Fully Complementary Interactions of LmiRNA with mRNA of Human Genes

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Article

Fully Complementary Interactions of LmiRNA with mRNA of Human Genes

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Abstract

Londin et al. discovered miRNAs (LmiRNAs) whose properties had been little studied for unknown reasons. In this study, we examined fully complementary interactions of LmiRNAs with mRNAs of human genes. Using the MirTarget program, a significant number of target genes with unique properties of interaction with LmiRNAs were identified. Of 3707 LmiRNAs in the mRNAs of 75 target genes, the sites of fully complementary LmiRNA binding were located in the 5'UTR with a high free energy of interaction. In the mRNAs of 81 target genes, the fully complementary LmiRNA binding sites (BSs) were located in the CDS. Only seven LmiRNAs bound in the 3'UTRs of target genes. The *KIFC3, PHF15, RPL15* and *SNX11* genes were identified, encoding LmiRNA-5p and LmiRNA-3p, which bind to the mRNAs of these genes. The mRNA of most genes were bound by only one LmiRNA. The *BMP8B, BMP8B, FGFRL1* and *SDC3* genes were identified, whose mRNAs bound the pair ID00121.5p and ID02992.5p. These results expand our knowledge of LmiRNAs and allow us to recommend these LmiRNAs as diagnostic and therapeutic agents for various diseases.

Keywords: miRNA; mRNA; human gene; disease; diagnostics

1. Introduction

For over thirty years, miRNAs from the NCBI database have been identified and used. Unfortunately, programs used to identify miRNA target genes have predominantly predicted false-positive target genes. Over the past few years, there has been a noticeable decline in publications studying the biological role of miRNAs. The group of Londin et al. identified 3,707 miRNAs [1], which are designated as LmiRNAs in this article. For unknown reasons, these LmiRNAs have not attracted much attention until now. We aimed to elucidate the potential of LmiRNAs to influence gene expression during protein synthesis. This resulted in significant biologically important findings regarding the properties of LmiRNAs. We obtained quantitative characteristics of miRNA interactions with human target genes.

2. Materials and Methods

The nucleotide (nt) sequence of 17,508 human genes were downloaded from National Center for Biotechnology Information (NCBI https://www.ncbi.nlm.nih.gov, 2022). The nucleotide sequences of 3707 LmiRNAs were taken from Londin et al [1]. The LmiRNA binding sites (BSs) in mRNA were predicted using the MirTarget program [2]. This program predicts the following features of miRNA interaction with mRNA: (a) the initiation of miRNA binding to the mRNA from the first nucleotide of the mRNA; (b) the localization of the miRNA BSs in the 5′-untranslated region (5′UTR), coding domain sequence (CDS), and 3′-untranslated region (3′UTR) of the mRNAs; (c) the schemes of nucleotide interactions between miRNA with mRNA, which clearly demonstrate the interactions of all nucleotides between miRNA with mRNA; (d) the free energy Gibbs (ΔG, kJ/mol) of the interaction

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between miRNA and the mRNA; and the ratio ΔG/ΔGm (%) is determined for each site. ΔGm equals the free energy of miRNA binding with its fully complementary canonical nucleotide sequence. Only miRNA whose nucleotides interacted with mRNA using canonical (G-C and A-U) nucleotides with a given ΔG value were selected from the calculated data. The MirTarget program finds hydrogen bonds between miRNA with mRNA according to the physicochemical characteristics of nucleotide interactions [3–6]. MirTarget differs from other programs in terms of finding miRNA BSs on mRNA in the following: it takes into account the interaction of miRNA with mRNA over the entire miRNA nucleotides sequence; and it calculates the free energy of the interaction of the miRNA with mRNA. Note that the G, A, C, and U nucleotides, which comprise the RNA structure of microorganisms, plants, and animals, interact identically under equal conditions. Therefore, the physicochemical properties of canonical nucleotide pairs given above do not require additional proof of the previously established physicochemical characteristics of their interaction The reliability of translation suppression by miRNAs that are fully complementary to mRNAs was proven by A. Fire and C.C. Mello [7], who were awarded the Nobel Prize in 2006 for this research. This year's Nobel Prize focuses on the discovery of a vital regulatory mechanism used in cells to control gene activity. Genetic information flows from DNA to messenger RNA (mRNA), via a process called transcription, and then on to the cellular machinery for protein production.

3. Results

Interaction of miRNA with mRNA in the 5′UTR. A study of the interactions of all 3,707 LmiRNAs with mRNA of 17,508 human genes revealed that 145 miRNAs can interact with 149 target genes by forming fully complementary pairs of canonical nucleotides G with C and A with U. The results of some of these calculations are presented in Table 1. The first important property of LmiRNA is that 75 LmiRNAs BSs are located in the 5′UTR of target gene mRNAs. The free energy of LmiRNA–mRNA interaction depends on both the GC content of LmiRNA and mRNA BSs and the number of LmiRNA nucleotides. A ΔG value greater than -140 kJ/mole was detected for the interaction of 15 LmiRNAs with target gene mRNAs. The location of LmiRNA BSs in the 5'UTR allows for translational termination early in the process, significantly reducing the energy expenditure on abortive protein synthesis. The mRNAs of the *ANKRD9, CERK* and *HYI* target genes each bound two ID00121.5p binding sites in the 5'UTR region (Table 2).

Table 1. Characteristics of LmiRNA interactions in the 5'UTR of mRNA of human genes.

mRNA of gene	LmiRNA	BS, nt	ΔG, kJ/mole	mRNA of gene	LmiRNA	BS, nt	ΔG, kJ/mole
ADRBK2	ID02187.5p	111	-138	*KIAA2018*	ID02368.3p	242	-140
ALK	ID01810.3p	52	-129	*KLHL15*	ID03422.3p	110	-123
ANKRD9	ID00943.3p	86	-127	*LANCL2*	ID02988.5p	425	-117
ARSI	ID02715.3p	511	-127	*LMX1A*	ID00230.5p	182	-129
ASTN2	ID03318.3p	334	-119	*LOXL4*	ID00411.5p	77	-144
C10orf25	ID00351.3p	70	-140	*MAP2K2*	ID01574.5p	221	-142
C11orf87	ID00616.5p	73	-134	*MAP3K1*	ID02634.3p	101	-129
CAMK1D	ID00323.3p	105	-123	*MSL1*	ID01311.3p	155	-149
CBLL1	ID03040.3p	184	-117	*N4BP1*	ID01157.5p	190	-125
CD164	ID02875.5p	68	-123	*NIPBL*	ID02626.5p	176	-123
CDYL	ID02781.3p	39	-125	*NLRX1*	ID00628.5p	167	-123
CERK	ID02992.5p	2	-115	*OGFOD3*	ID03179.5p	97	-138
CLDND2	D01758.3p	594	-138	*OXR1*	ID02217.5p	31	-134
CLIC4	ID00087.3p	34	-125	*PLA2G6*	ID02357.3p	242	-125
CMTM6	ID02300.3p	77	-144	*PROS1*	ID02292.5p	74	-134
DENND6A	ID02350.5p	11	-125	*RFTN1*	ID01075.3p	102	-140
DUSP15	ID02046.3p	315	-136	*RHBDL1*	ID01856.3p	243	-132

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E2F1	ID02052.5p	85	-149	*RNF103*	ID03367.5p	37	-125
ERCC6L2	ID03301.3p	131	-123	*RXRA*	ID02911.5p	244	-138
FAM131C	ID00064.3p	1	-123	*SCAF8*	ID02686.5p	77	-121
FAM178A	ID00414.3p	209	-117	*SEPT8*	ID01903.3p	313	-115
FAM81A	ID01009.5p	14	-125	*SFT2D3*	ID00185.5p	58	-144
GATAD2A	ID01675.5p	109	-132	*SLC22A15*	ID01492.3p	16	-129
GBA2	ID03260.3p	38	-115	*SMAD2*	ID01020.5p	193	-123
GSTZ1	ID00910.3p	34	-123	*SMAD3*	ID02853.3p	54	-125
HDAC4	ID02002.5p	236	-115	*SMAP1*	ID00053.5p	265	-115
HTRA3	D02487.5p	37	-117	*UBIAD1*	ID00921.3p	61	-121
ISL1	ID02632.5p	106	-117	*UBR7*	ID03019.5p	345	-125
KIAA1217	ID00330.5p	145	-140	*ZNF804B*			

Table 2. Characteristics of interaction of ID00121.5p and ID02992.5p with mRNA of target genes.

mRNA of gene	LmiRNA	BS, nt	ΔG, kJ/mole	Region of mRNA
ANKRD9	ID00121.5p	43	-115	5'UTR
ANKRD9	ID00121.5p	50	-115	5'UTR
CERK	ID00121.5p	1	-115	5'UTR
CERK	ID00121.5p	8	-115	5'UTR
HYI	ID00121.5p	126	-115	5'UTR
HYI	ID00121.5p	133	-115	5'UTR
HYI	ID02992.5p	127	-115	5'UTR
HYI	ID02992.5p	134	-115	5'UTR

2. **Interaction of LmiRNA with mRNA in the CDS**. 81 genes are miRNA targets with binding sites located in the CDS (Table 3). The free energy of interaction of LmiRNA with target gene mRNA greater than -140 kJ/mol was found for 12 target genes. This indicates a strong interaction of LmiRNA with target gene mRNA. Seven target genes contain binding sites in the 3′UTR (Table 3). The free energy of interaction of LmiRNA with mRNA ranged from -110 kJ/mol to -132 kJ/mol, which is lower than the interaction energy of LmiRNA with target gene mRNA in the 5′UTR and CDS.

Table 3. Characteristics of LmiRNA interactions in CDS and 3'UTR mRNAs of target genes.

mRNA of gene	LmiRNA	BS, nt	ΔG, kJ/mole	mRNA of gene	LmiRNA	BS, nt	ΔG, kJ/mole
AATK	ID01431.3p	2178	-142	*LHX4*	ID00245.3p	558	-123
ADAMTS8	ID00648.5p	821	-136	*LONRF2*	ID01873.3p	654	-132
ADRA1B	ID02729.5p	1320	-121	*LRRC26*	ID03389.3p	706	-121
ANGPTL4	ID01593.5p	259	-134	*MAP3K6*	ID00093.5p	542	-134
APC2	ID01540.3p	3174	-129	*METRNL*	ID01458.5p	187	-146
APRT	ID01212.5p	205	-110	*MIB2*	ID00017.3p	1432	-127
ARHGEF17	ID00592.3p	5054	-134	*MMP17*	ID00794.3p	223	-123
ARID1B	ID02914.3p	589	-125	*MMP24*	ID01804.3p	35	-146
BPTF	ID01377.3p	295	-127	*MORC4*	ID03448.3p	287	-136
C10orf95	ID00424.5p	845	-115	*MROH8*	ID02061.3p	188	-129
C19orf21	ID01521.3p	1055	-125	*MSH3*	ID02653.3p	464	-132
C2CD4D	ID00202.5p	1225	-146	*MXRA8*	ID00014.3p	210	-121
C9orf66	ID03226.3p	742	-123	*MYBBP1A*	ID01242.3p	2597	-138
CACNA1B	ID03398.5p	2997	-123	*MYO3A*	ID00333.3p	5184	-121
CAMSAP1	ID03370.5p	63	-121	*NEURL1B*	ID02740.3p	240	-119
CCDC6	ID00364.5p	246	-121	*PHLDA1*	ID00722.5p	282	-121
CDHR5	ID00474.3p	2204	-121	*PIK3IP1*	ID02201.3p	428	-119

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CEBPB	ID02084.3p	687	-151	*PIK3R2*	ID01662.3p	1273	-142
CELSR1	ID02250.3p	546	-142	*PLXNC1*	ID00731.5p	582	-121
CELSR2	ID00178.5p	8114	-129	*PLXND1*	ID02398.3p	1058	-123
CHADL	ID02231.3p	1228	-115	*POU3F1*	ID00117.5p	1220	-125
CTF1	ID01150.3p	610	-144	*PROB1*	ID02701.5p	808	-138
DCAF13	ID03178.5p	317	-134	*REPIN1*	ID03072.5p	1168	-115
DUSP28	ID02005.5p	669	-138	*RHBDD3*	ID02191.5p	943	-121
E2F1	ID02051.3p	291	-153	*RNF169*	ID00594.3p	281	-134
EPM2A	ID02899.3p	481	-149	*SETD9*	ID02635.5p	398	-117
F2	ID00524.3p	532	-119	*SHISA8*	ID02233.3p	952	-132
FAM160B2	ID03113.3p	2279	-125	*SLC44A2*	ID01602.5p	93	-121
FAM8A1	ID02789.5p	399	-132	*TONSL*	ID03220.3p	2763	-127
FBRSL1	ID00799.3p	2503	-129	*TPM1*	ID01010.5p	333	-123
FCRLB	ID00226.5p	772	-136	*TRIO*	ID02611.3p	6927	-138
FGFRL1	ID02002.5p	209	-115	*TTC39B*	ID03248.3p	57	-123
FGFRL1	ID02457.3p	206	-138	*USP22*	ID01275.5p	207	-123
GATA5	ID02103.5p	311	-129	*ZFP36L2*	ID01824.3p	1287	-115
GNAS	ID02093.5p	1626	-127	*FPM1*	ID01206.3p	2213	-142
GP1BB	ID02171.5p	384	-129	*ZNF488*	ID00356.3p	499	-123
GYS1	ID01747.5p	2354	-110	*ZNF628*	ID01775.3p	3586	-125
HCN2	D01804.3p	112	-146	*ZNF750*	ID01456.3p	2085	-125
HIC1	ID01236.5p	846	-125	*C9orf62*	ID03369.3p	1261*	-110
IGFBP3	ID02982.3p	435	-123	*MAPKAPK3*	ID02335.5p	2746*	-127
JUND	D01663.3p	298	-125	*MLL4*	ID01699.5p	8280*	-117
KCNC3	ID01755.3p	377	-127	*MLLT1*	ID01582.3p	4023*	-123
KDM1A	ID00081.3p	342	-136	*SFT2D3*	ID01905.5p	1157*	-132
KDM3B	ID02695.3p	242	-138	*SOX11*	ID01787.3p	3226*	-129
					ID01451.3p	1957*	-132

3. Features of some target genes. We identified four genes containing regions encoding LmiR-5p and LmiR-3p that bound to the mRNA of each of these genes (Fig. 1). We earlier identified the *RTL1* gene, which encoded four miRNA-5p and miRNA-3p that bind to the mRNA of this gene [8]. There is currently no explanation for this phenomenon, as it pertains to why a gene encodes LmiRNA that suppresses its expression.

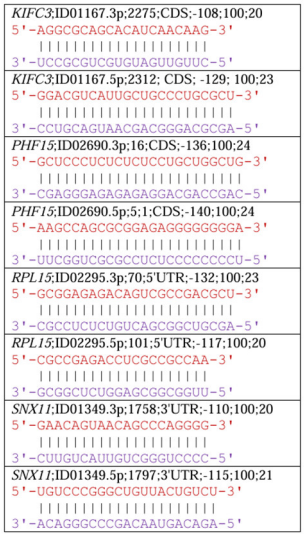

Figure 1. Schemes of mRNA interactions of the *KIFC3, PHF15, RPL15* and *SNX11* genes with LmiRNAs encoded by these genes.

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When predicting LmiRNA suppression of gene expression, it is possible to detect the suppression of multiple genes by a single LmiRNA. Such an example is shown in Figure 1. LmiRNA can suppress gene expression either alone or in combination with another LmiRNA (Fig. 2). In this article, we do not consider the function of the target genes studied by the miRNA, as this requires analyzing a large number of publications describing the involvement of genes in various diseases. Many of these publications only establish a correlation between gene expression and disease, without establishing the objective involvement of the gene in the development of a given disease.

The *HYI* gene's mRNA bound ID02992.5p at two sites. ID00121.5p and ID02992.5p were identified, binding to the miRNAs of three genes in a region of mRNA with a one-nucleotide difference in the onset of translational sequence (Fig. 2). This interaction between two LmiRNAs may mislead the proposal to use one miRNA as a disease prediction method by suppressing gene expression of only that miRNA.

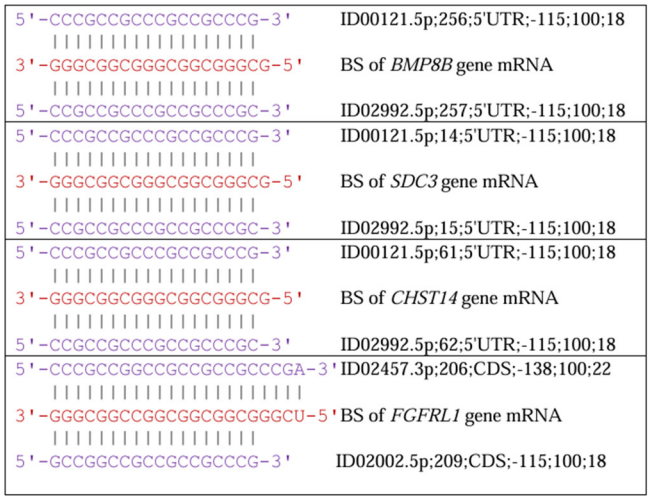

Figure 2. Schemes of interaction of two LmiRNAs with one mRNA.

4. Discussion

The above results characterize only the quantitative characteristics of LmiRNA interactions with target genes. The involvement of target genes and the corresponding LmiRNAs that suppress their expression requires specialized, labor-intensive, and expensive studies. A gene's involvement in a disease must be unambiguously determined, and then its expression can be suppressed or increased by increasing or decreasing the influence of LmiRNA. Regulation of LmiRNA expression is possible through the use of so-called miRNA-binding sponges [9].

LmiRNA targets 149 genes, which serves as the basis for studying their involvement in the development of various diseases. For example, five genes are transcription factors (Table 2). Increased expression of *ZNF488* promotes pancreatic cancer cell proliferation and tumor development [10], and LmiRNA ID00356.3p can be used to suppress *ZNF488* gene expression. The *ZNF628* gene may serve as a marker of follicular atresia, and its expression may be regulated by ID01775.3p [11]. *ZNF750* expression may serve as a reliable prognostic biomarker for metastatic prostate cancer, which lays the foundation for the development of new biological therapies [12]. *ZNF804B* was overexpressed on chromosome 7 and may serve as a tumor molecular marker [13]. This brief summary of the utility of the obtained data on the effect of LmiRNA on ZNF family transcription factors convincingly reflects the great utility of the obtained research results.

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