0.0216

miRNA: microRNA; hsa: Homo sapiens

Table 3: Predicted miRNA sequences within the 3′-untranslated region (3′-UTR) of human *PPARG* gene

miRNA	Stem Loop ID	Seed Length	Start	Position	End	P value
hsa-miR-559	hsa-mir-559	9	1879	2	1871	0.0008
hsa-miR-511	hsa-mir-511-1	8	1863	1	1856	0.0032
hsa-miR-548d-5p	hsa-mir-548d-2	8	1880	1	1873	0.0032
hsa-miR-24	hsa-mir-24-1	8	1725	1	1718	0.0032
hsa-miR-548i	hsa-mir-548i-1	8	1880	1	1873	0.0032
hsa-miR-511	hsa-mir-511-1	8	1863	1	1856	0.0032
hsa-miR-548c-5p	hsa-mir-548c	8	1880	1	1873	0.0032
hsa-miR-513a-3p	hsa-mir-513a-2	8	1790	1	1783	0.0032
hsa-miR-548n	hsa-mir-548n	8	1880	2	1873	0.0032
hsa-miR-24	hsa-mir-24-2	8	1725	1	1718	0.0032
hsa-miR-449a	hsa-mir-449a	8	1731	1	1724	0.0032
hsa-miR-548i	hsa-mir-548i-2	8	1880	1	1873	0.0032
hsa-miR-511	hsa-mir-511-2	8	1863	1	1856	0.0032
hsa-miR-545*	hsa-mir-545	8	1793	2	1786	0.0032
hsa-miR-548h	hsa-mir-548h-1	8	1880	1	1873	0.0032
hsa-miR-548b-5p	hsa-mir-548b	8	1880	1	1873	0.0032
hsa-miR-548j	hsa-mir-548j	8	1880	1	1873	0.0032
hsa-miR-27b	hsa-mir-27b	8	1797	1	1790	0.0032
hsa-miR-548i	hsa-mir-548i-3	8	1880	1	1873	0.0032
hsa-miR-27a	hsa-mir-27a	8	1797	1	1790	0.0032
hsa-miR-511	hsa-mir-511-2	8	1863	1	1856	0.0032
hsa-miR-34a	hsa-mir-34a	8	1731	1	1724	0.0032
hsa-miR-548h	hsa-mir-548h-2	8	1880	1	1873	0.0032
hsa-miR-338-5p	hsa-mir-338	8	1852	1	1845	0.0032
hsa-miR-548i	hsa-mir-548i-4	8	1880	1	1873	0.0032
hsa-miR-548h	hsa-mir-548h-3	8	1880	1	1873	0.0032
hsa-miR-548d-5p	hsa-mir-548d-1	8	1880	1	1873	0.0032
hsa-miR-454	hsa-mir-454	8	1757	1	1750	0.0032
hsa-miR-548a-5p	hsa-mir-548a-3	8	1880	1	1873	0.0032
hsa-miR-513a-3p	hsa-mir-513a-1	8	1790	1	1783	0.0032
hsa-miR-548h	hsa-mir-548h-4	8	1880	1	1873	0.0032
hsa-miR-548a-5p	hsa-mir-548a-3	7	1879	2	1873	0.0128
hsa-miR-513a-3p	hsa-mir-513a-1	7	1789	2	1783	0.0128

hsa-miR-1243	hsa-mir-1243	7	1751	1	1745	0.0128
hsa-miR-576-5p	hsa-mir-576	7	1828	1	1822	0.0128
hsa-miR-548h	hsa-mir-548h-4	7	1879	2	1873	0.0128
hsa-miR-511	hsa-mir-511-1	7	1862	2	1856	0.0128
hsa-miR-513a-5p	hsa-mir-513a-2	7	1797	1	1791	0.0128
hsa-miR-548d-5p	hsa-mir-548d-2	7	1879	2	1873	0.0128
hsa-miR-891b	hsa-mir-891b	7	1754	1	1748	0.0128
hsa-miR-24	hsa-mir-24-1	7	1724	2	1718	0.0128
hsa-miR-449b	hsa-mir-449b	7	1730	2	1724	0.0128
hsa-miR-548i	hsa-mir-548i-1	7	1879	2	1873	0.0128
hsa-miR-511	hsa-mir-511-1	7	1862	2	1856	0.0128
hsa-miR-548c-5p	hsa-mir-548c	7	1879	2	1873	0.0128
hsa-miR-7	hsa-mir-7-1	7	1748	1	1742	0.0128
hsa-miR-513a-3p	hsa-mir-513a-2	7	1789	2	1783	0.0128
hsa-miR-889	hsa-mir-889	7	1888	1	1882	0.0128
hsa-miR-586	hsa-mir-586	7	1847	1	1841	0.0128
hsa-miR-24	hsa-mir-24-2	7	1724	2	1718	0.0128
hsa-miR-128	hsa-mir-128-2	7	1796	1	1790	0.0128
hsa-miR-7	hsa-mir-7-2	7	1748	1	1742	0.0128
hsa-miR-340	hsa-mir-340	7	1857	1	1851	0.0128
hsa-miR-449a	hsa-mir-449a	7	1730	2	1724	0.0128
hsa-miR-548i	hsa-mir-548i-2	7	1879	2	1873	0.0128
hsa-miR-511	hsa-mir-511-2	7	1862	2	1856	0.0128
hsa-miR-7	hsa-mir-7-3	7	1748	1	1742	0.0128
hsa-miR-548h	hsa-mir-548h-1	7	1879	2	1873	0.0128
hsa-miR-656	hsa-mir-656	7	1886	1	1880	0.0128
hsa-miR-301b	hsa-mir-301b	7	1756	2	1750	0.0128
hsa-miR-548b-5p	hsa-mir-548b	7	1879	2	1873	0.0128
hsa-miR-548j	hsa-mir-548j	7	1879	2	1873	0.0128
hsa-miR-34c-5p	hsa-mir-34c	7	1730	2	1724	0.0128
hsa-miR-27b	hsa-mir-27b	7	1796	2	1790	0.0128
hsa-miR-548i	hsa-mir-548i-3	7	1879	2	1873	0.0128
hsa-miR-27a	hsa-mir-27a	7	1796	2	1790	0.0128
hsa-miR-511	hsa-mir-511-2	7	1862	2	1856	0.0128
hsa-miR-548k	hsa-mir-548k	7	1880	1	1874	0.0128
hsa-miR-34a	hsa-mir-34a	7	1730	2	1724	0.0128
hsa-miR-548h	hsa-mir-548h-2	7	1879	2	1873	0.0128
hsa-miR-128	hsa-mir-128-1	7	1796	1	1790	0.0128
hsa-miR-590-3p	hsa-mir-590	7	1894	1	1888	0.0128
hsa-miR-301a	hsa-mir-301a	7	1756	2	1750	0.0128
hsa-miR-338-5p	hsa-mir-338	7	1851	2	1845	0.0128
hsa-miR-409-3p	hsa-mir-409	7	1736	2	1730	0.0128
hsa-miR-548i	hsa-mir-548i-4	7	1879	2	1873	0.0128
hsa-miR-513a-5p	hsa-mir-513a-1	7	1797	1	1791	0.0128
hsa-miR-130b	hsa-mir-130b	7	1756	2	1750	0.0128
hsa-miR-335*	hsa-mir-335	7	1800	1	1794	0.0128
hsa-miR-548h	hsa-mir-548h-3	7	1879	2	1873	0.0128
hsa-miR-130a	hsa-mir-130a	7	1756	2	1750	0.0128

hsa-miR-1279	hsa-mir-1279	7	1832	1	1826	0.0128
hsa-miR-548l	hsa-mir-548l	7	1880	1	1874	0.0128
hsa-miR-548d-5p	hsa-mir-548d-1	7	1879	2	1873	0.0128
hsa-miR-454	hsa-mir-454	7	1756	2	1750	0.0128

miRNA: microRNA; hsa: Homo sapiens

Using miRWalk, number of potential target sites for miRNAs were identified within the sequences of 5′-UTR (5′-untranslated region), CDS (coding DNA sequence) and 3′ UTR (3′- untranslated region) of PPARG in the human genome. The functional regions of the PPARG gene cluster as possible sites for miRNA targeting were further analyzed. A unique target pattern was pointed within the genomic sequences representing the 5′ UTR, CDS and 3′ UTR of PPARG gene. Specific sequences within 5′ UTR, CDS and 3′ UTR of human PPARG gene along with seed sequences, its location and size respectively are shown in tables 1, 2 and 3. These experimental data show that the number of miRNA target sites ranges differently in different regions of *PPARG*. In the 5′ UTR of the screened gene, we found 29 different miRNA target sites with different *p* values. Among them, the target site for miRNA-181a-2 had the lowest *p* value (0.003), i.e. most significant value (Table 1). In case of CDS, we obtained 52 target sites, miRNA-367 being the most significant one (*p* value= 0.0014) (Table 2). Finally, 85 different miRNA target sites were identified within the 3′ UTR. We found miRNA-559 be the most significant one (*p*= 0.0080 amongst all within this region (Table 3). The findings would help when we want to select miRNAs for studying their role in *PPARG* regulation in laboratory conditions.

A number of computational miRNA-target prediction algorithms have been developed due to lack of high-throughput experimental methods but these programs still lacking sensitivity and specificity. The miRWalk database provides a comprehensive atlas of putative miRNA binding site prediction from multiple algorithms and therefore attracts researchers. These existing algorithms will become more accurate with more understanding of miRNA regulatory mechanism (Dweep et al. 2013). It can thus be concluded that a combination of both computational and experimental approaches would be required to unravel the complex networks of miRNA gene regulation and their expected therapeutic potentials.

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