

Original Research Article

Identification of potential target genes in *Homo sapiens*, by miRNA of *Triticum aestivum*: A cross kingdom computational approach

Daniel Sánchez-Romo, César I. Hernández-Vásquez, Benito Pereyra-Alfárez, Jorge H. García-García*

Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León, Av Universidad S/N, 66455, San Nicolás de los Garza, Nuevo León, Mexico



ARTICLE INFO

Keywords:

microRNA
mRNA
Triticum aestivum
Human
Functional enrichment

ABSTRACT

Plant-derived miRNAs can be found in the human body after dietary intake, and they can affect post-transcriptional gene regulation in human. It is important to identify targets to determine the possible effects in human genes by using computational approach. In this study, 787 possible mRNAs human targets were predicted by 84 miRNAs of wheat. A total of 14 miRNAs were identified with individual binding to 33 mRNAs associated with schizophrenia, epilepsy, neurodevelopmental disorders, and various cancers, located in the 3'UTR of the mRNA. A functional enrichment was carried out, where the results showed associations to pathways such as dopaminergic synapse (hsa04728), and signaling pathways, significantly associated with the target genes. The prediction of target mRNAs in humans by wheat miRNAs, offer candidates that could facilitate the search and verification, which could be of relevance for future projects and therefore contribute in the therapeutic treatment of various human diseases.

1. Introduction

The microRNAs (miRNAs) of plants and animals are an abundant class of small non-coding RNAs sequences around ~22 nucleotides long [1]. They are derived from hairpin precursors, which have remained evolutionarily similar in various species, and have the ability to negatively regulate gene expression at the post-transcriptional level by complementary base binding in various regions of the mRNA (3'UTR, 5'UTR and ORF) and via the RNA-induced silencing complex (RISC) whose binding causes mRNA cleavage or translation inhibition [2–4].

It is expected that 30–60% of the genes in humans are miRNA regulatory targets [5], involved in various developmental processes, molecular, cellular pathways and human disease [6]. When crossing the intestinal barrier after ingestion miRNAs can be packaged in microvesicles, circulate through the human body and be identified in fluids such as human plasma [7] and breast milk [8].

Identifying mammalian gene targets in a timely manner by plant miRNA offers a reliable, effective and economical way to determine the possible effects of interactions given by exogenous molecules, regardless of their origin, on human mRNA [9–14].

Recently, plant miRNAs and their interactions have been actively studied, contributing to the knowledge of coevolution mechanisms

between plant miRNAs and human mRNAs. For example, the *in silico* prediction on the possible regulation of plant miRNA on human mRNA was tested, coincided with an increase in cholesterol-LDL levels *in vivo* and *in vitro*, caused by the decrease of LDLRAP1 protein expression, providing the first evidence that food miRNA could bind to a target mRNA and influence the regulation of interspecies expression [15]. Similarly, through bioinformatics analysis, found a number of potential target genes for MIR2911 from the *Lonicera japonica* plant (honeysuckle) were found in different viruses such as H1N1, H3N2, H5N1 and H7N9. Later, in cultures of cellular lines infected with H1N1 and transfected with synthetic MIR2911, they managed to significantly decrease the viral count because the target genes: PB2 and NS1, are essential for replication. Subsequent functional studies showed that miR2911 inhibited several IAVs (influenza A viruses) including H1N1, H5N1 and H7N9, decreasing mortality in an animal model [16]. Suggesting the first reports that exogenous miRNAs regulate gene expression in endogenous mRNAs similar to mammalian miRNAs and in turn the use of the bioinformatics approach, playing an important role in target identification.

In the present study we have selected the monocotyledonous plant *Triticum aestivum* (wheat) because it is a common source in human nutrition, we performed an *in silico* prediction analysis of potential

* Corresponding author.

E-mail address: jorge.garciagr@uanl.edu.mx (J.H. García-García).

<https://doi.org/10.1016/j.ncrna.2022.03.002>

Received 10 December 2021; Received in revised form 31 January 2022; Accepted 14 March 2022

Available online 18 March 2022

2468-0540/© 2022 The Authors. Publishing services by Elsevier B.V. on behalf of KeAi Communications Co. Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

mRNAs target in *Homo sapiens* (human) by mature miRNAs of wheat, and its relationship to diseases through an enrichment of the possible target genes. In addition, we identified target genes with diverse functions in cellular processes, providing information on regulatory mechanisms given by wheat miRNAs. Also, a functional enrichment has been carried out to explore the participation of the target human mRNAs involved in biological processes and associated pathways, including apelin signaling pathway, fanconi anemia pathway and dopaminergic synapse.

2. Material and methods

2.1. Data collection

The complete set of known mature miRNA sequences, which includes a total of 122 miRNAs from wheat, were downloaded from the registered database (version 22.1) (<http://www.mirbase.org>) in FASTA format. To reduce errors in prediction and future detection, we applied rigorous filtering for mature miRNAs, i) The mature miRNA should have more than 1400 reads during sequencing reported in its bioinformatic analysis; ii) The miRNA size should be in the range of 19–25 nucleotides and iii) All mature miRNAs should be in the same direction from 5' to 3'. Following this, 84 miRNAs from wheat were selected as reference data set for the study.

2.2. Prediction of potential targets of miRNAs

The prediction of possible targets on human was carried out by the sequence similarity search tool BLASTn (2.9.0) on its NCBI website (<http://www.ncbi.nlm.nih.gov>) using the default settings, an E-value threshold of 10; and the search coordinates were set in the nucleotide sequence 2 to 13 of each miRNA. From the RefSeq RNA database the predicted miRNA target sites were identified by aligning the sequences in a complementary way to the miRNA of wheat with human mRNA. RNAhybrid [17] was used to scan the hybridization patterns of the individual miRNAs/mRNA following Zhou Z. et al. methodology with some modifications [16]. i) The seed region had to perfectly match the nucleotide 2 to 7 of the mature miRNA. ii) The minimum free energy (MFE) for the hybridization was below -25 kcal/mol iii) No G-U bonds in the seed region. We also used the MIR168a as a target prediction control [15].

2.3. miRNA homology

We searched for homology using MirCompare between 2042 *H. sapiens* and 84 *T. aestivum* matured miRNAs from miRBase was used [18]. The parameters used for this analysis were taken from Minutolo et al., 2018 [19], with modification in the threshold of the seed region according to the guidelines provided by the default settings of the tool. The possible target genes of the resulting human miRNAs with homology to wheat miRNAs as reported by MirCompare were found by an analysis with TargetScan 8.0 algorithms (<http://www.targetscan.org>).

2.4. Functional enrichment analysis

The functional analysis of the targets, including biological processes was done using clusterProfiler [20], package inside programming language R version 4.0.3 [21]; with only those target sequences with miRNA binding sites in the 5'UTR, 3'UTR and CDS regions. Designating the potential processes with an adjusted p-value lower than 0.05 (FDR <0.05) that involve the predicted mRNA sequences and suggesting the probable influence in the regulation of plant miRNAs in the human organism.

3. Results

3.1. Characteristics of the binding of wheat mature miRNAs with human mRNAs

According to the methodology previously mentioned, the results obtained were similar to the ones described by Zhang et al. 2012 [15]. We selected 84 mature miRNAs from wheat and predict putative targets genes in humans, based on the criteria specified in methods section, which resulted in a total 889 miRNA/target interactions with 787 genes with diverse functions, the characteristics and specific target sequences of the 84 miRNAs analyzed in this study are provided in the (Supplementary Table 1). 70 of these genes had interactions with more than one miRNA and 6 come from miRNAs families, as previously reported by Han R. et al. [22] (Table 1). These miRNAs were divided in two groups; in group one, 14 miRNAs identified with one to four target genes, giving a total of 30 target genes. In group two 70 miRNAs with five or more target genes, giving a total of 757 target genes. The tae-miR9652-3p and tae-miR9652-5p of group one, originated from the same precursor (pre-miRNA) in miRNA biogenesis, have binding sites in the mRNAs of different genes. In the second group the tae-miR9657b-3p and tae-miR9657b5-p, were found as a pair originating from the same pre-miRNA, obtaining 18 targets for miR-3p, and 10 for miR-5p strand, all being different. The miRNAs with the highest number of targets were tae-miR9657c (19 genes), tae-miR9654a (21 genes), tae-miR160 (22 genes), tae-miR396 (23 genes), tae-miR9662a (23 genes), tae-miR9666a (24 genes), tae-miR9664 (28 genes), tae-miR9655 (31 genes), tae-miR9666b-3p (34 genes), tae-miR164 (35 genes).

In order to show the targets and MFE for the binding between some wheat miRNAs with human mRNAs, data was filtered to show the targets genes with lower value of MFE and were the sites union are located in 3'UTR binding (Table 2). 10 of the miRNAs show 1 target gene, 2 miRNAs show two targets, and 2 miRNAs show three and six targets, with a MFE between -34.1 and -43 kcal/mol. The tae-miR160 shows 10 target genes with a free energy value between -36.9 and -45.9 kcal/mol.

Because genes are nucleotide sequences coding for diffusible elements that participate in all cellular processes, it is important to show information about some human target genes by wheat miRNAs, involved in various biological processes (Supplementary Table 2): RALY - lung cancer, CNTNAP4 - epilepsy, CCDC88C - neurodevelopment, PCARE - pigmentary retinosis, FRMD8 - inflammation, CRIBB33 - cataracts, PLEKHA6 - schizophrenia, BTNL9 - ocular melanoma, KAT5 - anaplastic thyroid cancer, PARBV - oral squamous cell carcinoma, PRKAG2 - nonalcoholic steatohepatitis, CHST10 - tumor suppressor, RTN4RL1 - nasopharyngeal carcinoma, MTCL1 - cerebellar ataxia, GNAI2 - ovarian cancer, SMARCC2 - embryogenesis and corticogenesis, CSRP1 - lung fibrosis, B3GNT7 - colon cancer, KCNA2 - epileptic encephalopathy, NOX5 - diabetic nephropathy, CBX7 - hepatocellular carcinoma, BCL7B - Wnt signaling, MBD3 - pancreatic cancer, CORO1B - TGF β 1 signaling, ATPAF2 - dementia risk, DIAPH3 - lung adenocarcinoma, and TPM2 - aortic dissection.

Interactions between wheat miRNA and human mRNA can show how efficiently these molecules act. Some tae-miRNA160/mRNAs binding patterns in the 3'UTR of the mRNA, resulting from the bioinformatic tool RNAhybrid are shown in (Fig. 1). Taking into consideration the nucleotide sequence after the seed region of the mature miRNA, in the *in silico* prediction through the RNAhybrid tool, it is possible to observe the type of interaction between the tae-miRNAs with their targets and provide an approach to the type of silencing like repression and/or degradation through the complementary base pairing. For this reason, when examining the type of binding or complementarity that was performed in each miRNA/mRNA result, in a complete or incomplete complementary binding, it was found that 100% of the 889 predicted tae-miRNA/target interactions, were shown as imperfect hybrids in at least one nucleotide of the complementary mRNA after the seed region.

Table 1
Potential target genes of 84 wheat miRNAs identified in human.

miRNA ID	Number of targets	miRNA family	Target Gene
Tae-miR1117	9		TULP4, CHSY3, SMARCC1, C5orf52, DSCAML1, BZW1, UBR5, LOC102724740, ACVR1B
Tae-miR1118	7		ARHGAP32, PRUNE2, XKRX, HOOK3, LOC105372263, ANXR2, MAP1B
Tae-miR1120b	1		RPAP2
Tae-miR1122a	6		SERINC5, ZBTB39, GDNF, SPAG9, ZC3H12C, CDC42BPB,
Tae-miR1122b	2		LOC105375144, SPTLC3
Tae-miR1122c	1		PPP2R3A
Tae-miR1123	9		EPO, LOC105375220, ASGR2, HPCAL1, CNFN, COL16A1, IGF2BP2, BEST1, LOC100507516
Tae-miR1125	5		CHRM3, SLC38A8, RALY, TIMM10, RYR2
Tae-miR1127a	4		MSRB3, IPO13, RRAD, MFAP3
Tae-miR1127b	9		XKR6, STXB5P5, NOL8, ZNF691, KRIT1, RP1L1, ZNF510, SNX13, RGS6
Tae-miR1128	12		CNTNAP4, LOC105371840, ESR1, RAPGEF6, LOC107986008, TMEM161B-AS1, BRINP1, INTS4, THAP9, ADCY1, RUSC2, KLHL36
Tae-miR1130b	2		PRORY, ASPH
Tae-miR1131	7		SMARCC1, DSCAML1, UBR5, CHSY3, BZW1, TULP4, STXB5P5
Tae-miR1133	7		WDR75, MALT1, RIMKLB, SKAP1, ACAD11, GPR176, ST8SIA4
Tae-miR1135	5		LOC112268243, LOC105371988, SPACA1, THSD7A, DDX5
Tae-miR1136	11		OBSCN, DHX37, MRC1, STAB1, LOC105379283, SNORA14B, ZNF174, CALN1, TMEFF2, CALN1, UQC22
Tae-miR1137a	3		ATP6V1B2, ZFPM2, ATXN1L
Tae-miR1137b	8		RBX1, STK35, CREB5, RNF121, KIF27, JAG1, PKHD1, ITGA11
Tae-miR1138	8		KDM3B, USP35, PRKACB, MAK, ZBED2, TESK2, BMP3, ZSCAN25,
Tae-miR1139	5		ZFH3, ZC3H8, CDON, MID1, DZIP1L
Tae-miR156	14		CCDC88C, F11R, FAM13A, KIF5A, LOC105369388, MAPK11, ZBTB40, LINC00312, CCDC15, TMEM201, ADGRD1, CAPN13, TGOLN2, CCNH
Tae-miR159a	8		CNOT9, GPR4, TEX13C, LOC105377862, MDH1B, LINC02789, MAN1A2, GPR135,
Tae-miR159b	8	miR159	CNOT9, GPR4, TEX13C, LOC105377862, MDH1B, LINC02789, MAN1A2, GPR135,
Tae-miR160	22	miR160	PCARE, FAM118B, BCAS1, BCAP29, TMEM241, CAMTA1, FMRD8, PSMD7, ASPDH, CRYBB3, PLEKHA6, BTNL9, ANKRD35, KAT5, DAAM2, PARVB, PRKAG2, RTN4RL1, CHST10, RHPN1, GHRL, LOC100996662
Tae-miR164	35	miR164	FGFRL1, ZNF701, SLC9A9, MTC1L, PPRC1, GREM1, SYNE1, C8orf33, GRM2, PCYT2, GLI3, SNED1, GNAI2, SMARCC2, CSRPI, B3GNT7, KCNA2, LOC107986317, RFPL4B, FSTL4, SLC25A1, RASSF6, KCNH6, APOBEC3B, APOBEC3CA, ABCB9, BIRC6, NCAPH, SETD4, SLC6A7, LRRCC55, MNT, ACBD4, TRIM41, PBXIP1
Tae-miR167a	13	miR167	PRICKLE2, WASHC2A, TM2D3, CACNA2D4, LOC105375492, NOX5, CBX7, TNFRSF10D, BCL7B, MBD3, NCBP3, SLC9B2, THBS3
Tae-miR167b	9		EME1, ZNF20, PLCE1, LOC107986558, CACNA1C-IT1, LOC440292, CAMK2D, GPD1, SNRNP200
Tae-miR167c	12		TM2D3, WASHC2A, CACNA2D4, NCBP3, BCL7B, LOC105375492, MBD3, NOX5, CBX7, TNFRSF10D, SLC9B2, THBS3
Tae-miR171a	2	miR171	IGF1, C15orf56
Tae-miR171b	11		TMEM8A, RNF112, TMEM151A, NAT16, RPAP1, LIMK2, GCC1, LOC107986282, MPRIP, RAPH1, LOC112268024
Tae-miR1847	15		CRISP2, CYP27A1, ALG3, LAMA5, N4BP1, ART3, ZNF619, UBQLN4, TMEM178B, LMOD1, LGI3, LOC105377347, SH3PXD2A, CRISPLD1, MRM1
Tae-miR319	14		SCART1, HOXB3, SLC1A4, TIGD7, FAT4, FAM205C, KLC2, KMT5A, ACLY, MUC16, CCDC134, LOC105375153, RAB5B, SUOX
Tae-miR395a	7		CLCN5, LOC107984275, VAPA, LOC105373908, CERS4, PPARGC1B, ITGB8
Tae-miR395b	11	miR395	CLCN5, USP6NL, LINC02739, FN1, CASP5, WDFY3, CEP63, METRN, UBAP1L, LOC112267986, LYPLAL1
Tae-miR396	23		SLC25A25, CPOG1, CYFIP1, NOTCH2NLB, ZNF814, KDM1A, PELI3, GCDH, ZNF32, ADAMTS18, ZNF776, CL11A1, PTCH2, UNC119B, ZBTB14, LOC107984192, REST, FOXP2, SLITRK9, CDC5L, EFHB, KIF2A, GRIN1
Tae-miR397	5		PALM2AKAP2, SURF6, BCAR1, C1orf67, RNF175
Tae-miR398	14		PPM1H, CORO1B, ANKK1, LOC105374534, FBXO10, DNMT1, SLC37A1, PCNX3, LOC105370363, LOC105378740, ZNF691, KIAA0408, SOGA3, PPIP5K2,
Tae-miR399	16		DRICH1, ANKRD36, SET, LINC02142, ARIH2, FAM166C, ZFP2, SCCPDH, ADPRM, LRRIQ1, AOC3, NUP210, CALM1, MANBA, MYLK4, ANKFN1
Tae-miR408	16		USP43, ATPAF2, PSMD11, ADGRF5, DIAPH3, AGO3, ABCG8, LOC105376475, ZNF518A, EPS15L1, ANXA11, ROS1, RYR3, KCNB2, LOC112268372, KSR1
Tae-miR444a	14		LOC112268280, RUND1, LOC105371800, ZC3H4, LOC107986334, CDC25A, ZC3H4, CCDC171, SUPT20HL1, LOC101929566, SYNJ2, TEKT3, FRMD3, YPEL5
Tae-miR444b	13		LOC112268280, RUND1, LOC107986334, LOC105371800, ZC3H4, CDC25A, CCDC171, SUPT20HL1, LOC101928566, SYNJ2, TEKT3, FRMD3, YPEL5
Tae-miR5048	5		CHRM3, TBC1D4, ADAM22, GRM4, LOC105378532
Tae-miR5049	2		LOC147004, PNPT1
Tae-miR5050	9		PEG3, IPPK, ITPA, TRIO, TEX30, SCFD2, FOSL2, LINC01226, BCLAF1
Tae-miR5062	9		ARMC9, YEATS2, LOC107985153, CD2BP2, ABI2, RNF183, CACNA2D4, AHNAK, HS6ST2
Tae-miR5175	16		SETBP1, RBBBP6, GPRC5D, CHST2, C4orf54, CENPS-CORT, CORT, RNLS, TRIM37, NSL1, LINC01588, SNRNP27, LOC101929270, SUCO, STK31, DNAJC6
Tae-miR5200	5		LINC02842, LOC101927610, LOC107986016, NRG4, LOC101928421
Tae-miR531	18		REPIN1, LOC107985320, SLC5A10, GBX2, GPC6, NINL, BACH2, B3GLCT, PLXNB3, DAB2IP, GCK, DCP2, ZNF282, NECTIN4, RFTN1, GPR22, ZNF618, GPR153
Tae-miR6197	3		EXOC6, ABCD2, ZNF519
Tae-miR7757	2		RPR6KA2, HDAC8
Tae-miR9652-3p	1		PSMD13
Tae-miR9652-5p	1		POU2F1
Tae-miR9653b	15		ADCK5, SHE, TPST2, CPNE2, FGGY, P4HA2, TPM2, P4HA2, LOC105372821, VPS26C, HES7, ABCG8, RAB37, MTOR, RAB37
Tae-miR9654a	21		DTX2, SGO2, LOC10537819, TBGR1, LOC107984429, NT5DC2, LOC107986811, ELP6, LYRM9, LOC105375206, CFAP61, CHI3L2, GEMIN5, AP3M2, ZSCAN26, FBXO40, BZW2, TNRC6B, SESTD1, EBF2, TBL2,
Tae-miR9654b	12		ZNF740, CNF740, CNST, HOXD4, ARHGAP1, SLITRK3, KALRN, UFL1-AS1, SLC04A1, PCDH8, MLYCD, PDE1A

(continued on next page)

Table 1 (continued)

miRNA ID	Number of targets	miRNA family	Target Gene
Tae-miR9655	31		KPNA6, ELMO1, GEMIN5, TOMM20, TRIM71, CCBE1, CMBL, TAX1BP3, FGFR10P, CCR9, ELOVL2, ADH6, ARHGAP33, RNFB, TTC39B, CHD1, HMBOX1, MYADM, PLSCR3, HIPK1, STX7, LOC105372520, LOC105377622, WBP2, NIPAL3, TRPC3, VAMP2, PRRT2, USP12, HOOK3, AP1S3
Tae-miR9656	7		NPSR1-AS1, DAAMI, LDLRAP1, TRIM72, FAM49B, TRAK1, FAM49B
Tae-miR9657a	18		MECR, C17orf80, PCNT, STOX1, MAGI2, MELTF, PITRM1, LASP1, EPHA10, FYCO1, RGS6, SNX29, LZTS3, ZNF395, LINC00473, TTF1, MYO10, CCDC40
Tae-miR9657b-3p	17		C17orf80, MELTF, PCNT, STOX1, MAGI2, LZTS3, PITRM1, EPHA10, RGS6, SMX29, LASP1, TTF1, ZNF395, FYCO1, LONC00473, MYO10, GAD1
Tae-miR9657b-5p	10		MAFB, SPON1, MAF, TBC1D8, MAFA, FSTL4, CEBPB, DMXL1, SCAMP4, ELFN2
Tae-miR9657c	19		MECR, C17orf80, MELTF, PCNT, STOX1, MAGI2, LZTS3, PITRM1, EPHA10, RGS6, SNX29, LASP1, TTF1, ZNF395, FYCO1, LINC00473, MYO10, CCDC40, GAD1
Tae-miR9662a	23		LOC101929698, LRCOL1, LOC105370502, KCNA7, RBCK1, CLDN20, HRH2, TAF5L, MIR155HG, LINC02568, RIBC2, PFKFB2, TOP3A, LOC107986445, MOB3C, CLTRN, JAKMIP2, HIRA, LOC105374262, PCNT, SS18L2, CASP9, ARNTL
Tae-miR9662b	12		LOC101929698, PIGV, FAM83H-AS1, LOC105370502, AOX3P-AOX2P, HRH2, LOC105371449, LINC02568, HIRA, LOC107986445, LOC102723784, GNB5
Tae-miR9663	5		TAF1, ZC3H7B, PSMB4, DHPS, SGF29
Tae-miR9664	28		IQGAP1, TNXB, RIN3, USP40, LOC112268055, TNC, SOX30, ABCC1, FAHD2B, ABCC1, FAHD2CP, TNR, MLST8, CLEC3B, CCN1, CASZ1, KRT31, COPG1, NTNG2, CORIN, ZBTB48, CFAP46, TENM3, TEO2, LOC112268408, ALPK1, DENND3, KIAA0586
Tae-miR9666a	24		RAPGED4-AS1, BOC, OVOL1, USH2A, NACA, C8orf34, ANXA11, DOK1, CDH11, MST1R, CSRN1P, MBD1, PRR23D1, STON2, DCHS1, MARC1, WDR46, MBD1, DEFB119, ZFAND5, CDH6, SFMBT1, SEC14L2, DLX5, RAD51AP2
Tae-miR9666b-3p	34		SEC14L2, DLX5, RAD51AP2, POLR2A, YEATS4, CC2D2A, PCDHA6, PCDHA2, PCDHA10, PCDHA4, PCDHA13, IFT22, RAET1G, BSN, PDLIM5, ADAMTS10, KCNIP3, GABBR1, PCDHA8, PDZD2, ATP13A1, RBM6, ARID5B, ARHGAP26, PHF2, PCDHA12, PCDHA5, SGPP2, PCDHA7, ARHGAP26, CLOCK, WDR1, SLC24A1, PEX5L
Tae-miR9668	6		LIN02362, TMEM185B, POLE2, LOC101928985, LOC105376956, SLX4
Tae-miR9669	8		TOP3A, RXRG, SSH2, ENGASE, LRP6, GAB1, SMUG1, BAHCC1
Tae-miR9670	7		LOC105372542, NCAPD3, TPM2, GDAP1, SMG7, SCTR, NRG1
Tae-miR9672a	12		FRG2C, DCTN5, BMP8A, PKHD1, PKHD1L1, LOC107987108, NELL2, CEP152, SEC22B, WDFY3, PLXNA4, TACR3
Tae-miR9672b	6		TOX4, LOC102724245, MYO18B, GNL2, APMAP, KIAA1217
Tae-miR9674a	18		ACSS1, FANK1, TJP2, LOC105370291, EIF3A, DYRK1A, ATP9B, ZNF629, PDZRN3, LIPG, RWDD1, PRMT3, TMEM246, ELF4, VPS33A, ERICH1, FAR1, LINGO2
Tae-miR9674b	15		TBX19, DSTYK, TXNDC9, FANCE, NSDHL, GALNT3, TCF4, FAAH2, TOP2B, LOC112268009, TCF4, TSTD2, GCLC, SBF2, LINC03094
Tae-miR9675	5		BTRC, TMEM135, MRRF, ATRIP, ATRIP-TREX1
Tae-miR9676	3		TMEM259, GRID1, FOXD1
Tae-miR9677a	6		MAN2A2, RRAS2, OLFML2B, LOC100132249, ABCC8, SLC8B1
Tae-miR9679	11		DPH7, ABLIM1, ZMIZ1, BCL7B, MBOAT2, CBX5, DRP2, INPP5B, ZNF704, TBC1D12, RELN
Tae-miR9772	5		AAK1, RAP1B, NUCB2, SRGAP2, LOC105374129
Tae-miR9774	6		CTB-3M24.3, H6PD, OTUD6B, EDNRB, RGS3, EXPH5
Tae-miR9775	3		MGAT4D, MED27, USP11
Tae-miR9776	5		OLIG2, HECA, ZBTB11, RNF213, LOC105370091
Tae-miR9782	16		LOC112267915, RCSD1, RNLS, ZNF184, SAMD12, CYP24A1, GASK1A, LOC105371867, LOC101928819, TRDMT1, LOC105375471, NIPAL2, SRGAP1, ALG10B, CD38, LOC107987129
Tae-miR9783	7		EIF4EBP2, FGFBP2, DHX8, GINS2, ARPC5, TRPC3, FAM209B

3.2. Analysis of human homologs of wheat miRNAs

In the analysis we found homology candidates in 9 human miRNA sequences, with 7 wheat miRNA sequences. Supporting this, we identified target gene predictions of human miRNAs using the TargetScan tool and determined those targets in common predicted in this study as a whole (Table 3).

3.3. Functional enrichment of target genes by wheat miRNAs

Functional annotation of target genes is an important step in understanding the respective functions. A gene ontology (GO) enrichment analysis was performed in the Kyoto Encyclopedia of Genes and Genomes (KEGG) to identify the functions that miRNAs could have on their predicted targets in humans, where all mRNAs that had binding sites in the prediction were used. From the complete target search data, 670 different genes were used, selected according to probable binding site, including only those in the 5' UTR, 3' UTR, and CDS region and excluding those in non-coding sequences for functional enrichment analysis. The main GO terms classified in biological process are shown, with

significance adjusted p-values (<0.05) indicating that a total of 17 mRNAs enriched the biological process of homophilic cell adhesion (GO:0007156), through adhesion molecules to the plasma membrane, and 22 mRNAs enriched the biological process of cell-cell adhesion through adhesion molecules to the plasma membrane (GO:0098742), by miRNAs known from wheat in the prediction (Fig. 2).

Enrichment analysis of the KEGG pathway showed that the 670 predicted genes of the 84 selected wheat miRNAs were significantly enriched in 8 pathways, with an adjusted p value (<0.05) (Supplementary Table 3). 18 genes were enriched in calcium signaling pathway (hsa04020), 10 genes were enriched in the gastric acid secretion pathway (hsa04971), 14 genes were enriched in the apelin signaling pathway (hsa04371), 9 genes were enriched in the long-term potentiation pathway (hsa04720), 11 genes were enriched in the circadian entrainment pathway (hsa04713), 8 genes were enriched in the fanconi anemia pathway (hsa03460), 13 genes were enriched in the dopaminergic synapse pathway (hsa04728), and 13 genes enriched the adrenergic signaling pathway in cardiomyocytes (hsa04261) (Fig. 3).

Table 2
Individual interaction between wheat miRNAs and human mRNAs at the 3' UTR site.

Gene	miRNA ID	kcal/mol
EPO	Tae-MIR1123	-39.1
RALY	Tae-MIR1125	-35.3
CNTNAP4	Tae-MIR1128	-38.7
CCDC88C	Tae-MIR156	-34.9
PCARE	Tae-MIR160	-45.9
FRMD8	Tae-MIR160	-40.3
CRYBB3	Tae-MIR160	-38.8
PLEKHA6	Tae-MIR160	-38.7
BTNL9	Tae-MIR160	-38.6
KAT5	Tae-MIR160	-37.7
PARVB	Tae-MIR160	-37.4
PRKAG2	Tae-MIR160	-37.2
CHST10	Tae-MIR160	-36.9
RTN4RL1	Tae-MIR160	-36.9
MTCL1	Tae-MIR164	-37.5
GNAI2	Tae-MIR164	-35
SMARCC2	Tae-MIR164	-34.9
CSRP1	Tae-MIR164	-34.3
B3GNT7	Tae-MIR164	-34.2
KCNA2	Tae-MIR164	-34
NOX5	Tae-MIR167a	-34.5
CBX7	Tae-MIR167a	-34.2
BCL7B	Tae-MIR167c	-35
MBD3	Tae-MIR167c	-34.6
SURF6	Tae-MIR397	-34.1
CORO1B	Tae-MIR398	-36.1
DRICH1	Tae-MIR399	-34.8
ATPAF2	Tae-MIR408	-36.8
DIAPH3	Tae-MIR408	-35.6
ZNF518A	Tae-MIR408	-34.2
LOC107985320	Tae-MIR531	-43
TPM2	Tae-MIR9653b	-37.4
LRCOL1	Tae-MIR9662a	-35.4

4. Discussion

Based on the results of this work and the extensive study on the mechanisms of plant miRNAs when entering the circulatory system through the diet, entering the cells to achieve a target and regulate the expression of a gene [15]. Some authors [23,24], suggest the use of available and evaluated tools for predictive analyses, since they recognize the fundamental importance of identifying the target genes in the human genome by exogenous miRNAs, in order to infer their functions and regulatory mechanisms, however they agree that it is convenient to explore and combine predictive tools because individually, they may not reflect the abundance and possible regulatory capacity of miRNAs, improving the speed and accuracy of the targets. In our research we used two search engines, that way we were able to simulate a possible interaction between species. By using BLASTn as the first instance as a search engine for complementary sequences with the updated version of the RefSeq human transcriptome database, we were able to find several targets and their variants, which is important because factors related to expression variation in target genes can provide a solid foundation and more systematic view of the genetic mechanisms that are related to human genetic variation [25].

The results obtained by the BLASTn analysis were filtered in a second computational method using RNAhybrid tool as used successfully used in several studies of cross kingdom miRNAs [16,26,27], which allows us to determine the MFE, characteristics and complementary regions of the miRNA/mRNA interaction, which allowed filtering of those interactions that we consider energetically favorable (MFE-25 kcal/mol). This

provided us with a combination of algorithms in the study that allowed us to obtain a total of 787 genes and their variants with different functions as shown in (Supplementary Table 1). In addition, Table S1 shows the transcripts of non-coding RNAs (ncRNAs) that were targeted by the 84 miRNAs, since miRNA targets are not limited to coding transcripts, but can also be found in ncRNAs, indicating that miRNA targeting mechanisms can be complex by binding to a wide variety of targets [28].

A total of target genes for 84 miRNA of wheat, divided into two groups according to the number of targets, and families that each miRNA obtained, since miRNAs with a larger number of target genes should be monitored in human fluids, due to their possible ability to significantly regulate human receptor cells [9]. These targets were located in various regions along the mRNA (5'UTR, CDS, 3'UTR), increasing the area of study on the interactions given by exogenous molecules, it could help understand the role of binding sites in the mRNA with the miRNAs [29]. We also evaluated the type of binding, obtaining 100% of the possible binding as imperfect base-pair by at least 1 base, however the analysis was made with the criterion of perfect binding of the seed region which would suggest that the possible mechanism of regulation of expression according to the reported silencing by mature miRNAs [2] could be translation inhibition and not degrading by cleavage the mRNA, which is sufficient for the miRNAs to suppress their targets [23,30]. In plants, miRNA bind to their targets with high complementarity, causing messenger degradation, however in animals, miRNA recognition in just their seed region is extremely important in target recognition, this complementarity can trigger expression regulation, [31,32]. Even in humans, the seed region of some miRNA can define toxicity or tumor-suppressive for some cancer cell lines [33]. This is due to seed region target recognition mechanisms [34,35], show that an initial search for target sites transitions from being transient to kinetically stable, when target complementarity binds to the full length of the seed region of miRNA [36]. Furthermore, several authors have identified and demonstrated regulation of target gene expression, even when miRNAs have not bound in a fully complementary manner, showing evidence of their function [16,37,38].

In this study we included a homology analysis, identifying 7 wheat-derived miRNAs show homology with 9 human miRNAs, revealing a series of target genes in common, through our prediction. Although plant sequences do not show high homology with human sequences, results obtained by author [39], show that they can perform similar functional activities to endogenous sequences, in a cross-kingdom interaction [19]. Therefore, we consider this dataset provides a solid basis for future projects.

In our mRNA and miRNA interaction analysis we aimed to identify the genes that are the target of multiple miRNAs and their possible health effect through KEGG pathways. We found that the target genes are associated with the calcium signaling pathway, showing participation of the CD38 gene, associated with diseases such as asthma, neoplasia and neuroimmune diseases [40], as well as the ADCY1 gene, involved in insulin secretion, related to diabetes mellitus and its correlation with pancreatic cancer [41].

Among the pathways, three stand out, the pathway of fanconi anemia (hsa03460), circadian entrainment (hsa04713), and dopaminergic synapse (hsa04728), which we have found a relation with a couple of publications [42,43](Gluten-Free Diet for the Treatment of ADHD; Pilot Study) where in a case study they describe the difficulty of finding a diagnosis to a series of neuropsychiatric manifestations, described as “non-celiac gluten sensitivity” (NCGS), where a series of symptoms related to the consumption of gluten-containing foods (wheat) are characterized in people not affected by celiac disease (CD) or wheat allergy (AW). Attention deficit hyperactivity disorder (ADHD) related

Table 3
Potential target genes in common by wheat and human miRNAs.

Tae-miRNA	Hsa-miRNA	Seed Score	Genes
Tae-miR9772	Hsa-miR-143-3p	1	AAK1
Tae-miR171a	Hsa-miR-8082	0.8125	IGF1
Tae-miR164	Hsa-miR-139-3p	0.8125	ZNF701, FSTL4, RASSF6, LRRC55, MNT
Tae-miR9674b-5p	Hsa-miR-4524b-5p	0.8125	DSTYK, TOP2B
Tae-miR164	Hsa-miR-5703	0.8125	ZNF701, SLC9A9, GREM1, GLI3, SNED1, SMARCC2, CSRP1, B3GNT7, RASSF6, SLC25A1, NCAPH, LRRC55, MNT, ACBD4, TRIM41, PBXIP1
Tae-miR9666b-3p	Hsa-miR-4259	0.8125	PCDHA6, PCDHA10, PCDHA13, PCDHA2, PCDHA4, PCDHAC2, PCDHA3, RBM33, ARID5B, PCDHA12, PCDHA5, PCDHA7, CLOCK
Tae-miR164	Hsa-miR-765	0.8125	SLC9A9, PPRC1, GREM1, SYNE1, C8orf33, GLI3, SNED1, B3GNT7, FSTL4, KCN6, LRCC55, ACBD4
Tae-miR5048-5p	Hsa-miR-1205	0.8125	CHRM3, ADAM22, GRM4
Tae-miR395a	Hsa-miR-203a-3p	0.8125	VAPA, PPARGC1B

health, such as cancer, schizophrenia, and metabolic diseases.

5. Conclusion

Considering that wheat and humans are different species, it is difficult to determine the possible alterations given by exogenous molecules caused by cross-kingdom interactions, however, it is necessary to highlight the importance of the use of bioinformatics tools, for the prediction of possible targets, because this study demonstrates the possible effects of wheat miRNAs in regulating complex disease networks like a schizophrenia, epilepsy, tumor suppressor, inflammation, and diverse type of cancer. Due to the fact that the information collected could facilitate the search and verification, that could be of importance for future projects. In addition, these findings can generate new lines of research in various areas, such as nutrition, medicine, biotechnology, agriculture, etc. It can also show possible miRNAs candidates that can further contribute in therapeutic treatment of various human diseases.

CRedit authorship contribution statement

Daniel Sánchez-Romo: Writing – original draft, Methodology, Investigation. **César I. Hernández-Vásquez:** Formal analysis. **Benito Pereyra-Alfárez:** Conceptualization, Resources. **Jorge H. García-García:** Project administration, Writing – review & editing.

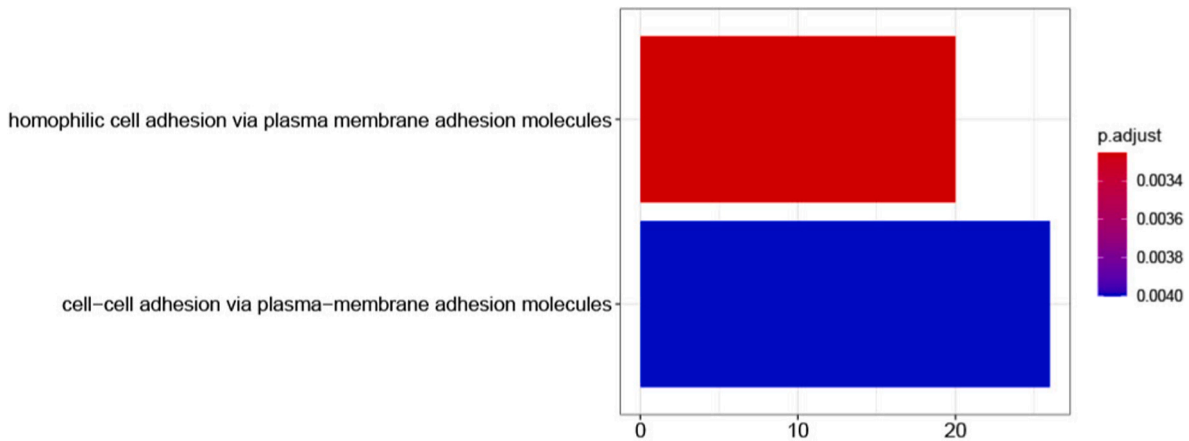


Fig. 2. Gene ontology analysis of predicted human target genes derived from miRNAs of wheat.

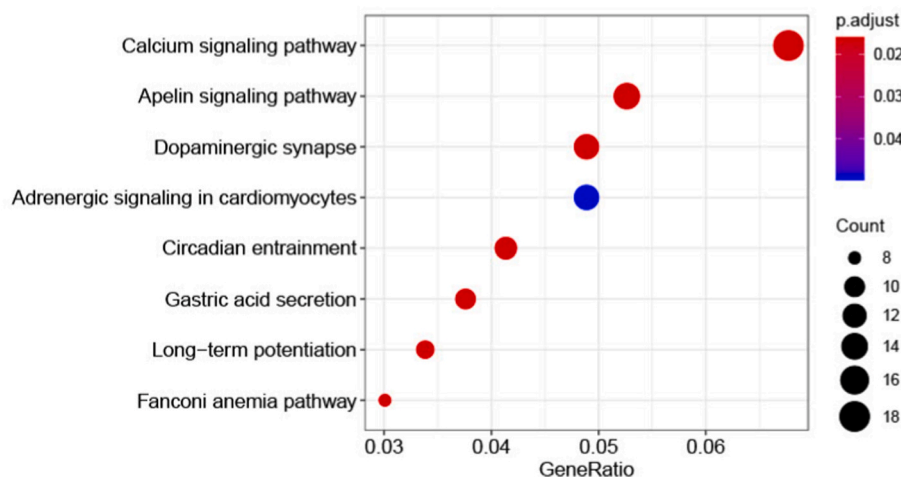


Fig. 3. Pathway enrichment of predicted human target genes derived from miRNAs of wheat in 8 significant KEGG categories.

Declaration of competing interest

None.

Acknowledgments

Non applicable.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ncrna.2022.03.002>.

References

- V. Ambros, The functions of animal microRNAs, *Nature* 431 (2004) 350–355, <https://doi.org/10.1038/nature02871>.
- R.C. Friedman, K.K.-H. Farh, C.B. Burge, D.P. Bartel, Most mammalian mRNAs are conserved targets of microRNAs, *Genome Res.* 19 (2009) 92–105, <https://doi.org/10.1101/gr.082701.108>.
- P. Graves, Y. Zeng, Biogenesis of mammalian MicroRNAs: a global view, *Genomics, Proteomics Bioinforma* 10 (2012) 239–245, <https://doi.org/10.1016/j.gpb.2012.06.004>.
- I. Lee, S.S. Ajay, I.Y. Jong, S.K. Hyun, H.H. Su, H.K. Nam, S.M. Dhanasekaran, A. M. Chinnaiyan, B.D. Athey, New class of microRNA targets containing simultaneous 5'-UTR and 3'-UTR interaction sites, *Genome Res.* 19 (2009) 1175–1183, <https://doi.org/10.1101/gr.089367.108>.
- P. Perge, Z. Nagy, A. Decmann, I. Igaz, P. Igaz, Potential relevance of microRNAs in inter-species epigenetic communication, and implications for disease pathogenesis, *RNA Biol.* 14 (2017) 391–401, <https://doi.org/10.1080/15476286.2016.1251001>.
- Y. Zhao, L. Cong, W.J. Lukiw, Plant and animal microRNAs (miRNAs) and their potential for inter-kingdom communication, *Cell. Mol. Neurobiol.* 38 (2018) 133–140, <https://doi.org/10.1007/s10571-017-0547-4>.
- H. Liang, S. Zhang, Z. Fu, Y. Wang, N. Wang, Y. Liu, C. Zhao, J. Wu, Y. Hu, J. Zhang, X. Chen, K. Zen, C.Y. Zhang, Effective detection and quantification of dietetically absorbed plant microRNAs in human plasma, *J. Nutr. Biochem.* 26 (2015) 505–512, <https://doi.org/10.1016/j.jnutbio.2014.12.002>.
- B.J. Stephen, N. Pareek, M. Saeed, M.A. Kausar, S. Rahman, M. Datta, Xeno-miRNA in maternal-infant immune crosstalk: an aid to disease alleviation, *Front. Immunol.* 11 (2020) 1–8, <https://doi.org/10.3389/fimmu.2020.00404>.
- A. Rakhmetullina, A. Pyrkova, D. Aisina, A. Ivashchenko, In silico prediction of human genes as potential targets for rice miRNAs, *Comput. Biol. Chem.* 87 (2020), 107305, <https://doi.org/10.1016/j.compbiolchem.2020.107305>.
- D. Pirim, B. Dogan, In silico identification of putative roles of food-derived xenomirs on diet-associated cancer, *Nutr. Cancer* 72 (2020) 481–488, <https://doi.org/10.1080/01635581.2019.1670854>.
- M. Patel, N. Mangukia, N. Jha, H. Gadhavi, K. Shah, S. Patel, A. Mankad, H. Pandya, R. Rawal, Computational identification of miRNA and their cross kingdom targets from expressed sequence tags of *Ocimum basilicum*, *Mol. Biol. Rep.* 46 (2019) 2979–2995, <https://doi.org/10.1007/s11033-019-04759-x>.
- J. He, T. Chen, Q. Xi, J. Sun, J. Luo, M. Li, H. Zhang, B. Zeng, J. Wu, Y. Zhang, Identification of microRNA in *Houttuynia cordata* Thunb and prediction of cross kingdom functions, *ExRNA* 1 (2019), <https://doi.org/10.1186/s41544-019-0028-7>.
- D. Kumar, S. Kumar, G. Ayachit, S.B. Bhairappanavar, A. Ansari, P. Sharma, S. Soni, J. Das, Cross-kingdom regulation of putative miRNAs derived from happy tree in cancer pathway: a systems biology approach, *Int. J. Mol. Sci.* 18 (2017) 1–21, <https://doi.org/10.3390/ijms18061191>.
- H. Gadhavi, M. Patel, N. Mangukia, K. Shah, K. Bhadrasha, S.K. Patel, R.M. Rawal, H.A. Pandya, Transcriptome-wide miRNA identification of *Bacopa monnieri*: a cross-kingdom approach, *Plant Signal. Behav.* 15 (2020), <https://doi.org/10.1080/15592324.2019.1699265>.
- L. Zhang, D. Hou, X. Chen, D. Li, L. Zhu, Y. Zhang, J. Li, Z. Bian, X. Liang, X. Cai, Y. Yin, C. Wang, T. Zhang, D. Zhu, D. Zhang, J. Xu, Exogenous Plant MIR168a Specifically Targets Mammalian LDLRAP1: Evidence of Cross-Kingdom Regulation by microRNA, 2012, pp. 107–126, <https://doi.org/10.1038/cr.2011.158>.
- Z. Zhou, X. Li, J. Liu, L. Dong, Q. Chen, J. Liu, H. Kong, Q. Zhang, X. Qi, D. Hou, L. Zhang, G. Zhang, Y. Liu, Y. Zhang, J. Li, J. Wang, X. Chen, H. Wang, J. Zhang, H. Chen, K. Zen, C.Y. Zhang, Honeysuckle-encoded atypical microRNA2911 directly targets influenza A viruses, *Cell Res.* 25 (2015) 39–49, <https://doi.org/10.1038/cr.2014.130>.
- R. Giegerich, M. Rehmsmeier, P. Steffen, M. Ho, Fast and effective prediction of microRNA/target duplexes, *RNA* 10 (2004) 1507–1517, <https://doi.org/10.1261/rna.5248604.and>.
- S. Pirrò, A. Minutolo, A. Galgani, M. Potestà, V. Colizzi, C. Montesano, Bioinformatics prediction and experimental validation of MicroRNAs involved in cross-kingdom interaction, *J. Comput. Biol.* 23 (2016) 976–989, <https://doi.org/10.1089/CMB.2016.0059>.
- A. Minutolo, M. Potestà, A. Gismondi, S. Pirrò, M. Cirilli, F. Gattabria, A. Galgani, L. Sessa, M. Mattei, A. Canini, R. Muleo, V. Colizzi, C. Montesano, Olea europaea small RNA with functional homology to human miR34a in cross-kingdom interaction of anti-tumoral response, *Sci. Rep.* 8 (2018) 1–14, <https://doi.org/10.1038/s41598-018-30718-w>.
- G. Yu, L.G. Wang, Y. Han, Q.Y. He, ClusterProfiler: an R package for comparing biological themes among gene clusters, *OMICS A J. Integr. Biol.* 16 (2012) 284–287, <https://doi.org/10.1089/omi.2011.0118>.
- R. R core Team, A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna., 2018. <https://www.r-project.org/>.
- R. Han, C. Jian, J. Lv, Y. Yan, Q. Chi, Z. Li, Q. Wang, J. Zhang, X. Liu, H. Zhao, Identification and characterization of microRNAs in the flag leaf and developing seed of wheat (*Triticum aestivum* L.), *BMC Genom.* 15 (2014) 289, <https://doi.org/10.1186/1471-2164-15-289>.
- B. Liu, J. Li, M.J. Cairns, Identifying miRNAs, targets and functions, *Briefings Bioinf.* 15 (2014) 1–19, <https://doi.org/10.1093/bib/bbs075>.
- H. Min, S. Yoon, Got target?: computational methods for microRNA target prediction and their extension, *Exp. Mol. Med.* 42 (2010) 233–244, <https://doi.org/10.3858/emmm.2010.42.4.032>.
- C. Liu, W.A. Rennie, C.S. Carmack, S. Kanoria, J. Cheng, J. Lu, Y. Ding, Effects of genetic variations on microRNA: target interactions, *Nucleic Acids Res.* 42 (2014) 9543–9552, <https://doi.org/10.1093/nar/gku675>.
- H. Zhang, Y. Li, Y. Liu, H. Liu, H. Wang, W. Jin, Y. Zhang, C. Zhang, D. Xu, Role of plant MicroRNA in cross-species regulatory networks of humans, *BMC Syst. Biol.* 10 (2016) 1–10, <https://doi.org/10.1186/s12918-016-0292-1>.
- Y. Luo, P. Wang, X. Wang, Y. Wang, Z. Mu, Q. Li, Y. Fu, J. Xiao, G. Li, Y. Ma, Y. Gu, L. Jin, J. Ma, Q. Tang, A. Jiang, X. Li, M. Li, Detection of dietetically absorbed maize-derived microRNAs in pigs, *Sci. Rep.* 7 (2017) 1–10, <https://doi.org/10.1038/s41598-017-00488-y>.
- A. Helwak, G. Kudla, T. Dudnakova, D. Tollervey, Mapping the human miRNA interactome by CLASH reveals frequent noncanonical binding, *Cell* 153 (2013) 654–665, <https://doi.org/10.1016/j.cell.2013.03.043>.
- W. Gu, Y. Xu, X. Xie, T. Wang, J.H. Ko, T. Zhou, The role of RNA structure at 5' untranslated region in microRNA-mediated gene regulation, *RNA* 20 (2014) 1369–1375, <https://doi.org/10.1261/ma.044792.114>.
- J.K.W. Lam, M.Y.T. Chow, Y. Zhang, S.W.S. Leung, siRNA versus miRNA as therapeutics for gene silencing, *Mol. Ther. Nucleic Acids* 4 (2015) e252, <https://doi.org/10.1038/mtna.2015.23>.
- A.F.A. Samad, M.F. Kamaroddin, M. Sajad, Cross-kingdom regulation by plant microRNAs provides novel insight into gene regulation, *Adv. Nutr.* (2020) 1–15, <https://doi.org/10.1093/advances/nmaa095>.
- E. Huntzinger, E. Izaurralde, Gene silencing by microRNAs: contributions of translational repression and mRNA decay, *Nat. Rev. Genet.* 12 (2011) 99–110, <https://doi.org/10.1038/nrg2936>.
- Q.Q. Gao, W.E. Putzbach, A.E. Murmann, S. Chen, A.A. Sarshad, J.M. Peter, E. T. Bartom, M. Hafner, M.E. Peter, 6mer seed toxicity in tumor suppressive microRNAs, *Nat. Commun.* 9 (2018), <https://doi.org/10.1038/s41467-018-06526-1>.
- S.M. Klum, S.D. Chandradoss, N.T. Schirle, C. Joo, I.J. Macrae, Helix-7 in Argonaute2 shapes the microRNA seed region for rapid target recognition, *EMBO J.* 37 (2018) 75–88, <https://doi.org/10.15252/embj.201796474>.
- M. Klein, S.D. Chandradoss, M. Depken, C. Joo, Why Argonaute is needed to make microRNA target search fast and reliable, *Semin. Cell Dev. Biol.* 65 (2017) 20–28, <https://doi.org/10.1016/j.semcdb.2016.05.017>.
- S.D. Chandradoss, N.T. Schirle, M. Szczepaniak, I.J. Macrae, C. Joo, A dynamic search process underlies microRNA targeting, *Cell* 162 (2015) 96–107, <https://doi.org/10.1016/j.cell.2015.06.032>.
- M. Li, T. Chen, J.J. He, J.H. Wu, J.Y. Luo, R.S. Ye, M.Y. Xie, H.J. Zhang, B. Zeng, J. Liu, Q.Y. Xi, Q.Y. Jiang, J.J. Sun, Y.L. Zhang, Plant MIR167e-5p inhibits enterocyte proliferation by targeting β -catenin, *Cells* 8 (2019) 1–14, <https://doi.org/10.3390/cells8111385>.
- A.R. Chin, M.Y. Fong, G. Somlo, J. Wu, P. Swiderski, X. Wu, S.E. Wang, Cross-kingdom inhibition of breast cancer growth by plant miR159, *Cell Res.* 26 (2016) 217–228, <https://doi.org/10.1038/cr.2016.13>.
- B. Avsar, Y. Zhao, W. Li, W.J. Lukiw, *Atropa belladonna* expresses a microRNA (aba-miRNA-9497) highly homologous to *Homo sapiens* miRNA-378 (hsa-miRNA-378); both miRNAs target the 3'-untranslated region (3'-UTR) of the mRNA encoding the neurologically relevant, zinc-finger transcription factor, *Cell. Mol. Neurobiol.* 40 (2020) 179–188, <https://doi.org/10.1007/s10571-019-00729-w>.
- D.A. Deshpande, A.G.P. Guedes, R. Graeff, S. Dogan, S. Subramanian, T.F. Walseth, M.S. Kannan, CD38/cADPR Signaling Pathway in Airway Disease: Regulatory Mechanisms, *Mediators Inflamm.* 2018, <https://doi.org/10.1155/2018/8942042>, 2018.
- W. Pang, W. Yao, X. Dai, A. Zhang, L. Hou, L. Wang, Y. Wang, X. Huang, X. Meng, L. Li, Pancreatic cancer-derived exosomal miRNA-19a induces β -cell dysfunction by targeting adcy1 and epac2, *Int. J. Biol. Sci.* 17 (2021) 3622–3633, <https://doi.org/10.7150/ijbs.56271>.
- C. Catassi, J.C. Bai, B. Bonaz, G. Bouma, A. Calabrò, A. Carroccio, G. Castillejo, C. Ciacci, F. Cristofori, J. Dolinsek, R. Francavilla, L. Elli, P. Green, W. Holtmeier, P. Koehler, S. Koletzko, C. Meinhold, D. Sanders, M. Schumann, D. Schuppan, R. Ullrich, A. Vécsei, U. Volta, V. Zevallos, A. Sapone, A. Fasano, Non-celiac gluten

- sensitivity: the new frontier of gluten related disorders, *Nutrients* 5 (2013) 3839–3853, <https://doi.org/10.3390/nu5103839>.
- [43] E. Lionetti, S. Leonardi, C. Franzonello, M. Mancardi, M. Ruggieri, C. Catassi, Gluten psychosis: confirmation of a new clinical entity, *Nutrients* 7 (2015) 5532–5539, <https://doi.org/10.3390/nu7075235>.
- [44] D. Palm, A. Uzoni, F. Simon, M. Fischer, A. Coogan, O. Tucha, J. Thome, F. Faltraco, Evolutionary conservations, changes of circadian rhythms and their effect on circadian disturbances and therapeutic approaches, *Neurosci. Biobehav. Rev.* 128 (2021) 21–34, <https://doi.org/10.1016/j.neubiorev.2021.06.007>.
- [45] W. Wang, D. Liu, X. Zhang, D. Chen, Y. Cheng, F. Shen, Plant microRNAs in cross-kingdom regulation of gene expression, *Int. J. Mol. Sci.* 19 (2018), <https://doi.org/10.3390/ijms19072007>.