Published in final edited form as:

Parasitology. 2019 February ; 146(2): 197–205. doi:10.1017/S0031182018001002.

A novel miRNA, miR-13664, targets *CpCYP314A1* to regulate deltamethrin resistance in *Culex pipiens pallens*

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SUMMARY

Extensive insecticide use has led to the resistance of mosquitoes to these insecticides, posing a major barrier to mosquito control. Previous Solexa high-throughput sequencing of *Culex pipiens pallens* in the laboratory has revealed that the abundance of a novel microRNA (miRNA), miR-13664, was higher in a deltamethrin-sensitive (DS) strain than a deltamethrin-resistant (DR) strain. Real-time quantitative PCR revealed that the miR-13664 transcript level was lower in the DR strain than in the DS strain. miR-13664 oversupply in the DR strain increased the susceptibility of these mosquitoes to deltamethrin, whereas inhibition of miR-13664 made the DS strain more resistant to deltamethrin. Results of bioinformatic analysis, quantitative reverse-transcriptase polymerase chain reaction (qRT-PCR), luciferase assay, and miR mimic/inhibitor microinjection revealed *CpCYP314A1* to be a target of miR-13664. In addition, downregulation of *CpCYP314A1* expression in the DR strain reduced the resistance of mosquitoes to deltamethrin. Taken together, our results indicate that miR-13664 could regulate deltamethrin resistance by interacting with *CpCYP314A1*, providing new insights into mosquito resistance mechanisms.

Keywords

Culex pipiens pallens; miR-13664; *CpCYP314A1*; deltamethrin resistance

INTRODUCTION

Mosquitoes are blood-sucking insects that can transmit many mosquito-borne diseases such as malaria, dengue fever, Zika, hemorrhagic fever, encephalitis, and meningitis. They are

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CONFLICTS OF INTEREST

All authors declare no conflict of interest.

ETHICAL STANDARDS

Not applicable

responsible for infecting hundreds of millions of people and a large number of deaths each year (Benelli, 2016; Liang et al., 2015; Pastula et al., 2016; Zhu et al., 2017). The World Health Organization reported that in 2016, there were 216 million cases of malaria and 445000 deaths across 91 countries (WHO, 2017). The worldwide incidence of dengue fever has risen sharply in recent decades, and 390 million people get infected with dengue fever each year (Bhatt et al., 2013). *Culex pipiens pallens* is the most abundant and widely distributed species of mosquito in China. This species is the main blood-sucking mosquito species in northern China and is primarily responsible for West Nile fever (Jiang et al., 2014), bancroftian filariasis, and type B encephalitis (Cui et al., 2013). Recently, a number of studies of *C. pipiens pallens* resistance to insecticides, especially pyrethroid insecticides, have been published (Liu et al., 2011; Wang et al. 2012).

There is an urgent need to devise effective methods of mosquito control to reduce the incidence and spread of these diseases. Chemical control is efficient and convenient for large-scale applications (Ma et al., 2017). In recent years, deltamethrin has been commonly used for indoor residual spraying and in insecticide-treated nets due to its low mammalian toxicity and high efficiency (White et al., 2014). However, mosquitoes are gradually acquiring deltamethrin resistance, which has become a major obstacle in the control of mosquito-borne disease (Benelli, 2015; Siegwart et al., 2015). There are three main causes for the development of resistance: metabolic resistance due to increased enzymatic activity, target-site resistance due to altered target protein bound to the pesticide, and cuticular resistance caused by decreased epidermal penetration (Hemingway et al., 2004), with studies showing the importance of metabolic resistance (Zhang et al. 2016). Cytochromes P450 are often shown to be vital for the detoxification of pesticides in the metabolic resistance mechanism. A number of *P450* genes have been reported to be overexpressed in resistant mosquitoes (Guo et al., 2017). Recent studies on cytochromes P450 have focused on transcriptional regulation, but few have studied the post-transcriptional regulation.

MicroRNAs are a group of non-coding single-stranded RNA molecules that are widely found in animals and plants. MiRNAs are encoded by endogenous genes and are approximately 22 nucleotides in length (Carthew et al., 2006). They negatively regulate gene transcription levels via base pairing with target sites in messenger RNA (mRNA) (Ding et al., 2015; Lucas & Raikhel, 2013; Sebastiani et al., 2015). Generally, the miRNA “seed sequence” (nucleotides 2–8 at the 5′-end) binds to a complementary seed-matching site within the 3′- or 5′-untranslated regions of the mRNA, resulting in translational inhibition or mRNA degradation (Kanokudom et al., 2017; Lee et al., 2017; Lucas & Raikhel, 2013). Previous studies on miRNAs and their role in deltamethrin resistance have found that miR-932 and miR-285 regulate deltamethrin resistance by targeting the cytochromes P450 family (Liu et al., 2016; Tian et al., 2016). The miR-2~13~71 cluster (miR-2, miR-13, and miR-71) has been reported to be involved in deltamethrin resistance in mosquitoes (Guo et al., 2017). In previous research in our laboratory (Hong et al. 2014) which profiled miRNAs in a deltamethrin resistant strain of *C. pipiens* we identified miR-13664 to be highly expressed using Solexa high-throughput sequencing and miRDeep predictive analysis. However, the role of miR-13664 in insecticide resistance in mosquitoes remains unclear.

In the present study, we validated the role of miR-13664 in the DR strain by demonstrating that over-supply of miR-13664 decreased the transcript levels of a putative target gene *cytochromes P450 314A1* (*CpCYP314A1*) in the DR strain. These results indicate that miR-13664 may be involved in the mechanism of pyrethroid resistance by regulating *CpCYP314A1* expression.

MATERIALS AND METHODS

Mosquito strains

Two strains of *C. pipiens pallens* were used: the DS strain (50% lethal concentration of deltamethrin $LC_{50} = 0.04$ mg/L) and the DR strain ($LC_{50} = 3.42$ mg/L). The DS strain was collected in the field from Tangkou in Shandong Province, and maintained in our laboratory without exposure to any insecticides. The DR strain was screened by culturing the field strains obtained from Tangkou for 80 generations. Prior to screening, the LC_{50} concentration was measured by larval impregnation, and the LC_{50} concentration was selected as the screening concentration for each subsequent generation. Deltamethrin solution was used immediately after preparation. In brief, the prepared deltamethrin solution was poured into a 1000-mL porcelain bowl, and approximately 1000 4th instar larvae were placed in. After 24 hours, the surviving larvae were transferred and reared in fresh dechlorinated water. Judgment of dead larvae: gently touching the larvae, those that did not move were considered as dead. To obtain DR strain, DS strain larvae were selected and exposed constantly to deltamethrin (LC_{50} concentration) and screened over 80 generations. All laboratory strains were fed at 28–30°C at a relative humidity of 70–80% and with a 16-h light/8-h dark cycle. All adult mosquitoes were fed 10% glucose.

In addition, We collected mosquito larvae in the fields of Pingyin, Shanghe and Jining and raised them to adult mosquitoes in the laboratory. The feeding conditions were the same as above. After three days post adult emergence, the female mosquitoes were selected and measured with WHO discriminating dose (0.05% deltamethrin film) according to standard WHO cones test. The mosquitoes were exposed to the film for 60 min. After 24 hours recovery in the recovery cylinder, knocked down group were served as field-sensitive strains (FDS) and the survival group were served as field-resistant strain (FDR).

RNA extraction and cDNA synthesis

According to the manufacturer's protocols, total RNA extraction was performed from pools of 10 individuals from adult female DR and DS mosquitoes at three days post-eclosion using TRIzol reagent (TaKaRa, Japan). RNA integrity was assessed by 1% agarose gel electrophoresis. The quality and quantity of the extracted RNA were determined using a Thermo Scientific™ NanoDrop 2000 spectrophotometer (NanoDrop, Wilmington, DE). cDNAs were synthesized from mRNAs using a PrimeScript™ RT Master Kit (TaKaRa, Japan) and stem loop primers were added using stem - loop method to synthesize cDNA from miRNA using the PrimeScript RT Kit (TaKaRa, Japan) according to the manufacturer's protocols, respectively (Tang et al., 2006).

Quantitative qRT-PCR analysis

Quantitative real-time PCR (qRT-PCR) was carried out on a LightCycler® 96 instrument (Roche, Switzerland) based on the SYBR Green method, according to EvaGreen 2× qPCR MasterMix-No Dye (ABM, Canada) protocols. The total reaction system consisted of a 20-μL mixture containing 10 μL of EvaGreen 2× qPCR Master Mix-No Dye, 1 μL each of upstream and downstream primers, and 8 μL of cDNA. The relative expression levels of *CpCYP314A1* and miR-13664 were calculated and compared to the expression levels of β -actin or U6 small nuclear RNA, respectively, which were used as the internal reference standards. Gene-specific primers were designed by Primer Premier 5.0 software and are listed in Table 1. The miRNA expression levels were calculated using the stem-loop RT-PCR method (Salone & Rederstorff, 2015). Assays were conducted in triplicate and repeated three times. Expression levels were calculated using $2^{-\Delta\Delta C_t}$ (Schmittgen and Livak, 2008) with the transcript level in DS strain set as 1.

Plasmid construction and luciferase assay

The 3'-UTR region of *CpCYP314A1* contained a potential miR-13664-binding site (CGGATCA). Two pairs of primers (Table 1) were designed to amplify this region with one pair introducing a mutation into this binding site. Primers were designed according to the transcript sequences of *C. quinquefasciatus* (CPIJ001380) to amplify a 100-bp region upstream of *CpCYP314A1*. The 3'-UTR primer pair amplifies the native sequence whilst in the 3'-UTR-MUT primer pair four bases were altered (GGCAACT) in the seed region. The region of the *CpCYP314A1* 3'-UTR (*CpCYP314A1* 3'-UTR-WT) and mutant 3'-UTR (*CpCYP314A1* 3'-UTR-MUT) were PCR amplified from *C. pipiens pallens* cDNA. PCR was executed using the following cycle protocol: 94°C for 5 min; 35 cycles of 94°C for 30 s, 52°C for 30 s, and 72°C for 30 s; with a final step of 72°C for 7 min. The amplified PCR products were electrophoresed on 2% agarose gel, purified using a MiniBEST Agarose Gel DNA Extraction Kit Ver.4.0 (TaKaRa, Japan), and sequenced by Invitrogen.

The 3'-UTR-WT and 3'-UTR-MUT of *CpCYP314A1* were cloned into pMIR-Report miRNA report vector using *SacI* and *HindIII*. miR-13664 mimics were manufactured by GenePharma (Gene-Pharma, Shanghai, China). The negative control (NC) was an unrelated sequence designed according to other species, which would not interfere with the target genes in our study and were synthesized by GenePharma. HEK-293T cells were seeded in 24-well plates at a concentration of 1×10^5 cells per well and incubated for 24 h. The recombinant plasmids (pMIR-UTR and pMIR-MUT) and miRNA mimics or NC were transfected into cells using FuGENE® HD Transfection Reagent (Promega, WI, USA). Briefly, the recombinant plasmids, miRNA mimics/NC, and cell culture medium were gently mixed and incubated for 15 min at room temperature. The mixture was gently spread onto cells. After transfection for 48 h, the cells were lysed and subjected to luciferase assay that was implemented using the Dual-Luciferase Reporter Assay System (Promega, WI) (Guo et al., 2017).

Microinjection

The miR-13664 mimic/inhibitor and dsRNAs of *CpArgonaute 1* (*CpAgo1*), *CpArgonaute 3* (*CpAgo3*), and *CpCYP314A1* genes were procured from GenePharma (GenePharma,

Shanghai; Table 2). The NC was designed to contain the same amount of scrambled small RNA (GenePharma; Table 2). One-day-old non-blood-fed female mosquitoes of both the DS and DR strains were anesthetized on ice and then microinjected in the thorax. According to standard methodology (Blandin et al., 2002), the DR strain received injection of dsRNA against *CpCYP314A1* or miR-13664 mimic (0.07 μ l, 333uM), while the DS strain received injection of miR-13664 inhibitor, *CpAgo1*, or *CpAgo3*. Mosquitoes that received an equivalent volume of diethylpyrocarbonate (DEPC)-treated water or NC were used for the two controls. Each group had three biological replicates. Three days after injection, total RNA was extracted from the injected female mosquitoes, and the efficiency of microinjection was verified by qRT-PCR. CDC bottle bioassays were performed to test the mosquito's resistance to deltamethrin.

CDC bottle bioassay

The Centers for Disease Control and Prevention (CDC) bottle bioassay is used as a monitoring tool to detect pesticide resistance (Aizoun et al., 2013). According to the laboratory protocol (Guo et al., 2017), deltamethrin was dissolved in acetone to obtain a concentration of 10 mg/mL and diluted to a final concentration of 7.5 mg/mL for the DR strain and 2 μ g/mL for the DS strain. Then, 1 mL of liquid was evenly applied to the surface of each experimental bottle. Another bottle coated with the same volume of acetone served as the control. For each bioassay, approximately 20 microinjected mosquitoes per bottle were exposed to deltamethrin. Then, mosquito mortality was recorded every 15 min for 2 h or until all the mosquitoes were dead. Each experiment was performed three times, with three biological replicates each.

Statistical analysis

In this study, statistical calculations were performed using SPSS (Version 20.0). Differences between populations were statistically analyzed by the Student's *t* test (all values were represented as means $\pm$ SD). The chi-square test was used to assess differences among the three independent experiments. A *P* value of <0.05 was considered statistically significant.

RESULTS

Identification of the novel miRNA miR-13664

Based on the results of the Solexa high-throughput sequencing data obtained in a previous study in our laboratory (Hong et al., 2014), we used miRDeep prediction methods to identify novel miRNAs (Friedländer et al., 2008). Briefly, nine miRNAs with relatively high scores from the miRNAs obtained from the miRDeep prediction analysis were selected, and their relative expression levels in DS and DR mosquitoes were analyzed using quantitative PCR (Table 3). The results of the analysis revealed a significant difference in miR-13664 expression between the DS and DR strains; therefore, we chose to study the relationship between miR-13664 and deltamethrin resistance.

Ago1 is essential for miR-13664 biosynthesis

Argonaute 1 (*Ago1*) has been found to be involved in the miRNA pathways in insects (Fu et al. 2017; Hussain et al., 2013; Lewis et al., 2016). *Argonaute 3* (*Ago3*) is known to

participate in the piRNA pathway (Campbell et al., 2008; S. Luo & Lu, 2017; Rogers et al., 2017). We found that the miR-13664 transcript levels were 38% less in *dsCpAgo1*-injected mosquitoes compared to the NC control (Fig. 1A and B), whereas knockdown of *dsCpAgo3* did not alter the expression of miR-13664 (Fig. 1C and D), indicating that *Ago1* is necessary for the biosynthesis of the novel miRNA identified in this study, miR-13664.

Increased miR-13664 abundance in laboratory and field-collected DS strains

To verify the importance of miR-13664 in deltamethrin resistance in *C. pipiens pallens*, we used stem-loop qRT-PCR to verify the miR-13664 abundance in female DS and DR mosquitoes. As shown in Fig. 2A, miR-13664 expression was 1.92× higher in the laboratory DS strain than in the DR strain. To further analyze miR-13664 abundance in the field-collected *C. pipiens pallens* population, Pingyin, Shanghe, and Jining strains were used in our experiment. As shown in Fig. 2B, the miR-13664 expression levels in FDS (field deltamethrin sensitive) strains from Pingyin, Shanghe, and Jining were 1.93, 2.70, and 4.42-fold higher than the corresponding FDR (field deltamethrin resistant) strains from the same regions, suggesting that miR-13664 is related to deltamethrin resistance.

MiR-13664 regulates deltamethrin resistance in mosquitoes

To investigate the role of miR-13664 in insecticide resistance, female DR and DS mosquitoes received thoracic microinjections of miR-13664 mimics and inhibitors one day after eclosion. After three days of recovery, the CDC bottle bioassay was conducted after microinjection and the miR-13664 expression level in the mosquitoes was determined by qRT-PCR to verify the injection efficiency (Fig. 3A and Fig. 3C). miR-13664 oversupply in the DR strain was found to reduce the resistance of mosquitoes to deltamethrin (Fig. 3B), while knockdown of miR-13664 in the DS strain increased resistance to deltamethrin (Fig. 3D). Together, these results indicate that miR-13664 may be a potential regulator of insecticide resistance in *C. pipiens pallens*.

MiR-13664 target gene prediction and identification

Mature miRNAs interact with the 3'-UTR or 5'-UTR to modulate target mRNA (Carthew et al., 2006). To identify the miRNA target gene in *C. pipiens pallens*, we aligned the 2–8 complementarily paired nucleotides at the 5'-end of miR-13664 with the known mRNAs of *C. quinquefasciatus* (Yan et al., 2012). A potential binding site for miR-13664 was found in the *CpCYP314A1* 3'-UTR region (Fig. 4A). Then, we measured the *CpCYP314A1* transcript level in the DS and DR strains. Unlike the miR-13664 expression level, *CpCYP314A1* expression was 2.32-fold higher in the DR strains than in the DS strains (Fig. 4C). MiR-13664 mimics induced a decrease in the *CpCYP314A1* mRNA levels, while miR-13664 inhibitors increased their abundance (Fig. 4D, Fig. 4E). These results suggest that *CpCYP314A1* may be the target gene of miR-13664. To confirm this, plasmids were inserted with the *CpCYP314A1* 3'-UTR and MUT of and designated *CpCYP314A1*-3'-UTR (WT) and *CpCYP314A1*-3'-MUT (MUT), respectively, then cotransfected with the pGL4.7 control plasmid into HEK293T cells together with miR-13664 mimics or NC. Results of the luciferase assay revealed that miR-13664 reduced *CpCYP314A1*-3'-UTR expression by 37%, but did not affect the expression levels of *CpCYP314A1*-3'-MUT

relative to the levels in the NC (Fig. 4B). The above results provide further evidence that *CpCYP314A1* is the direct target of miR-13664.

CpCYP314A1 plays a role in deltamethrin resistance of mosquitoes

We microinjected DR strain mosquitoes with *CpCYP314A1* dsRNA, performed a CDC bottle bioassay, and determined the *CpCYP314A1* expression levels by qRT-PCR to verify injection efficiency. The qRT-PCR results showed that knockdown efficiency of *CpCYP314A1* was 59% compared to the NC (Fig. 5A). CDC bottle bioassays showed that knockdown of *CpCYP314A1* can reduce resistance of mosquitoes to deltamethrin (Fig. 5B). As shown in Fig. 5B, the mortality in the knockdown group was increased by 11.83%, 20.03%, 26.5%, 33.97%, and 33.97% at 60, 75, 90, 115, and 120 min, respectively, compared to the control group.

DISCUSSION

The vast majority of miRNAs originate in the endogenous locus and are transcribed into primary miRNA (pri-miRNA) transcripts by RNA polymerase II. The classical miRNA pathway converts pri-miRNA hairpins into approximately 22 nucleotide (nt) miRNAs by sequential cleavage with the help of two dsRNA-binding proteins by two RNase III enzymes, Drosha and Dicer. One of the strands of a mature duplex miRNA is loaded onto the Argonaute protein, which is responsible for the activity of the small RNA, to form the ribonucleoprotein complex miRNA-mediated silencing complex (miRISC). The bound strand in miRISC is called the guide strand, while the unbound strand that is subsequently degraded is called the passenger strand. The guide strand directs miRISC to the messenger RNA (mRNA) target via incomplete base pairing, resulting in decreased gene expression through mRNA destabilization and/or translational inhibition (Chorostecki et al., 2017; Fu et al., 2017). *Ago1* is reported to be involved in miRNA pathways (Fu et al., 2017; Hussain et al., 2013; Lewis et al., 2016). In our study, we found that miR-13664 is involved in deltamethrin resistance and that the miR-13664 expression level is decreased following microinjection of mosquitoes with *dsCpAGO1*, but microinjection of mosquitoes with *dsCpAGO3* did not alter miR-13664 expression. Therefore, we predicted miR-13664 to be a miRNA.

In recent years, miRNA has become the focus of life science research. In humans, chemoresistance mechanisms are closely related to miRNA regulation (Chen, Cao, & Feng, 2017; Han & Chen, 2015; Li et al., 2017). As important post-transcriptional regulators, miRNAs themselves are regulated by other genes. Studies have found numerous binding sites for transcription factors in the promoter regions of some miRNAs, which themselves are the main targets of other miRNAs. Interestingly, these transcription factor promoter regions contain active sites that interact with hormones (such as ecdysone and auxin), and a network relationship is established between the physiological needs of biology and the regulation of genes, constituting a delicate feedback path, which coordinates the constant application of biological changes to the external environment (Liu et al. 2013). In insects, miRNAs, miR-7a and miR-8519 are involved in diamide insecticide resistance in *Plutella xylostella* and they function by upregulating *ryanodine receptor* expression (Li et al., 2015).

In *C. pipiens pallens*, our laboratory found that miR-92a is involved in deltamethrin resistance by targeting the epidermal protein *CpCPR4* (Ma et al., 2017), and for the first time reported the relationship between miR-2~13~71 clusters and deltamethrin resistance (Guo et al., 2017). Pesticide resistance is a complex multi-gene genetic phenomenon (Zou et al., 2015), and here we explored the relationship between miR-13664 and deltamethrin resistance in *C. pipiens pallens* to explore in detail the mechanism underlying resistance to insecticides. We found that microinjecting DR mosquitoes with miR-13664 mimics resulted in an increased susceptibility of these mosquitoes to deltamethrin, whereas microinjecting DS mosquitoes with the miR-13664 inhibitor resulted in increased resistance to deltamethrin. These findings indicate that miR-13664 is involved in the regulation of deltamethrin resistance in *C. pipiens pallens*.

The “seed sequence” (5′-nucleotides 2–8) of an miRNA is known to modulate the inhibition or degradation of targeted mRNA translation by interacting with the 3′- or 5′-UTR (Breving & Esquela-Kerscher, 2010; Shi et al., 2017). In this study, we compared the seed sequence of miR-13664 with the *C. quinquefasciatus* database and found that *CpCYP314A1* was completely complementary to the seed sequence. Cytochromes P450 are involved in the metabolism of compounds in conjunction with endogenous or exogenous compounds, including insecticides (Elzaki et al., 2017). Cytochromes P450 protect insects from insecticides by metabolizing insecticides (Gong et al., 2017). *CYP6Z1* overexpression has been detected in pyrethroid and DDT-resistant strains that metabolize DDT (Chiu et al., 2008). *CYP6P3* and *CYP6M2* have appeared to be most widely overexpressed in resistant field populations (Djouaka et al., 2008; Muller et al., 2008). Importantly, they have been shown to metabolize permethrin and deltamethrin (Muller et al., 2008; Stevenson et al., 2011). Previous studies have shown that three of the four *CYP6F* subfamily genes are associated with deltamethrin or cisplatin detoxification in *Locusta migratoria* (Guo et al., 2016). In the present study, *CpCYP314A1* was overexpressed in DR strains and *CpCYP314A1* knockdown *C. pipiens pallens* mosquitoes were more susceptible to deltamethrin compared to those with this gene. These results indicate the involvement of *CpCYP314A1* in deltamethrin resistance in *C. pipiens pallens*.

MiRNAs are involved in the regulation of target gene expression levels by post-transcriptional mechanisms. Here, miR-13664 mimics were found to reduce luciferase activity in HEK293T cells. Meanwhile, miR-13664 knockdown resulted in increased *CpCYP314A1* expression, indicating that miR-13664 could regulate *CpCYP314A1* in vitro or in vivo. In summary, miR-13664 expression is decreased in *C. pipiens pallens* DR strains and potentially plays a role in deltamethrin resistance by regulating *CpCYP314A1*. This study provides new information on the mechanism by which *C. pipiens pallens* individuals exhibit resistance to deltamethrin, providing a new scientific basis for the treatment of mosquito resistance.

Acknowledgments

We would like to thank the native English speaking scientists of Elixigen Company (Huntington Beach, California) for editing our manuscript.

FINANCIAL SUPPORT

This work was supported by the National Institutes of Health of US (NIH) [grant number 2R01AI075746]; the National Natural Science Foundation of China [grant numbers 81772227, 81471984, 81672056, 81672058]; and the Priority Academic Program Development of Jiangsu Higher Education Institutions.

References

- Aizoun N, Aikpon R, Padonou GG, Oussou O, Oke-Agbo F, Gnanguenon V, Ossè R, Akogbeto M. 2013; Mixed-function oxidases and esterases associated with permethrin, deltamethrin and bendiocarb resistance in *Anopheles gambiae* s.l. in the south-north transect Benin, West Africa. *Parasites & Vectors*. 6:223.doi: 10.1186/1756-3305-6-223 [PubMed: 23919515]
- Blandin S, Moita LF, Kocher T, Wilm M, Kafatos FC, Levashina EA. 2002; Reverse genetics in the mosquito *Anopheles gambiae*: targeted disruption of the Defensin gene. *EMBO reports*. 3:852–856. DOI: 10.1007/s00438-015-1109-4 [PubMed: 12189180]
- Benelli G. 2015; Research in mosquito control: current challenges for a brighter future. *Parasitology Research*. 114:2801–2805. DOI: 10.1007/s00436-015-4586-9 [PubMed: 26093499]
- Benelli G. 2016; Green synthesized nanoparticles in the fight against mosquito-borne diseases and cancer-a brief review. *Enzyme and Microbial Technology*. 95:58–68. DOI: 10.1016/j.enzmictec.2016.08.022 [PubMed: 27866627]
- Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, Drake JM, Brownstein JS, Hoen AG, Sankoh O, Myers MF, George DB, Jaenisch T, Wint GRW, Simmons CP, Scott TW, Farrar JJ, Hay SI. 2013; The global distribution and burden of dengue. *Nature*. 496:504–507. DOI: 10.1038/nature12060 [PubMed: 23563266]
- Brevig K, Esquela-Kerscher A. 2010; The complexities of microRNA regulation: mirandering around the rules. *International Journal of Biochemistry & Cell Biology*. 42:1316–1329. DOI: 10.1016/j.biocel.2009.09.016 [PubMed: 19800023]
- Campbell CL, Black WC, Hess AM, Foy BD. 2008; Comparative genomics of small RNA regulatory pathway components in vector mosquitoes. *BMC Genomics*. 9:425.doi: 10.1186/1471-2164-9-425 [PubMed: 18801182]
- Carthew RW. 2006; Gene regulation by microRNAs. *Curr Opin Genet Dev*. 16:203–208. [PubMed: 16503132]
- Chen L, Cao H, Feng Y. 2017; MiR-199a suppresses prostate cancer paclitaxel resistance by targeting *YES1*. *World Journal of Urology*. doi: 10.1007/s00345-017-2143-0
- Chorostecki U, Moro B, Rojas AML, Debernardi JM, Schapire AL, Notredame C, Palatnik JF. 2017; Evolutionary Footprints Reveal Insights into Plant MicroRNA Biogenesis. *Plant Cell*. 29:1248–1261. DOI: 10.1105/tpc.17.00272 [PubMed: 28550151]
- Chiu TL, Wen Z, Rupasinghe SG, Schuler MA. 2008; Comparative molecular modeling of *Anopheles gambiae* *CYP6Z1*, a mosquito P450 capable of metabolizing DDT. *Proceedings of the National Academy of Sciences of the United States of America*. 105:8855–8860.10.1073. [PubMed: 18577597]
- Cui J, Li S, Zhao P, Zou F. 2013; Flight capacity of adult *Culex pipiens pallens* (Diptera: Culicidae) in relation to gender and day-age. *Journal of Medical Entomology*. 50:1055–1058. [PubMed: 24180110]
- Ding N, Wang S, Yang Q, Li Y, Cheng H, Wang J, Wang D, Deng Y, Yang Y, Hua S, Zhao H, Fang X. 2015; Deep sequencing analysis of microRNA expression in human melanocyte and melanoma cell lines. *Gene*. 572:135–145. DOI: 10.1016/j.gene.2015.07.013 [PubMed: 26164755]
- Djouaka RF, Bakare AA, Coulibaly ON, Akogbeto MC, Ranson H, Hemingway J, Strode C. 2008; Expression of the cytochrome P450s, *CYP6P3* and *CYP6M2* are significantly elevated in multiple pyrethroid resistant populations of *Anopheles gambiae* s.s. from Southern Benin and Nigeria. *BMC Genomics*. 9
- Elzaki MEA, Miah MA, Wu M, Zhang H, Pu J, Jiang L, Han Z. 2017; Imidacloprid is degraded by *CYP353D1v2*, a cytochrome P450 overexpressed in a resistant strain of *Laodelphax striatellus*. *Pest Management Science*. 73:1358–1363. DOI: 10.1002/ps.4570 [PubMed: 28296046]
- Friedländer MR, Chen W, Adamidi C, Maaskola J, Einspanier R, Knespel S, Rajewsky N. 2008; Discovering microRNAs from deep sequencing data using miRDeep. *Nature Biotechnology*. 26:407–415.

- Fu X, Dimopoulos G, Zhu J. 2017; Association of microRNAs with Argonaute proteins in the malaria mosquito *Anopheles gambiae* after blood ingestion. *Scientific Reports*. 7:6493.doi: 10.1038/s41598-017-07013-1 [PubMed: 28747726]
- Gong Y, Li T, Feng Y, Liu N. 2017; The function of two P450s, CYP9M10 and CYP6AA7, in the permethrin resistance of *Culex quinquefasciatus*. *Scientific Reports*. 7:587.doi: 10.1038/s41598-017-00486-0 [PubMed: 28373679]
- Guo Q, Huang Y, Zou F, Liu B, Tian M, Ye W, Guo J, Sun X, Zhou D, Sun Y, Ma L, Shen B, Zhu C. 2017; The role of miR-2 ~ 13 ~ 71 cluster in resistance to deltamethrin in *Culex pipiens pallens*. *Insect Biochemistry and Molecular Biology*. 84:15–22. DOI: 10.1016/j.ibmb.2017.03.006 [PubMed: 28342977]
- Guo Y, Wu H, Zhang X, Ma E, Guo Y, Zhu KY, Zhang J. 2016; RNA interference of *cytochrome P450 CYP6F* subfamily genes affects susceptibility to different insecticides in *Locusta migratoria*. *Pest Management Science*. 72:2154–2165. DOI: 10.1002/ps.4248 [PubMed: 26853074]
- Han J, Chen Q. 2015; MiR-16 modulate temozolomide resistance by regulating BCL-2 in human glioma cells. *International Journal of Clinical and Experimental Pathology*. 8:12698–12707. [PubMed: 26722459]
- Hemingway J, Hawkes NJ, McCarroll L, Ranson H. 2004; The molecular basis of insecticide resistance in mosquitoes. *Insect Biochemistry and Molecular Biology*. 34:653–665. [PubMed: 15242706]
- Hong S, Guo Q, Wang W, Hu S, Fang F, Lv Y, Yu J, Zou F, Lei Z, Ma K, Ma L, Zhou D, Sun Y, Zhang D, Shen B, Zhu C. 2014; Identification of differentially expressed microRNAs in *Culex pipiens* and their potential roles in pyrethroid resistance. *Insect Biochemistry and Molecular Biology*. 55:39–50. DOI: 10.1016/j.ibmb.2014.10.007 [PubMed: 25446390]
- Hussain M, O'Neill SL, Asgari S. 2013; Wolbachia interferes with the intracellular distribution of *Argonaute 1* in the dengue vector *Aedes aegypti* by manipulating the host microRNAs. *RNA Biology*. 10:1868–1875. DOI: 10.4161/rna.27392 [PubMed: 24351659]
- Jiang S, Wang Z, Guo X, Zhang Y, Li C, Dong Y, Xing D, Zhao T. 2014; Infection and dissemination of West Nile virus in China by the potential vector, *Culex pipiens pallens*. *Journal of Vector Ecology*. 39:78–82. [PubMed: 24820559]
- Kanokudom S, Vilaivan T, Wikan N, Thepparit C, Smith DR, Assavalapsakul W. 2017; miR-21 promotes dengue virus serotype 2 replication in HepG2 cells. *Antiviral Research*. 142:169–177. DOI: 10.1016/j.antiviral.2017.03.020 [PubMed: 28365456]
- Lee YR, Yeh SF, Ruan XM, Zhang H, Hsu SD, Huang HD, Hsieh CC, Lin YS, Yeh TM, Liu HS, Gan DD. 2017; Honeysuckle aqueous extract and induced let-7a suppress dengue virus type 2 replication and pathogenesis. *Journal of Ethnopharmacology*. 198:109–121. DOI: 10.1016/j.jep.2016.12.049 [PubMed: 28052239]
- Letelier P, Riquelme I, Hernandez AH, Guzman N, Farias JG, Roa JC. 2016; Circulating MicroRNAs as Biomarkers in Biliary Tract Cancers. *International Journal of Molecular Sciences*. 17doi: 10.3390/ijms17050791
- Lewis SH, Salmela H, Obbard DJ. 2016; Duplication and Diversification of Dipteran Argonaute Genes, and the Evolutionary Divergence of Piwi and Aubergine. *Genome Biology and Evolution*. 8:507–518. DOI: 10.1093/gbe/evw018 [PubMed: 26868596]
- Li S, Zeng A, Hu Q, Yan W, Liu Y, You Y. 2017; miR-423-5p contributes to a malignant phenotype and temozolomide chemoresistance in glioblastomas. *Journal of Neuro-oncology*. 19:55–65. DOI: 10.1093/neuonc/now129
- Li X, Guo L, Zhou X, Gao X, Liang P. 2015; miRNAs regulated overexpression of ryanodine receptor is involved in chlorantraniliprole resistance in *Plutella xylostella* (L.). *Scientific Reports*. 5:14095.doi: 10.1038/srep14095 [PubMed: 26370154]
- Liang G, Gao X, Gould EA. 2015; Factors responsible for the emergence of arboviruses; strategies, challenges and limitations for their control. *Emerging Microbes & Infections*. 4:e18.doi: 10.1038/emi.2015.18 [PubMed: 26038768]
- Liu B, Tian M, Guo Q, Ma L, Zhou D, Shen B, Sun Y, Zhu C. 2016; MiR-932 Regulates Pyrethroid Resistance in *Culex pipiens pallens* (Diptera: Culicidae). *Journal of Medical Entomology*. doi: 10.1093/jme/tjw083

- Liu Y, Zhang H, Qiao C, Lu X, Cui F. 2011; Correlation between carboxylesterase alleles and insecticide resistance in *Culex pipiens* complex from China. *Parasites & Vectors*. 4:236. [PubMed: 22177233]
- Liu YP, Yang J, Liu Y. 2013; Research progress in microRNAs in insects. *Acta Entomologica Sinica*. 56:1026–1037.
- Lucas K, Raikhel AS. 2013; Insect microRNAs: biogenesis, expression profiling and biological functions. *Insect Biochemistry and Molecular Biology*. 43:24–38. DOI: 10.1016/j.ibmb.2012.10.009 [PubMed: 23165178]
- Luo S, Lu J. 2017; Silencing of Transposable Elements by piRNAs in *Drosophila*: An Evolutionary Perspective. *Genomics Proteomics Bioinformatics*. 15:164–176. DOI: 10.1016/j.gpb.2017.01.006 [PubMed: 28602845]
- Lu J, Getz G, Miska EA, Alvarez-Saavedra E, Lamb J, Peck D, Sweet-Cordero A, Ebert BL, Mak RH, Ferrando AA, Downing JR, Jacks T, Horvitz HR, Golub TR. 2005; MicroRNA expression profiles classify human cancers. *Nature*. 435:834–838. [PubMed: 15944708]
- Luo Y, Wang P, Wang X, Wang Y, Mu Z, Li Q, Fu Y, Xiao J, Li G, Ma Y, Gu Y, Jin L, Ma J, Tang Q, Jiang A, Li X, Li M. 2017; Detection of dietetically absorbed maize-derived microRNAs in pigs. *Scientific Reports*. 7:645.doi: 10.1038/s41598-017-00488-y [PubMed: 28381865]
- Ma K, Li X, Hu H, Zhou D, Sun Y, Ma L, Zhu C, Shen B. 2017; Pyrethroid-resistance is modulated by miR-92a by targeting *CpCPR4* in *Culex pipiens pallens*. *Comparative Biochemistry and Physiology - B Biochemistry and Molecular Biology*. 203:20–24. DOI: 10.1016/j.cbpb.2016.09.002 [PubMed: 27627779]
- Muller P, Warr E, Stevenson BJ, Pignatelli PM, Morgan JC, Steven A, Yawson AE, Mitchell SN, Ranson H, Hemingway J, Paine MJ, Donnelly MJ. 2008; Field-caught permethrin-resistant *Anopheles gambiae* overexpress *CYP6P3*, a P450 that metabolises pyrethroids. *PLoS Genetics*. 4:e1000286.10.1371. [PubMed: 19043575]
- Muller P, Chouaibou M, Pignatelli P, Etang J, Walker ED, Donnelly MJ, Simard F, Ranson H. 2008; Pyrethroid tolerance is associated with elevated expression of antioxidants and agricultural practice in *Anopheles arabiensis* sampled from an area of cotton fields in Northern Cameroon. *Molecular Ecology*. 17:1145–1155.10.1111. [PubMed: 18179425]
- Pastula DM, Smith DE, Beckham JD, Tyler KL. 2016; Four emerging arboviral diseases in North America: Jamestown Canyon, Powassan, chikungunya, and Zika virus diseases. *Journal of Neurovirology*. 22:257–260. DOI: 10.1007/s13365-016-0428-5 [PubMed: 26903031]
- Rogers AK, Situ K, Perkins EM, Toth KF. 2017; Zucchini-dependent piRNA processing is triggered by recruitment to the cytoplasmic processing machinery. *Genes & Development*. 31:1858–1869. DOI: 10.1101/gad.303214.117 [PubMed: 29021243]
- Salone V, Rederstorff M. 2015; Stem-loop RT-PCR based quantification of small non-coding RNAs. *Methods in Molecular Biology*. 1296:103–108. DOI: 10.1007/978-1-4939-2547-6_10 [PubMed: 25791594]
- Schmittgen TD, Livak KJ. 2008; Analyzing real-time PCR data by the comparative C(T) method. *Nature Protocols*. 3:1101–1108. [PubMed: 18546601]
- Sebastiani G, Po A, Miele E, Ventriglia G, Ceccarelli E, Bugliani M, Marselli L, Marchetti P, Gulino A, Ferretti E, Dotta F. 2015; MicroRNA-124a is hyperexpressed in type 2 diabetic human pancreatic islets and negatively regulates insulin secretion. *Acta Diabetologica*. 52:523–530. DOI: 10.1007/s00592-014-0675-y [PubMed: 25408296]
- Shi TF, Wang YF, Liu F, Qi L, Yu LS. 2017; Influence of the Neonicotinoid Insecticide Thiamethoxam on miRNA Expression in the *Honey Bee* (Hymenoptera: Apidae). *Journal of Insect Science*. 17doi: 10.1093/jisesa/iex074
- Sieglwart M, Graillot B, Blachere Lopez C, Besse S, Bardin M, Nicot PC, Lopez-Ferber M. 2015; Resistance to bio-insecticides or how to enhance their sustainability: a review. *Frontiers in Plant Science*. 6:381.doi: 10.3389/fpls.2015.00381 [PubMed: 26150820]
- Stevenson BJ, Bibby J, Pignatelli P, Muangnoicharoen S, O'Neill PM, Lian LY, Müller P, Nikou D, Steven A, Hemingway J, Sutcliffe MJ, Paine MJ. 2011; Cytochrome P450 6M2 from the malaria vector *Anopheles gambiae* metabolizes pyrethroids: sequential metabolism of deltamethrin revealed. *Insect Biochemistry and Molecular Biology*. 41:492–502.10.1016. [PubMed: 21324359]

- Tang F, Hajkova P, Barton SC, Lao K, Surani MA. 2006; MicroRNA expression profiling of single whole embryonic stem cells. *Nucleic Acids Research*. 34(2):e9.doi: 10.1093/nar/gnj009 [PubMed: 16434699]
- Tian M, Liu B, Hu H, Li X, Guo Q, Zou F, Liu X, Hu M, Guo J, Ma L, Zhou D, Sun Y, Shen B, Zhu C. 2016; MiR-285 targets P450 (CYP6N23) to regulate pyrethroid resistance in *Culex pipiens pallens*. *Parasitology Research*. 115:4511–4517. DOI: 10.1007/s00436-016-5238-4 [PubMed: 27651043]
- Wang Z, Li C, Xing D, Yu Y, Liu N, Xue R, Dong Y, Zhao T. 2012; Detection and widespread distribution of sodium channel alleles characteristic of insecticide resistance in *Culex pipiens* complex mosquitoes in China. *Medical and Veterinary Entomology*. 26:228–232. [PubMed: 22070231]
- White MT, Lwetoijera D, Marshall J, Caron-Lormier G, Bohan DA, Denholm I, Devine GJ. 2014; Negative cross resistance mediated by co-treated bed nets: a potential means of restoring pyrethroid-susceptibility to malaria vectors. *PLoS One*. 9:e95640.doi: 10.1371/journal.pone.0095640 [PubMed: 24788951]
- WHO. World malaria report 2017. 2017.
- Wu N, Zhu Q, Chen B, Gao J, Xu Z, Li D. 2017; High-throughput sequencing of pituitary and hypothalamic microRNA transcriptome associated with high rate of egg production. *BMC Genomics*. 18:255.doi: 10.1186/s12864-017-3644-3 [PubMed: 28335741]
- Yan L, Yang P, Jiang F, Cui N, Ma E, Qiao C, Cui F. 2012; Transcriptomic and phylogenetic analysis of *Culex pipiens quinquefasciatus* for three detoxification gene families. *BMC Genomics*. 13:609. [PubMed: 23140097]
- Ye W, Liu X, Guo J, Sun X, Sun Y, Shen B, Zhou D, Zhu C. 2017; piRNA-3878 targets P450 (CpCYP307B1) to regulate pyrethroid resistance in *Culex pipiens pallens*. *Parasitology Research*. doi: 10.1007/s00436-017-5554-3
- Zhang Y, Meng X, Yang Y, Li H, Wang X, Yang B, Zhang J, Li C, Millar NS, Liu Z. 2016; Synergistic and compensatory effects of two point mutations conferring target-site resistance to fipronil in the insect GABA receptor RDL. *Scientific Reports*. 6:32335.doi: 10.1038/srep32335 [PubMed: 27557781]
- Zhao B, Lucas KJ, Saha TT, Ha J, Ling L, Kokoza VA, Roy S, Raikhel AS. 2017; MicroRNA-275 targets sarco/endoplasmic reticulum Ca²⁺ adenosine triphosphatase (SERCA) to control key functions in the mosquito gut. *PLoS Genetics*. 13:e1006943.doi: 10.1371/journal.pgen.1006943 [PubMed: 28787446]
- Zhu Y, Zhang R, Zhang B, Zhao T, Wang P, Liang G, Cheng G. 2017; Blood meal acquisition enhances arbovirus replication in mosquitoes through activation of the GABAergic system. *Nature Communications*. 8:1262.doi: 10.1038/s41467-017-01244-6
- Zou F, Chen C, Zhong D, Shen B, Zhang D, Guo Q, Wang W, Yu J, Lv Y, Lei Z, Ma K, Ma L, Zhu C, Yan G. 2015; Identification of QTLs Conferring Resistance to Deltamethrin in *Culex pipiens pallens*. *PLoS One*. 10:e0140923.doi: 10.1371/journal.pone.0140923 [PubMed: 26484540]

Fig.1. miR-13664 abundance is influenced by *Ago1*. (A) *Ago1* transcript levels after *dsCpAgo1* injection. (B) miR-13664 abundance level after *dsCpAgo1* injection. (C) *Ago3* transcript level after *dsCpAgo3* injection. (D) miR-13664 abundance levels after *dsCpAgo3* injection. Data are presented as means $\pm$ standard error (SE) of three independent experiments. ** $P < 0.01$ compared with the negative control (NC).

Fig.2.

MiR-13664 abundance levels in the DS and DR strains of *C. pipiens pallens*. (A) Laboratory mosquito populations. (B) Field-collected mosquito populations. Data are presented as means $\pm$ SE of three independent experiments. *** $P < 0.001$ compared with the DS strain.

Fig.3.

MiR-13664 is involved in mediating mosquito resistance to deltamethrin. (A) MiR-13664 abundance levels after miR-13664 mimic injection. (B) Mortality of mosquitoes exposed to deltamethrin in CDC bottles after 72 h by injection of miR-13664 mimic. (C) MiR-13664 abundance levels after miR-13664 inhibitor injection. (D) Mortality of mosquitoes exposed to deltamethrin in CDC bottles after 72 h by injection of miR-13664 inhibitor. Data are presented as mean $\pm$ SE of three independent experiments. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Fig.4.

CpCYP314A1 is a direct target of miR-13664. (A) The miR-13664 binding in the 3'-UTR of *CpCYP314A1* mRNA and the constructed mutated vector sequence. (B) Luciferase activity in HEK293T cells cotransfected with *CpCYP314A1*-3'-UTR (WT) or mutant *CpCYP314A1*-3'-MUT (MUT) construct together with miR-13664. (C) *CpCYP314A1* transcript levels in the DS and DR strains of *C. pipiens pallens*. (D) *CpCYP314A1* transcript levels after injection with miR-13664 mimic. (E) *CpCYP314A1* transcript levels after miR-13664 inhibitor injection. Luciferase activity was assayed in HEK293T cells cotransfected with *CpCYP314A1*-3'-UTR (WT) or the mutant *CpCYP314A1*-3'-MUT

(MUT) construct with miR-13664. Data are presented as means $\pm$ SE of three independent experiments. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

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Fig.5.

CpCYP314A1 affects deltamethrin resistance in DR mosquitoes. (A) Expression levels of *CpCYP314A1* after microinjection with *CpCYP314A1* dsRNA. (B) Microinjected mosquito mortality observed after exposure to deltamethrin in CDC bottles for 2 h. Data are presented as the means $\pm$ SE of three independent experiments. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Table 1

List of primers for qRT-PCR and vector constructs

Primers	Forward primer (5' to 3')	Reverse primer (5' to 3')
<i>AGO1</i>	GAGCACAAGCACACCTACCT	CCCACGGACCTCCATCATAC
<i>AGO3</i>	AAACGGTGCAGACGGTAGAG	GGAACAGCTTAACCCCGTCA
miR13664	ACACTCCAGCTGGGCCGGATCAGGTT	TGGTGTCTGGGAGTCG
U6	GCTTCGGCTGGACATATACTAAAAT	GAACGCTTCACGATTTTGCG
<i>CYP314A1</i>	GGAGGATGATGTGCGGAGTAT	AGGTTGTGCAGCAGGAATGA
<i>β-actin</i>	AGCGTGAACTGACGGCTCTTG	ACTCGTCGTACTCCTGCTTGG
CpCYP314A1–3' UTR	CGAGCTCCTATTACTACTCCTTGATACGA	CCAAGCTTTAATCGTACACGGATCAAGAC
CpCYP314A1–3' MUT	CGAGCTCCTATTACTACTCCTTGATACGA	CCAAGCTTTAATCGTACAGGCAACTAGAC

Table 2

List of the miR-13664 mimic/inhibitor and *dsCpCYP314A1*, *dsCpAgo1*, and *dsCpAgo3* primers.

Primers	Forward primer (5' to 3')	Reverse primer (5' to 3')
miR-13664 mimic	CCGGAUCAGGUUGAAGUC	CUUCAACCUGAUCCGGUU
miR-13664 inhibitor	GACUUCAACCUGAUCCGG	
<i>dsCpCYP314A1</i>	GCUGCGAGGAGAUCAUUUATT	UAAAUGAUCUCCUCGCAGCTT
<i>dsCpAgo1</i>	GCAUGCCGUCGAUGCAUUUTT	AAAUGCAUCGACGGCAUGCTT
<i>dsCpAgo3</i>	GGGUCGGAAGAACUUCGAUTT	AUCGAAGUUCUCCGACCCTT
NC	UUCUCCGAACGUGUCACGUTT	ACGUGACACGUUCGGAGAATT
Inhibitor NC	CAGUACUUUUGUGUAGUACAA	

Table 3

Relative expression levels of 9 miRNAs with relatively high scores from the miRNAs obtained from the miRDeep prediction analysis in DS-strain and DR-strain mosquitoes.

miRNA Name	Sequence	miRDeep.score	Expression(DS/DR)
miR-1133	GTAGCTCAGTCGGTAGAG	0.7	No difference
miR-6194	CGCGGCGCCGTACAGCAC	0.8	No difference
miR-7529	CTTCGGTGGGTGGCAAAAC	0.5	No expression
miR-9322	CAAATCCGGCTCGAAGGACC	0.6	No difference
miR-12362	TTTTCCTGGTCTGGGGTT	4.1	No expression
miR-13664	CCGGATCAGGTTGAAGTC	1.6	1.92-fold
miR-14091	CAGTACTTCTGCAATGCAACCC	16.3	0.4-fold
miR-14325	ACACACACGTACTGCCCC	0.2	No expression
miR-17823	GGTTTTTTGTTTCAGCGTTCA	0.2	1.61-fold