Simulation of the Gαq /Phospholipase Cβ1 Signaling Pathway Returns Differentiated PC12 Cells to a Stem-like State

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ABSTRACT

Phospholipase Cβ1 is activated by Gαq to generate calcium signals in response to hormones and neurotransmitters, and is found at high levels in mammalian neuronal tissue. Besides carrying out this key plasma membrane function, PLCβ1 has a cytosolic population that helps, in part, to drive the differentiation of PC12 cells by inhibiting a nuclease that promotes RNA-induced silencing (C3PO). Here, we show that down-regulating PLCβ1 or reducing its cytosolic population by activating Gαq to drive it to the plasma membrane, returns differentiated PC12 cells to an undifferentiated state. In this state, the cells return to a spherical morphology, resume proliferation and express the stem cell transcription factors nanog and Oct4. Similar changes are seen with C3PO down-regulation. This return to a stem-like state is accompanied by shifts in multiple miR populations, such as increased levels of rno-miR-21 and rno-miR-26a. Surprisingly, we find that de-differentiation can also be induced by extended stimulation of the Gαq. In this case, the neurites completely retract over a 10-minute period, and while levels of nanog remain unchanged, the levels of some miRs begin to return to their undifferentiated values. In complementary studies, we followed the real time hydrolysis of a fluorescent-tagged miR in cells where PLCβ1 or C3PO were down-regulated. These samples showed substantial differences in miR processing in cells both the undifferentiated and differentiated states. Taken together, our studies suggest that PLCβ1, through its ability to regulate C3PO and endogenous miR populations, plays a key role in mediating PC12 cell differentiation.

INTRODUCTION

Differentiation of a stem cell to a dedicated lineage occurs through a series of carefully orchestrated transcriptional events that generate the transcription of specific mRNAs and alter the distribution and amounts of proteins in the cell. While many of these events occur in rapid

succession, some occur over longer periods resulting in cells that may have a narrow or broad a distribution of differentiation. Reversal of differentiation often occurs in cells that have transformed to cancerous states [1]. Thus, the ability to control differentiation and induce de-differentiation would greatly aid in optimizing cancer therapies and other therapies where inducing or reversing differentiation would be beneficial.

In this study, we show that we can manipulate the differentiated state of PC12 cells, a cultured cell line that is commonly used as a model for neuronal cells, through the $G\alpha_q$ /PLC β 1 signaling pathway [2, 3]. This pathway is initiated when neurotransmitters such as acetylcholine, serotonin, dopamine and melatonin bind to their specific transmembrane receptor. The ligand-bound receptor then activates $G\alpha_q$ which in turn activates phospholipase C β , leading to an increase in intracellular calcium. There are four known families of PLC β , and PLC β 1 is the most responsive to $G\alpha_q$, and is highly expressed in neuronal cells, such as PC12.

Aside from its classic role on the plasma membrane, PLC β 1 also has an atypical cytosolic population that binds to unique partners. Specifically, we have found that PLC β 1 binds to and inhibits component 3 promoter of RISC activity (C3PO), which promotes RNA-induced silencing by accelerating degradation of the passenger strand of the silencing RNA [4, 5]. Binding to C3PO occurs through the non-catalytic C-terminal region of PLC β 1, which is required for binding and activation by $G\alpha_q$. The lipase activity of PLC β is not required for C3PO inhibition, and C3PO does not inhibit PLC β . However, C3PO competes for the same site on PLC β as $G\alpha_q$ and increasing its cellular levels quenches calcium signaling mediated through $G\alpha_q$ receptors [6-8]. It is notable that increasing the level of activated $G\alpha_q$ by transfection or stimulation, will result in dissociation of PLC β from C3PO and allow C3PO to promote RNA-induced silencing [7, 8].

In previous studies, we found that cytosolic PLC β 1 is required for differentiation of PC12 cells [9]. PC12 cells are a widely used model for neuronal cell differentiation [10]. In their undifferentiated state, they are round, rapidly dividing cells. After exposure to the neurotropic factor NGF (nerve growth factor), they stop dividing, sprout neurites, and form and release synaptic vesicles within ~72 hours. Although changes induced by NGF are initiated by TrkA receptors that signal through pathways associated with growth factors [11], we have shown that PLC β 1 and C3PO are absolutely necessary for the morphological changes that accompany differentiation [9]. Additionally, in early stages of differentiation, PLC β 1 binds and inhibits CDK16 [12], a testis and neuronal-specific cyclin dependent kinase that promotes proliferation. The bifunctional effects of PLC β 1 on differentiation can be reversed by increasing the level of activated $G\alpha_q$ to drive the cytosolic population of PLC β 1 associated with CDK16 and C3PO to the plasma membrane thereby returning cells to a round, proliferative state.

Since the level of $G\alpha_q$ impacts the level of cytosolic PLC β 1 which is required for PC12 cell differentiation, then external signals that activate $G\alpha_q$ may impact the state of differentiation. Here, we show that down-regulating PLC β 1 in fully differentiated PC12 cells returns cells to a stem-like state in terms of morphology, division and stem cell markers. Our data suggest that the mechanism responsible for de-differentiation is lies in the reduction of PLC β 1-C3PO complexes

that affect specific cellular miR populations and in turn, expressed proteins. Prolonging the activated state of Gαq also returns PC12 cells to an undifferentiated state. Our results suggest that PLCβ1 and its constructs might be useful in modulating the differentiated state of cells.

MATERIALS AND METHODS

PC12 Cell Culture and Differentiation—PC12 cells were purchased from American Tissue Cell Culture (ATCC) and cultured in DMEM (Gibco) with 10% horse serum from PAA (Ontario, Canada), 5% FBS (Atlanta Biological, Atlanta, GA), and 1% penicillin/streptomycin. Cells were incubated in 37 °C, 5% CO₂, and 95% humidity. Differentiation was carried out a medium of 1% horse serum, 1% penicillin/streptomycin and initiated by the addition of nerve growth factor (NGF) from Novo protein. Medium was changed every 24 h. Cells from 8 passages or less were used in these studies.

siRNA knock-down. Down-regulation of PLCβ1 was accomplished using SmartPool rat siRNA(PLCβ1) from Dharmacon Cat# L-010280-00-0005 to give greater than 90% down-regulation as estimated by western blotting. siRNA(CDK16) from Dharmacon cat gave 86±3% down-regulation. siRNA(TRAX) was purchased from Ambion (cat# AM16708) to give 73 ± 8% knock-down, and siRNA(translin, human) from Dharmacon (cat# L-011897-01) gave 68 ± 5%. Transfection with both siRNA(TRAX) and siRNA(translin) gave (95 ± 2%). siRNA negative control sigma cat# SIC001.

Western Blotting. Samples were placed in 6-well plates and collected in lysis buffer that included Nonidet P-40 and protease inhibitors. After SDS-PAGE electrophoresis, protein bands were transfer to nitrocellulose membrane (Bio-Rad, Inc). Primary antibodies to PLCβ1 (D-8), Gαq (E-17) were from Santa Cruz Biotechnology. Antibodies for actin and GAPDH, TRAX, CDK16 from were from Abcam, Inc. Membranes were treated with antibodies diluted 1:1000 in 0.5% BSA and washed 3 times for 5 min before applying secondary antibiotic (anti-mouse or anti-goat from Santa Cruz Biotechnology and Anti-Rabbit from Abcam at a concentration of 1:1000. Membranes were washed 3 times for 10 minutes before imaging on a Bio-Rad imager to determine the band intensities. Bands were measured at several sensitivities to ensure the intensities were in a linear range and avoid detector saturation. Data were analyzed using Image J in grayscale plot profile. Bands were normalized to loading control.

Transfection. Plasmid transfections were accomplished using Lipofectamine 3000 (Invitrogen, Inc) as recommended by the manufacturer. Transfection of siRNAs was also carried out using Lipofectamine 3000.

Proliferation assay. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Thermo Fisher Scientific) was added to cells at a final concentration of 2mM and incubated at 37°C in 5%CO₂ for 1 h. Medium was carefully aspirated from each well and replaced with 200 ml DMSO. The contents of each well were mixed by pipetting up and down and horizontally shaking the plates until the color was homogeneous. Absorbance data were obtained from PerkinElmer Victor 3 Plate Reader (Waltham, MA,USA) at 570 nm.

miRNA sequencing. We knocked down PLC β 1 using the smartpool mixture of 4 target miRNAs from Dharmacon. The following samples were tested: Control (mock transfected), PLC β 1 knock-down, Control (mock transfected) +NGF, PLC β 1 knock-down +NGF. Each experiment had three (3) plates/sample. PC12 cells were cultured as described [9] and transfection was performed with the RNAiMAX reagent. NGF (1/1000) was added to the appropriate samples for 24 hours. Total RNA was extracted using the Qiagen miRNA Mini kit and run on a formaldehyde denaturing gel to check integrity. cDNA was synthesized for each sample using 5 μ g of RNA with the Qiagen MiScript RT Kit, according to the manufacturer's instructions. PLC β 1 knock-down was verified in each experiment by real-time PCR, using the QuantiFast SyBr Green PCR Kit (Qiagen) and the following primers: forward 5' CGGCCAGGCTATCACTACAT 3'; reverse 3' ATTCACCCCATTCTCTGCTG 5'. Only samples from experiments with at least 80% knock-down of PLC β 1 mRNA were used for RNA sequencing. Aliquots from the selected samples of each experimental group from three separate experiments were pooled and submitted to the University of Cincinnati Genomics Core for RNA sequencing.

Fluorescent Oligonucleotides. A fluorescent double stranded DNA oligonucleotide based on the miR103 sequence, AGCAGCAUUGUACAGGGCUAUGA, labeled on 3' end with 6-carbofluorescein (FAM) and the 5' end with 5-carboxytetramethylrhodamine (TAMRA) was purchased from Genewiz. The small length of the oligonucleotide, upon excitation of FAM, Forster Resonance Energy Transfer (FRET). An oligonucleotide with 3 mismatches on the 5' strand, AGCGATAUUTUACGAACUAUGA also containing the same FRET pair on the 5' and 3' ends were also prepared.

Confocal Imaging. Cells were seeded in poly-D-lysine coated glass-covered dishes from Mat-Tek. Images were acquired using Zeiss 510 meta confocal microscope. Data were analyzed using, LSM software and ImageJ software.

RT-PCR. Cells were seeded in 6 well plates and transfected with siRNA of each target protein as described above. After 48 hours, cells were washed twice with PBS then lysed using Qiazol (Qiagen). Total RNA including miRNA was extracted using miRNeasy kit from Qiagen following the manufacturer's protocol.

Reverse transcription were performed using TaqManTM Advanced miRNA cDNA Synthesis Kit Cat# A28007 as recommended by the manufacturer. RT-PCR measurements were performed using TaqManTM Fast Advanced Master Mix. Assays were carried out using Taqman reagents and U6 as internal control on a Roche Light cycler 96 as follows (90C for 20 Seconds then 45 cycles of 95C for 3 seconds and 60C for 30 seconds) and analyzed using Light cycler software.

FRET/FLIM imaging. Images of cells in MatTek chambers (MatTek, Ashland, MA, USA) were acquired using a 2-photon Mai Tai laser (Spectra-Physics, Santa Clara, CA, USA) and a Nikon inverted confocal microscope in an ISS Alba System (Champaign, IL, USA). Data were analyzed using ISS Vista Vision and ImageJ (National Institutes of Health, Bethesda, MD, USA)

software packages. Fluorescence lifetime imaging microscopy (FLIM) was carried out on an ISS instrument. Atto 425 ($t = 3.61\text{ns}$) was used to calibrate the sample lifetimes.

Microinjection studies. Cells were washed with Hanks' Balanced Salt Solution (HBSS) media before carrying out FRET/FLIM imaging on the ISS ALBA system described above. Fluorescence lifetimes of 6-carbofluorescein (FAM) labeled oligonucleotides were excited at 780 nm. Microinjections were performed using Femtotip capillaries (Eppendorf) with 100 μM solution of the oligonucleotides injected with an injection pressure of 40 hPa over 0.65 seconds, with an Eppendorf FemtoJet 4i microinjection apparatus.

Retraction studies. Cells were differentiated following the protocol above. Cells were stimulated by adding 5 μM carbachol (Sigma) directly into the cell media while imaging.

RESULTS

Down-regulation of PLC β reverses the differentiation of PC12 cells. Under typical conditions, treatment of undifferentiated PC12 cells with NGF halts proliferation and induces the growth of neurites over 36-72 hours. Here, we treated PC12 cells with NGF for 72 hours to achieve full differentiation as assessed morphologically (i.e. when the length of the neurite are greater than three times the length of the cell diameter). Cells were then washed and transfected with siRNA(PLC β 1) to reduce the level of PLC β 1 by 80-90% as determined by western blotting. In mock transfected cells, the neurite length remained constant over 72 hours but in cells treated with siRNA(PLC β 1), we find a complete retraction of neurites into the soma (**Fig. 1A-B**). Alternately, transfecting cells with siRNA(PLC β 1) 24-48 hours before NGF treatment prevented neurite growth (**Fig. 1B**) as previously noted [9].

Differentiated PC12 cells are non-proliferative and so we determined whether down-regulating PLC β 1 would transition the cells back to a proliferative state. Thus, we monitored the proliferation of PC12 cells using an MTT assay. Down-regulating PLC β 1 before NGF treatment did not affect proliferation in contrast to cells with endogenous PLC β 1 levels (**Fig. 1C**). However, transfecting non-proliferative differentiated cells with siRNA(PLC β 1) allowed the cells to resume proliferation at a rate similar to undifferentiated cells (**Fig. 1C**). Taken together, the results in **Fig. 1A-C** show that PLC β 1 is required to maintain the differentiated state.

To better characterize the de-differentiated state resulting from reduced PLC β 1 levels, we monitored changes in the amounts of two stem-cell marker proteins: nanog and Oct4. In **Fig. 1D** we show that the expression levels of these proteins are high in undifferentiated cells and are greatly reduced upon NGF treatment, as expected. However, in differentiated cells where PLC β 1 is down-regulated, the levels of both nanog and Oct4 are recovered. Taken together, these studies show that loss of PLC β 1 in differentiated PC12 cells causes the cells to adapt a stem-cell like state.

Loss of C3PO also induces PC12 cell de-differentiation. PLC β 1 has been found on the plasma membrane, the cytosol and the nucleus and we wondered which population impacts differentiation. We noted that since we are not working under conditions that activate G α_q , then it is probably not the plasma membrane population of PLC β 1, and siRNA down-regulation should effect the cytosolic PLC β 1 pool and not the nuclear one. These observations suggest that cytosolic PLC β 1 is promoting de-differentiation. As mentioned, PLC β 1 halts PC12 cell proliferation by inhibiting the mitotic kinase, CDK16, and PLC β 1 helps to induce the neurite growth by inhibiting C3PO [9, 12]. Thus, loss of PLC β 1 may promote de-differentiation due to interactions with CDK16 and/or C3PO.

We first determined whether CDK16 is involved in de-differentiation. CDK16 is primarily expressed in neuronal tissues and testis [13]. During neuronal differentiation, CDK16 transitions from its pre-mitotic role of inhibiting the proliferation inhibitor p27kip, to a post-mitotic role in synaptic vesicle trafficking (see [13]). In previous studies, we showed that PLC β 1-CDK16 associates in undifferentiated PC12 cells using Förster resonance energy transfer (FRET) [12]. However, in differentiated PC12 cells transfected with eGFP-PLC β 1 / mCherry-CDK16, we could not detect FRET (0.002 ± 0.003 , $n=8$). Since PLC β 1-CDK16 complexes are not present in differentiated cells, it is unlikely they promote de-differentiation. In a supporting study, we de-differentiated PC12 cells expressing mCherry-CDK16 by down-regulating PLC β 1, and then transfecting with eGFP-PLC β 1. After 24 hrs we could detect both mCherry-CDK16 and eGFP-PLC β 1, but could not detect FRET suggesting that PLC β 1-CDK16 complexes do not form during de-differentiation. In a second series of studies we down-regulated CDK16 and found that treatment with NGF halted proliferation (**Fig. 2A**). These results suggest that CDK16, either alone or in complex with PLC β 1, does not play a role in de-differentiation.

We then tested whether changes in interactions between PLC β 1 and C3PO are associated with de-differentiation. In initial studies, we down-regulated C3PO in differentiated PC12 cells. C3PO is an oligomer of two TRAX and six translin subunits, and we find that down-regulating either TRAX or translin alone results in a ~70% reduction of C3PO. In **Fig. 2B** we show that knocking down C3PO reverses de-differentiating similar to the behavior seen for PLC β 1. These results suggest that both PLC β and C3PO are required to maintain differentiation, and that their absence promotes de-differentiation.

If loss of PLC β 1 is associated with proliferation, we would expect to see an increase in enzymes that mediate key proliferation signaling pathways. To test this idea, we monitored the level of ERK1/2 which plays a central role in proliferative MAPK pathways [14]. We find that down-regulating PLC β 1 results in a large and significant increase in ERK1/2, supporting the direct relationship between PLC β 1 and proliferation (**Fig. 2C**).

De-differentiation is associated with shifts in the distribution of microRNAs. C3PO has been reported to promote RNA-induced silencing [5] and its activity is inhibited by PLC β 1 [15]. With this in mind, we tested whether differentiation and subsequent de-differentiation is reflected in shifts in miR populations of PC12 cells, and whether some of these changes correlate

to PLC β 1 levels. To do this, we characterized miR populations of four sets of PC12 cell samples: undifferentiated cells, undifferentiated where PLC β 1 was almost fully knocked-down (i.e. >95%), differentiated cells and differentiated cells treated with siRNA(PLC β 1) for 2 days where the cells are estimated to be ~40% de-differentiated (*supplemental*).

In comparing the miR populations of the four samples, we were surprised to find that NGF treatment did not change the total number of miRs (3.00 vs 3.04 million). However, siRNA(PLC β 1) treatment reduced the number of miRs by ~50% in undifferentiated cells to 2.04 million and by ~17% in de-differentiating cells to 2.57 million (*supplemental*). This loss in the number of miRs with PLC β 1 down-regulation is consistent with higher C3PO nuclease activity and greater miR processing due to the loss of inhibition of PLC β 1 (see Discussion). It is notable that the overall loss in the number of miRs with PLC β 1 is very high considering the relatively low expression of PLC β 1 in undifferentiated cells.

RNAseq studies identified 393 different miRNAs in untreated, undifferentiated cells, and of these, only 49 showed more than 10,000 copies. These are listed in **Fig. 3A**. **Fig. 3B** shows the changes in the levels of these miRs with NGF treatment. We find that the miRs whose populations change overlap with those previously identified in neuronal cell differentiation [16]. While most of these miRs showed a 10-40% reduction in the number of miRs, two of these, miR-21 and -26a, showed an increase (**Fig. 3B**). It is notable that these miRs have been associated with neuronal tissue and show a strong sequence homology (**Fig. 4A**).

In **Fig. 3C**, we compare the miRs that are impacted when PLC β 1 is down-regulated in undifferentiated and de-differentiated cells. In undifferentiated cells, loss of PLC β 1 decreases the number of individual miRs between -10 and 30% (black circles) consistent with increased C3PO activity. In de-differentiating cells, down-regulating the enzyme decreases the levels of almost all miRs between 5-50% (blue triangles). To more easily compare these data, we subtracted the transcripts reduced by PLC β 1 down-regulation in de-differentiating cells from undifferentiated cells (**Fig. 3D**) (i.e. the blue minus the black points in **3C**). While we see that almost all the transcripts increase during the de-differentiation process, miR-21 and miR-26a, are upregulated and miR-22 and -29a to a lesser extent (**Fig. 3D**).

The data in **Fig. 3** only focus on the miRs with high copy numbers. Many miRs with lower copy numbers change with differentiation and PLC β 1 knock-down. A few miRs show higher than a two-fold change with differentiation and/or PLC β 1 knock-down but their copy numbers are very low (i.e. <50) which may accentuate changes that are not significant, and so these miRs are not considered. In a separate analysis, we screened the data for families of miRs that change at least 25% and are present at 200 copy number or higher (counting the -5p and -3p as a single type) (*supplemental*). By analyzing this expanded set of data, we find that 17 members of the let-7 family are reduced with PLC β 1 down-regulation, and the eight with the highest copy number are shown in red boxes in **Fig. 3C**. Additionally, the miR-30 family has 10 members that change, miR-125 has 5 members, miR-344 has 5 members, and miR-99, miR-181 and miR-130 families all have 4 members that change with PLC β 1 down-regulation.

We aligned the mature miRs of these different families to determine common sequences. These families include one group of up-regulated miRs, the *let-7* family, and two other down-regulated groups. While there was no overall homology, if we separate these into different groups, some homologous groups emerge (**Fig. 3D**). It is notable that none of these groups are significantly different in terms of physical properties, such as Tm or GC content, and none fit into specific miR clusters. While some sequence homology in the groups are seen, in general, this analysis indicates a lack of strong sequence specificity in miRs and their changes in levels with de-differentiation.

We used RT-PCR to verify the results for a few representative miRs (**Fig. 4**). We find that in control cells, miR-22-3p increases ~1.8 fold when treated with NGF where the number of transcripts significantly increase with NGF treatment, and these levels return to basal with PLC β 1 or C3PO down-regulation. These results closely follow the RNAseq results. We also tested a representative miR that does not change greatly with differentiation, *let-7i-5p*, and find that these levels show a small increase with differentiation, and a reduction with PLC β and TRAX knock-down, again in accord with the RNAseq. In addition, miR-103 shows a small increase with differentiation but a significant decrease with both PLC β 1 and C3PO down-regulation. Taken together, these results corroborate the RNAseq studies that show only minor changes in miR levels with differentiation except for a few miRs, such as miR-22, and down-regulation of PLC β 1 greatly reduces the number of miRs. Additionally, our studies show that C3PO down-regulation produces the same changes as PLC β 1 supporting the idea that PLC β 1 works with C3PO in regulating miR populations to control the differentiated state.

Cross-regulation of PLC β by *let-7*. Because PLC β 1 levels are inversely correlated to proliferation, but positively correlated to the *let7* levels, which in turn represses proliferation, we tested the possibility of reciprocal regulation of PLC β 1 by members of the *let-7* family. This study was carried out by transfecting cells with *let-7a* and monitoring the relative changes in the levels of PLC β 1. We simultaneously monitored H2B levels to follow proliferation. We find that treating cells with siRNA(PLC β 1) for 2 days increases the amount of H2B consistent with increased proliferation (**Fig.5**). However, transfecting control cells with *let-7a* reduces PLC β 1 levels and this reduction no longer occurs when C3PO is down-regulated. Taken together, these results suggest an interdependence between PLC β 1, C3PO and *let-7a* in cell proliferation.

Neurotransmitter stimulation can promote the return of differentiated PC12 cells to a stem-like state. We determined whether stimulation of G α_q could shift the cytosolic population of PLC β 1 to the plasma membrane and away from C3PO to reverse differentiation. Stimulation of G α_q results in an increase in intracellular calcium within the first minute followed by recovery over the next hour. Concurrent with this recovery is internalization of muscarinic receptors allowing for desensitization [17].

Starting with fully differentiated PC12 cells, we followed changes in morphology over 30 minutes with under continuous exposure to carbachol (a derivative of acetylcholine). Immediately

upon stimulation, we see retraction of neurites towards the soma membrane resulting in full retraction after ~10 minutes resulting in cells with an undifferentiated-like morphology (**Fig 6A**). To better understand the properties of these retracted cells, we measured changes in the levels of nanog in cells stimulated with carbachol for 30 minutes. We find a slight recovery in nanog levels consistent with cells that are transitioning out of the differentiated state (**Fig. 6B**). In a corroborative study, we measured changes in two different miRs (miR-22 and 23a) with differentiation and under carbachol stimulation (**Fig. 6C**). In line with the RNAseq studies, the levels of both miRs both change with differentiation, and carbachol stimulation causes their levels to shift towards those seen in undifferentiated cells.

Live cell imaging of miR processing. Thus far, our measurements have assessed changes in miR levels in cell lysates and we wanted to assess miR processing in intact, living cells. Our approach was to monitor the degradation of miR-103 labeled on the 5' and 3' ends with a FRET donor / acceptor pair (FAM/TAMRA) and introduce them into cells by microinjection. This oligonucleotide has a high degree of FRET that decreases as it degrades with nuclease activity. In these measurements, we monitored FRET by the decrease in fluorescence lifetime of the donor (FAM) due to energy transfer to acceptor (TAMRA). Hydrolysis of the oligonucleotide is seen by a net increase in fluorescence lifetime. For comparison, we also constructed an imperfect miR103 analog with 3 mismatched nucleotides. This imperfect nucleotide should result in stalled Ago2 complexes[18] and should not be sensitive to changes in the levels of PLC β 1 or C3PO.

In **Fig. 7** we show the shift in the fluorescence lifetime of miR103-3p labeled with the FAM/TAMRA FRET pair at 3 minutes in differentiated cells and note that similar results are observed in undifferentiated cells. Additionally, the values remain constant over the 3-20 minutes viewed. Cell images show that the oligonucleotide is not distributed uniformly in the cytosol but appears to sequester in aggregates, which may correspond to RISC. Microinjecting the tagged miR into PC12 cells showed a clear increase in lifetimes from 1.4 to 2.8 ± 0.13 ns in undifferentiated cells and 1.8 to 3.2 ± 0.097 ns in differentiated cells showing that this miR is processed slightly more efficiently in differentiated cells. When we down-regulate PLC β 1, we find a pronounced shift to longer lifetimes, consistent with increased C3PO activity. In contrast, when we down-regulate TRAX (**Fig. 7**), hydrolysis is reduced. Processing was greatly reduced with carbachol stimulation, which is consistent with reduced levels of cytosolic PLC β 1 and sequestration of the miRs in stress granules (*see discussion*). In contrast, the imperfect miR tended to sequester in the nucleus, and both the cytosolic and nuclear populations were not impacted by changes in the levels of PLC β 1, C3PO or carbachol.

DISCUSSION

In this study, we show that we can control the differentiation of a model neuronal cell line by changing the levels of cytosolic PLC β 1 either by varying its expression or by driving it to the membrane with stimulation of G α q. Specifically, our studies suggest that while PLC β 1-C3PO complexes are required to promote the transition of PC12 cells to the differentiated state, a basal amount is required to maintain differentiation. Our studies show that PLC β 1 levels regulate miRs associated with differentiation, and this regulation is in part associated with PLC β 1's ability to inhibit C3PO.

A yeast two-hybrid study found that C3PO is a binding partner of PLC β 1 and this association was verified by coIP, pull-downs, co-immunofluorescence and FRET in all cell lines tested (HEK293, SK-N-SH, HeLa and PC12 cells [7, 8]). C3PO has been reported to promote RNA-induced silencing by processing the passenger strand of silencing RNA after an initial nick by Ago2 which leaves the guide strand free to hybridize to its target mRNA and be degraded by Ago2 of RISC [5]. Because the nuclease activity of Ago2 is operative when the miR-mRNA is perfectly paired, C3PO will only promote silencing in cases of perfect pairing between miR and mRNA, or in exogenous siRNAs. Support for this model comes from our live cell imaging data showing that hydrolysis of perfectly paired miR103-3 depends on the level of C3PO, PLC β 1 but imperfectly paired miR103-3 does not. It is notable that PLC β 1 inhibits C3PO processing of miRs with higher Tms that are more slowly hydrolyzed by C3PO [15]. Thus, the impact of PLC β 1 on RNA-induced silencing by C3PO inhibition is limited to specific endogenous miRs.

In previous work, we followed the differentiation of PC12 cells stimulated by the neurotropic agent NGF, and found that both PLC β 1 and C3PO are required for neurite growth. Undifferentiated PC12 cells, like most stem cells, have a very low expression of G α q and PLC β 1 since these cells have not developed mature response networks to neurotransmitters. Within the first 24 hours after NGF treatment, the level of PLC β 1 increases while G α q and C3PO remain constant. Our studies show that this increased PLC β 1 associates with C3PO. This period is then followed by increased G α q production and a loss of PLC β 1-C3PO complexes. However, some PLC β 1-C3PO complexes remain in the differentiated state. Although other mechanisms are possible, we postulate these complexes help maintain the differentiated state by modulating miR populations.

We propose that the ability of PLC β 1 to regulate differentiation can be traced to its regulation of C3PO, and in turn, modulation of silencing by miRs. Therefore, we carried out RNAseq in undifferentiated and differentiated cells, and in undifferentiated cells where PLC β 1 was almost completely absent, in cells undergoing de-differentiation through down-regulation of PLC β 1. While the total amount of cellular miR was unchanged with differentiation, there were some shifts in the populations of several species (see **Fig. 3B**), and these are in line with those reported in other studies of developing rat and monkey brains [16]. Notably, two species showed a substantial increase with differentiation, miR-21 and mi-26a. In contrast to our data, miR-21

levels are positively correlated with proliferation in cardiac smooth muscle tissue [19], while miR-26a is inversely correlated with proliferation in a number of tissues correlation with our results (for review see [20]). The levels of many other miRs were found to decrease slightly with differentiation. Of note is the relatively large decrease in miR-93 whose overexpression diminishes PTEN activity and promotes carcinogenesis through dysregulation of the PI3K/AKT pathway [21].

In general, our data show that reducing PLC β 1 substantially decreases the total amount of cellular miRs. Reduction of total cellular miRs with PLC β 1 down-regulation is in line with the large increase in small RNAs seen when PLC β 1 is overexpressed in HEK293 cells [7]. While this observation is consistent with increased C3PO activity, it is probable that other downstream mechanisms contribute considering the limited set of miRs that are expected to be impacted by PLC β 1-C3PO association. Of note is the strong dependence of the *let-7* family with PLC β 1 expression is in accord with the impact of *let-7* on signaling pathways (e.g. [22]). We find that the levels of 17 members of the *let-7* family changed with PLC β 1 expression. In nematodes and drosophila, *let-7* is a key regulator in differentiation and development, and in vertebrates, *let-7* levels are associated with differentiated state [23] which correlates well their involvement in de-differentiation. The interdependence between PLC β 1 and *let-7* implies a feedback mechanism that asserts multiple controls on proliferation and provides a roadmap into potential mechanisms that might drive differentiation processes initiated by changes in PLC β 1 expression. It is notable that C3PO down-regulation does not impact proliferation, as seen by H2B levels.

In addition to *let-7*, several other miR families are associated with PLC β 1-induced de-differentiation. We searched for commonalities between the miRs that increase or decrease with PLC β 1 down-regulation, we could not find a strikingly strong correlation even when divided into groups. However, we note that the local copy number of these miRs and the presence of competitive ones may override absolute sequence dependence making it unclear whether common regions found in the alignment of these groups these are true targets of C3PO-PLC β 1. Broadly, we propose that the global shift in the population of these miR families that not only affect the translation of specific mRNAs, but also function to compete with other miR to indirect affect protein production. Future systems biology studies that probe the mRNAs and proteins regulated by these miRs will offer better insight.

Our studies show a surprising relationship between activation of G α q and de-differentiation through PLC β 1. Upon G α q activation by carbachol, we find that the neurites of differentiated PC12 cells rapidly retract resulting in a morphology that resembles undifferentiated cells. Our studies indicate that the levels of miRs begin to return to undifferentiated ones although the timeline appears to be much longer. Our studies also show that carbachol reduces the hydrolysis of the fluorescent-tagged miR103 and this observation correlates well with studies showing that reduction of carbachol stimulation increases the number and size of stress granule particles [24]. The link between G α q and stress granules is through the ability of cytosolic PLC β 1 to bind stress granule proteins inhibiting their aggregation. We propose that the formation of stress granules

caused by Gαq activation incorporates miR103 into the aggregates thereby protecting the nucleotide from hydrolysis. It is likely that a similar mechanism occurs for other miRs impacted by PLCβ1. Taken together, our results show a clear connection between external agents that stimulate and differentiation.

Our studies used PC12 cells as a model for differentiation and since the Gαq/PLCβ pathway is ubiquitous in mammalian cells, and PLCβ1 and C3PO have been found to associated in other cell lines then it is possible that link between PLCβ1 and stemness, and feedback from the extracellular environment can control the level of differentiation is a general. These finding suggest that PLCβ1 constructs could be used a method to control the degree of the level of differentiation.

ACKNOWLEDGEMENTS

The authors would like to thank Dr. V. Siddartha Yerramilli for his helpful comments throughout this work and to Dr. David McKinnon for help with the RNAseq studies. This work was supported by NIH GM116187. OG was supported by funds from the Richard Whitcomb award.

FIGURE LEGENDS

1-Reversal of PC12 cell differentiation with siRNA(PLCβ1). **A** - DIC images of PC12 cells (*top panels*) where the time refers to the hours after NGF treatment showing the growth of neurites. The *bottom panels* show images from the same set of cells at 48hr post NGF that were treated with siRNA(PLCβ1) showing retraction of neurites. **B** – Graph showing changes in neurite length during differentiation induced by NGF and subsequent treatment with siRNA(PLCβ1). Data are from 4 independent experiments. ANOVA comparison between 0 - 3 days and between 3- 6 days is statistically significant $p \leq 0.001$. Error bars indicate SEM. **C**- MTT proliferation assay showing that NGF treated cells (orange circles) lose their ability to proliferate as they differentiate and produce neurites. However, NGF treated cells return to proliferation after siRNA(PLCβ1) (blue circles). Down-regulation of PLCβ1 before NGF treatment allows the cells to continuously proliferate (red triangles). Data are from two independent experiments and SEM is shown. **D** - Western blot showing changes in the levels of two stem cell markers, Oct4 and nanog after 48 hrs of NGF treatments, and after treating the siRNA(PLCβ1).

2- PLC β 1 down-regulation allows differentiated PC12 cells to become proliferative. **A** – Proliferation as monitored by an increase in 570nm absorption using an MTT assay. PC12 cells were treated with NGF for 48 hours to induce differentiation, and then treated with siRNA(PLC β 1) for 24 hours (orange circles), or siRNA(CDK16) (red). In an alternate study, undifferentiated cells were treated with siRNA(CDK16) on day 2 and NGF was added at day 3 (yellow triangles). These data indicate that, unlike PLC β 1, down-regulation of CDK16 does not overcome reduced proliferation by NGF treatment. **B** – The effect of TRAX down-regulation on neurite length in PC12 cells over a 2 days period where n=12. **C**- The effect of PLC β 1 down-regulation on ERK1/2 expression as determined by western blotting.

3- RNAseq results of PC12 cells with differentiation and PLC β 1 down-regulation. **A** – Copy number of the 49 miRs expressed in undifferentiated PC12 cells present in copy numbers greater than 10,000. **B** – Changes in the levels of the miR listed in 3A with NGF treatment as expressed as the ratio of undifferentiated / differentiated. Values below 1.0 correspond to miRs that increase with differentiation and values above 1.0 decrease with differentiation. **C**- Changes in miR levels with siRNA(PLC β 1) where the X axis is the ratio of mock/si(PLC β 1) transfected in undifferentiated (black) and differentiated (blue). Members of the *let-7* family are identified by red boxes. **D** - To better identify the miRs involved in differentiation affected by PLC β 1, we subtracted of the undifferentiated minus differentiated values in 3C (i.e. the blue symbols minus the black). **E** – Alignment of miRs shown to be affected by PLC β 1 down-regulation. The first group (blue) are the miRs that shown an increase with differentiation. Nucleotides in red are homologous.

4. RT-PCR results of selected miRs. RT-PCR measurements were carried out as described in undifferentiated PC12 cells (UN), differentiated PC12 cells (NGF) cells and in differentiated cells treated with siRNA(PLC β 1) or siRNA(TRAX) for 2 hours and where n= 4 and $P \leq 0.001$ and SD is shown..

5. *Let-7a* influences PLC β 1 expression. Compilation of band intensities from western blots showing that transfection of *let-7a* in undifferentiated PC12 cells reduces endogenous levels of PLC β 1 and down-regulation of TRAX restores PLC β 1 levels, where n=8, * $P \leq 0.001$ and SD is shown.

6. *Gaq* stimulation causes neurite retraction. **A** – Images of differentiated cells that have been transfected with mCherry actin, at time 0, time ~10 minutes and ~30 minutes after stimulation with 5 μ M carbachol. Scale bar is 20 μ M. **B**- Normalized band intensities from western blots of differentiated PC12 cells showing changes in nanog expression 30 min after carbachol (5 μ M) where n=2 and SD are shown. **C**- Changes in $\Delta\Delta$ Ct values for miR-22 (*left*) and miR-23a (*right*)

of differentiated PC12 cells (DIFF) under basal conditions and 30 min 5 μ M carbachol stimulation (CARB) normalized to undifferentiated controls (UN).

7. FLIM measurements in live cells show miR processing. Studies showing live processing of miR-103-3p labeled on the 3' and 5' ends with the FRET pair FAM/TAMRA. Here, hydrolysis of the oligonucleotide is followed by the increase in FAM lifetime from dequenching by TAMRA. In all graph, microinjection into untransfected cells is labeled *Ctrl*, stimulation with 2 μ M carbachol after microinjection is labeled as *Carb*. Graphs representing lifetimes of fluorescently labeled miR-103 in differentiated (**A**) and (**B**) undifferentiated PC12 cells. **C** – Identical study as in (**A**) but using an imperfectly paired version of FAM-miR-103-TAMRA. **D** – Example of a phasor plot and the corresponding image from a FLIM measurement of FAM-miR-103-TAMRA expressed in wild type undifferentiated PC12 cells. The heat map indicates FAM and TAMARA signal intensity. **E** – Similar study as (**D**) but with undifferentiated cells stimulated with 2 μ M carbachol after injection. **F** – Similar study as (**D**) but with undifferentiated cells transfected with PLC β 1 siRNA before injection.

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Fig.1

A

Hours after NGF treatment

Hours after siRNA(PLC β 1) treatment

B

Neurite Length with NGF and siRNA(PLC β 1)

Fig.1 cont

C

D

Fig. 2

A

B

Fig. 2C

Effect of down regulation of PLCβ1 on ERK 1/2

Fig.3A miR populations in Undifferentiated PC12 cells

Fig.3B Changes in miR with Differentiation

Fig. 3C Effect of PLC β 1 in undifferentiated and differentiated cells

Fig. 3D Differences in miRNAs content in undifferentiated minus differentiated cells treated with siRNA(PLC β)

Fig. 3D – Alignment of miRs affected by differentiation and PLCβ1 down-regulation

rno-miR26a	CCUAUUC-UUGGUUACUUGCAC
rno-miR21	UAGCUUAUCAGACUGAUGUUGA
rno-miR-22	AAGCUGCCAGUUGAAGAACUGU
rno-miR-24-3p	UGGCUAGUUCAGCAGGAACAG
rno-miR-23a-3p	AUCACAUUGCCAGGGAUUUC
rno-miR-23b-3p	AUCACAUUGCCAGGGAUUACC
rno-miR-103-3p	AGCAGCAUUGUACAGGGCUAUGA
rno-miR-27a-3p	UUCACAGUGGCUAAGUUCCGC
rno-miR-27b-3p	UUCACAGUGGCUAAGUUCUGC
rno-miR-375-3p	UUUGUUCGUUCGGCUCGCGUGA
rno-let-7b-5p	UGAGGUAGUAGGUUGUGUGGUU
rno-let-7f-5p	UGAGGUAGUAGAUUGUAUAGUU
rno-let-7i-5p	UGAGGUAGUAGUUUGUGCUGUU
rno-miR-10b-5p	CCCUGUAGAACCGAAUUUGUGU
rno-miR-99b	CACCCGUAGAACCGACCUUGCG
rno-miR-218a	UUGUGCUUGAUCUAACCAUGU
rno-miR-93	AGUG-CUGUUCGUGCAGGUAG
rno-miR-532	CAUGCCUUGAG-UGUAGGACUGU
rno-miR328a	----GC- ----GGGGG GCAGGA

Fig. 4

A

B

C

Fig. 5

Fig. 6

Fig. 7

Supplementary Data for *Simulation of the Gαq /Phospholipase Cβ1 Signaling Pathway Returns Differentiated PC12 Cells to a Stem-like State*

RNAseq results

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#miRNA	DM14.1 (Control)	DM14.2(Control + NGF)	DM14.3(PLCb1 KO)	DM14.4(PLCb1 KO + NGF)	1vs3(%)	2vs4(%)	1v3(log2)	2v4(log2)
rno-miR-375-3p	312450	305234	207853	260195	0.40206188	0.15930913	0.58806177	0.23032243
rno-miR-218a-5p	248358	266035	176830	200345	0.33645352	0.28170162	0.49005816	0.40912956
rno-miR-218a-5p	239358	257236	171309	193346	0.3314072	0.28358878	0.48256908	0.41190764
rno-miR-27b-3p	216880	237451	163146	194758	0.28279118	0.1975572	0.4107334	0.28594724
rno-miR-30d-5p	180048	160389	112969	141869	0.45785057	0.12254432	0.67245464	0.17701582
rno-miR-10b-5p	133718	149239	92864	126030	0.36061117	0.16862778	0.52600235	0.24385741
rno-let-7i-5p	128640	122557	84470	110262	0.41452771	0.10561853	0.60682836	0.15251721
rno-miR-24-3p	91543	94548	59641	68990	0.42202879	0.31256344	0.61814512	0.45465968
rno-miR-24-3p	91536	94543	59640	68986	0.42197174	0.31256841	0.61805899	0.45466703
rno-let-7f-5p	83682	89941	52824	77301	0.452112	0.1511582	0.66372378	0.21849185
rno-let-7f-5p	82726	88626	51871	76158	0.45847976	0.15132537	0.67341264	0.21873441
rno-let-7g-5p	74580	66680	50190	68009	0.39095937	-0.0197344	0.57138885	-0.0284716
rno-miR-99b-5p	53187	56734	32649	47991	0.47854047	0.16697064	0.70403486	0.24144971
rno-miR-22-3p	41795	47486	37611	38508	0.10538246	0.20880527	0.1521757	0.30234404
rno-miR-21-5p	36746	56315	28590	37129	0.24966328	0.41064167	0.36207659	0.60097279
rno-miR-103-3p	34065	35400	20827	27032	0.48232894	0.26806766	0.70983516	0.3890811
rno-miR-103-3p	33773	35046	20578	26699	0.48554764	0.27037007	0.71476757	0.39246408
rno-miR-532-5p	31957	27067	20884	28951	0.41910638	-0.0672641	0.61373392	-0.0970782
rno-miR-30a-5p	29428	27481	20103	25892	0.37653187	0.05954321	0.54977868	0.08592809
rno-let-7a-5p	28115	29099	18426	27691	0.41636407	0.04958619	0.60959713	0.07155242
rno-let-7a-5p	28105	29069	18434	27695	0.41560841	0.04841096	0.60845766	0.0698559
rno-let-7c-5p	27824	27785	18525	26665	0.40126001	0.04113866	0.5868563	0.05935891
rno-let-7c-5p	27661	27609	18433	26419	0.40039918	0.04405123	0.58556242	0.06356277
rno-miR-191a-5p	26357	24322	16659	20926	0.45090199	0.15010608	0.66188437	0.2169653
rno-miR-29a-3p	25661	27745	22461	23580	0.1329953	0.16229907	0.19215523	0.23466408
rno-miR-378a-3p	24555	21499	20531	23588	0.1785033	-0.0926653	0.25821292	-0.1337835
rno-miR-23b-3p	20242	20905	12637	15542	0.46260531	0.29429034	0.67969783	0.42767588
rno-let-7d-5p	19450	16710	11824	16185	0.48768945	0.03191974	0.71805198	0.04605437
rno-miR-125a-5p	19253	17243	14327	19076	0.29338892	-0.1009389	0.42634672	-0.1457479
rno-miR-7a-5p	18826	16987	12312	16555	0.41839553	0.02575875	0.61266136	0.03716408
rno-miR-7a-5p	18687	16809	12200	16411	0.42004727	0.02396147	0.61515383	0.03457075
rno-miR-320-3p	17715	17793	13065	16373	0.30214425	0.08312357	0.43926434	0.11999109
rno-miR-93-5p	15885	11953	9034	10695	0.54986155	0.11109149	0.81422827	0.16043627
rno-miR-340-5p	15756	15240	9455	13164	0.49986117	0.14617659	0.73675196	0.21126497
rno-miR-340-5p	15756	15240	9455	13164	0.49986117	0.14617659	0.73675196	0.21126497

rno-miR-27a-3p	15571	13800	10798	12947	0.362016	0.06378285	0.52809748	0.09205042
rno-miR-25-3p	14240	12138	10169	13157	0.33356549	-0.0805693	0.48577133	-0.1162998
rno-miR-9a-5p	14074	13247	9321	12691	0.40632614	0.04287146	0.59447577	0.06185992
rno-miR-9a-5p	14073	13247	9327	12693	0.40564103	0.04271396	0.59344488	0.06163258
rno-miR-9a-5p	14073	13247	9326	12693	0.40574384	0.04271396	0.59359957	0.06163258
rno-miR-128-3p	13685	12454	8718	11449	0.44342276	0.08408986	0.65052633	0.12138759
rno-let-7b-5p	13177	11801	8818	11614	0.39636281	0.01597267	0.57949857	0.02304418
rno-miR-101a-3p	12684	12845	9264	11300	0.31164571	0.12797681	0.45330262	0.18488412
rno-miR-128-3p	11661	10405	7174	9610	0.47645341	0.07944042	0.70084186	0.11466863
rno-miR-26a-5p	11420	15231	9186	10206	0.21683005	0.39509376	0.31405396	0.57759312
rno-miR-30e-5p	11408	10494	7150	9050	0.45888566	0.14776914	0.67403074	0.213575
rno-miR-101b-3p	11406	10803	7964	9904	0.35539494	0.08683054	0.51822781	0.12534879
rno-miR-423-3p	10487	9579	6707	9080	0.43968826	0.05348625	0.64486252	0.07718276
rno-miR-328a-3p	10307	8647	5978	7708	0.5316549	0.11482727	0.78588967	0.16584312
rno-miR-152-3p	9073	8472	6525	7967	0.32670855	0.06143926	0.47560176	0.08866601
rno-miR-30c-5p	8805	8162	6352	8054	0.32367883	0.01332018	0.47111209	0.01921724
rno-miR-30c-5p	8798	8157	6343	8047	0.32428505	0.01357689	0.47201026	0.01958762
rno-miR-183-5p	8227	9352	5289	7122	0.43474401	0.27072963	0.63737147	0.39299249
rno-miR-185-5p	8202	8538	5616	7244	0.3742944	0.16398429	0.54643281	0.23711162
rno-miR-351-5p	7985	7802	6029	7269	0.27914942	0.07073187	0.40537368	0.10208709
rno-miR-351-5p	7985	7802	6029	7269	0.27914942	0.07073187	0.40537368	0.10208709
rno-let-7e-5p	7883	8143	5306	7287	0.3907802	0.11095269	0.5711201	0.16023541
rno-miR-344a-3p	7789	7725	5210	7008	0.39679975	0.09733252	0.58015475	0.14053216
rno-miR-344a-3p	7789	7725	5210	7008	0.39679975	0.09733252	0.58015475	0.14053216
rno-miR-210-3p	7358	5324	4035	6322	0.58334065	-0.1713893	0.866745	-0.2478705
rno-miR-140-3p	7212	7467	5875	7019	0.2043249	0.06185282	0.29581055	0.08926323
rno-miR-186-5p	7181	6671	5151	5721	0.32922478	0.15332473	0.47933222	0.2216357
rno-miR-182	6438	7009	4676	5627	0.31707756	0.21874011	0.46133765	0.31684266
rno-miR-98-5p	5494	5241	3447	4863	0.45789062	0.07482185	0.67251561	0.10799552
rno-miR-92a-3p	5412	4267	3119	4505	0.53756887	-0.0542636	0.79507829	-0.078305
rno-miR-139-5p	5169	5758	3089	4014	0.50375394	0.35693819	0.74274533	0.52052717
rno-miR-20a-5p	4908	4511	2809	3679	0.54399378	0.2031746	0.80507862	0.29413361
rno-miR-148b-3p	4454	3941	2800	3541	0.45602426	0.10692328	0.66967473	0.15440491
rno-miR-16-5p	4260	4149	3141	3826	0.30239157	0.08100313	0.43962949	0.11692678
rno-miR-23a-3p	4258	4889	3278	3428	0.26008493	0.3513286	0.3773601	0.5121723
rno-miR-30b-5p	4142	3818	2856	3129	0.36753358	0.198359	0.53633157	0.28711544

rno-miR-151-3p	3832	4035	2569	3128	0.39462584	0.25324585	0.57689067	0.36732816
rno-miR-149-5p	3819	4319	2201	2607	0.53754153	0.49436904	0.79503577	0.72830673
rno-miR-374-5p	3740	4040	2430	3120	0.42463533	0.25698324	0.62208196	0.37280926
rno-miR-181d-5p	3639	3308	2567	3261	0.34547212	0.01430964	0.50345876	0.02064479
rno-miR-130b-5p	3596	2912	1941	2954	0.59779664	-0.0143198	0.8895929	-0.0206595
rno-miR-125b-5p	3479	3939	2607	3433	0.28655932	0.13727618	0.4162821	0.19835956
rno-miR-125b-5p	3439	3849	2543	3345	0.29956536	0.14011676	0.43545765	0.20247746
rno-miR-106b-3p	3394	2808	2171	2837	0.43953279	-0.0102746	0.64462684	-0.0148232
rno-miR-146b-5p	3276	3808	2471	3078	0.28014616	0.2120244	0.40684035	0.30704025
rno-miR-672-5p	3258	2670	2350	3090	0.32382311	-0.1458333	0.47132585	-0.2107671
rno-miR-322-3p	3192	3400	2824	3178	0.12234043	0.06749772	0.17672056	0.09741562
rno-miR-322-3p	3192	3400	2824	3178	0.12234043	0.06749772	0.17672056	0.09741562
rno-miR-598-3p	3153	3411	1976	2705	0.45895886	0.23086985	0.67414222	0.33456616
rno-miR-598-3p	3153	3411	1976	2705	0.45895886	0.23086985	0.67414222	0.33456616
rno-miR-298-5p	3002	3326	1899	2404	0.45011222	0.32181501	0.66068407	0.46835127
rno-miR-26b-5p	2938	3182	2223	2519	0.27707809	0.23259077	0.40232645	0.33708271
rno-miR-107-3p	2879	2586	1812	2148	0.45491366	0.18504436	0.66798483	0.26772828
rno-miR-378b	2707	2326	2065	2439	0.26906957	-0.0474292	0.39055311	-0.0684387
rno-miR-361-3p	2577	2368	1522	2244	0.5147597	0.05377277	0.75972418	0.07759641
rno-miR-450a-5p	2533	2595	2004	2454	0.23319374	0.05585264	0.33796457	0.08059929
rno-miR-872-5p	2444	2390	1688	2145	0.36592449	0.10804851	0.53392938	0.15603297
rno-miR-344b-3p	2358	2050	1480	1921	0.45752996	0.06497104	0.67196654	0.09376639
rno-miR-344b-3p	2358	2050	1480	1921	0.45752996	0.06497104	0.67196654	0.09376639
rno-miR-96-5p	2267	2431	1707	1951	0.28183191	0.21907805	0.40932133	0.31733612
rno-miR-106b-5p	2219	1945	1422	1871	0.43779182	0.03878407	0.6419882	0.0559606
rno-miR-423-5p	2185	2009	1386	1845	0.4474937	0.08510638	0.65670602	0.12285675
rno-miR-340-3p	2072	1957	1444	1818	0.35722412	0.07364238	0.52095326	0.10629156
rno-miR-340-3p	2072	1957	1444	1818	0.35722412	0.07364238	0.52095326	0.10629156
rno-miR-501-3p	2050	1521	1165	1512	0.55054432	0.00593472	0.81529395	0.00856201
rno-miR-484	1964	1840	1020	1522	0.63270777	0.18917311	0.94522578	0.27373741
rno-miR-17-5p	1922	1578	999	1264	0.63197535	0.22097115	0.94405175	0.32010074
rno-let-7d-3p	1856	1544	1146	1417	0.47301799	0.08578183	0.69558967	0.12383299
rno-miR-1839-5p	1843	1643	1147	1272	0.46555184	0.25454545	0.68419068	0.36923381
rno-miR-17-5p	1836	1516	959	1204	0.62754919	0.22941176	0.93696334	0.33243436
rno-miR-29b-3p	1728	1631	1201	1340	0.35984978	0.19589364	0.52486707	0.28352378
rno-miR-29b-3p	1728	1631	1201	1340	0.35984978	0.19589364	0.52486707	0.28352378

rno-miR-29b-3p	1726	1631	1202	1339	0.3579235	0.196633	0.52199557	0.28460082
rno-miR-24-2-5p	1712	2047	1332	1488	0.24967148	0.31626591	0.36208862	0.46013658
rno-miR-99b-3p	1658	1351	1005	1445	0.49042433	-0.0672389	0.72224851	-0.0970418
rno-miR-425-5p	1647	1515	965	1215	0.52220521	0.21978022	0.77123971	0.31836148
rno-miR-450b-5p	1537	1686	1298	1625	0.1686067	0.03684687	0.24382678	0.05316482
rno-miR-450b-5p	1537	1686	1298	1625	0.1686067	0.03684687	0.24382678	0.05316482
rno-let-7a-1-3p	1537	1238	1016	1346	0.40814728	-0.0835913	0.59721676	-0.1206671
rno-let-7c-2-3p	1536	1237	1016	1346	0.40752351	-0.084398	0.59627781	-0.1218329
rno-miR-362-5p	1527	988	719	1064	0.71950134	-0.0740741	1.08663639	-0.1069152
rno-miR-195-5p	1473	1510	1101	1269	0.28904429	0.17344368	0.41994296	0.25085648
rno-miR-92a-3p	1447	837	671	994	0.73276676	-0.171491	1.10868025	-0.2480182
rno-miR-325-5p	1443	1622	1092	1307	0.27692308	0.21509047	0.40209844	0.31151468
rno-miR-192-5p	1417	1452	1104	1410	0.24831416	0.0293501	0.36009959	0.04234629
rno-miR-344b-1-3p	1412	1474	811	1113	0.54071075	0.27908775	0.79996627	0.40528293
rno-miR-143-3p	1390	1347	1290	1570	0.07462687	-0.1528968	0.10771382	-0.2210147
rno-miR-10a-5p	1390	1459	1010	1492	0.31666667	-0.0223653	0.46072959	-0.0322677
rno-miR-296-3p	1388	1783	915	1222	0.41076856	0.3733777	0.60116392	0.54506242
rno-miR-126a-3p	1253	1253	965	1143	0.25969342	0.0918197	0.37678557	0.13256101
rno-miR-140-5p	1214	1123	904	1007	0.29272899	0.10892019	0.42537374	0.15729424
rno-miR-384-5p	1209	1286	838	1051	0.36248168	0.20111254	0.5287921	0.29112797
rno-miR-195-3p	1187	963	822	1156	0.36336486	-0.1821614	0.53010964	-0.2635337
rno-miR-542-3p	1130	1103	791	960	0.35294118	0.13863306	0.51457317	0.20032648
rno-miR-542-3p	1130	1103	791	960	0.35294118	0.13863306	0.51457317	0.20032648
rno-miR-542-3p	1130	1103	791	960	0.35294118	0.13863306	0.51457317	0.20032648
rno-miR-503-5p	1128	1176	1111	1137	0.01518535	0.03372244	0.02190825	0.04865581
rno-miR-503-5p	1128	1176	1111	1137	0.01518535	0.03372244	0.02190825	0.04865581
rno-miR-384-3p	1121	1178	729	899	0.42378378	0.26865672	0.62079556	0.38994652
rno-miR-15b-5p	1094	994	708	874	0.42841287	0.12847966	0.62779147	0.18561257
rno-miR-361-5p	1046	955	669	873	0.43965015	0.08971554	0.64480474	0.12951908
rno-miR-181c-3p	1008	937	712	870	0.34418605	0.07415606	0.50154649	0.10703365
rno-miR-652-3p	965	1230	557	712	0.53613666	0.53347065	0.79285161	0.78870917
rno-miR-342-3p	899	951	460	633	0.64606328	0.40151515	0.96668725	0.58723984
rno-miR-325-3p	892	846	576	686	0.43051771	0.20887728	0.6309749	0.30244909
rno-miR-374-3p	866	1095	576	946	0.40221914	0.14600686	0.58829821	0.21101878
rno-miR-532-3p	862	709	626	763	0.3172043	-0.0733696	0.46152521	-0.1058974
rno-miR-322-5p	825	790	713	757	0.14564369	0.04266322	0.21049204	0.06155935

rno-miR-322-5p	825	790	713	757	0.14564369	0.04266322	0.21049204	0.06155935
rno-miR-203a-3p	803	998	584	697	0.31578947	0.35516224	0.45943162	0.51788116
rno-miR-335	801	851	545	637	0.38038633	0.28763441	0.55554601	0.41786576
rno-miR-339-5p	786	729	633	690	0.21564482	0.05496829	0.31232381	0.07932245
rno-miR-421-3p	785	836	494	563	0.455043	0.39027877	0.66818161	0.57036802
rno-miR-499-5p	745	872	610	738	0.19926199	0.16645963	0.28843118	0.24070732
rno-miR-3559-5p	724	761	493	685	0.37962202	0.10511757	0.55440205	0.15179247
rno-miR-132-3p	684	1135	562	675	0.19582665	0.50828729	0.28342619	0.74973289
rno-miR-674-3p	645	695	430	572	0.4	0.19415943	0.5849625	0.28099783
rno-miR-301a-5p	636	487	386	496	0.48923679	-0.0183113	0.72042592	-0.0264183
rno-miR-378a-5p	614	520	458	547	0.29104478	-0.0506092	0.42289106	-0.0730292
rno-miR-702-3p	612	534	294	430	0.70198675	0.21576763	1.0577155	0.31250308
rno-miR-29c-3p	608	601	453	496	0.29217719	0.19143118	0.42456027	0.27702487
rno-miR-29c-3p	608	601	453	496	0.29217719	0.19143118	0.42456027	0.27702487
rno-miR-760-3p	587	965	335	472	0.54663774	0.6861517	0.80919941	1.03174208
rno-miR-362-3p	574	462	476	524	0.18666667	-0.1257606	0.27008916	-0.181674
rno-miR-181c-5p	560	467	474	512	0.16634429	-0.0919305	0.24053977	-0.1327213
rno-miR-19b-3p	546	439	393	494	0.32587859	-0.1178992	0.47437164	-0.1702901
rno-miR-19b-3p	546	442	393	495	0.32587859	-0.113127	0.47437164	-0.1633822
rno-miR-330-5p	546	509	367	460	0.39211391	0.10113519	0.57312089	0.1460318
rno-miR-296-5p	531	463	285	394	0.60294118	0.16102684	0.89774994	0.23281656
rno-miR-27b-5p	528	624	502	560	0.05048544	0.10810811	0.07285057	0.1561192
rno-miR-15a-5p	523	454	328	426	0.45828437	0.06363636	0.67311513	0.09183887
rno-miR-132-5p	492	713	444	504	0.1025641	0.34346754	0.14809864	0.50047834
rno-miR-1843a-5p	488	483	315	447	0.43088418	0.07741935	0.63152932	0.11174836
rno-miR-30a-3p	484	439	361	483	0.29112426	-0.0954447	0.42300821	-0.1378022
rno-miR-15b-3p	471	358	253	312	0.60220994	0.13731343	0.89658967	0.19841356
rno-miR-130a-3p	467	433	336	368	0.32627646	0.16229713	0.47496132	0.23466126
rno-miR-30c-2-3p	453	388	290	398	0.43876178	-0.0254453	0.64345815	-0.0367118
rno-miR-24-1-5p	440	424	318	368	0.32189974	0.14141414	0.46847676	0.2043585
rno-miR-505-3p	438	346	195	280	0.76777251	0.21086262	1.16745675	0.30534521
rno-miR-152-5p	432	416	281	449	0.42356241	-0.0763006	0.62046118	-0.1101319
rno-miR-365-3p	414	452	207	275	0.66666667	0.4869326	1	0.71689115
rno-let-7i-3p	397	267	239	286	0.49685535	-0.0687161	0.73212839	-0.0991754
rno-miR-10b-3p	371	376	242	317	0.42088091	0.17027417	0.61641214	0.24624982
rno-miR-324-3p	355	283	209	273	0.5177305	0.03597122	0.76431608	0.0519011

rno-miR-32-5p	354	370	244	309	0.36789298	0.17967599	0.53686821	0.25991843
rno-miR-497-5p	353	337	262	266	0.29593496	0.23548922	0.43010137	0.34132235
rno-miR-872-3p	351	288	215	269	0.48056537	0.06822262	0.70713437	0.09846264
rno-miR-330-3p	350	300	248	287	0.34113712	0.04429302	0.4970148	0.06391176
rno-miR-193b-3p	339	428	290	295	0.15580286	0.36791148	0.22523237	0.53689584
rno-miR-151-5p	324	290	207	241	0.44067797	0.18455744	0.64636305	0.26701975
rno-miR-147	315	330	236	278	0.28675136	0.17105263	0.41656497	0.24738114
rno-miR-30d-3p	311	284	237	251	0.27007299	0.12336449	0.39202752	0.17820357
rno-miR-27a-5p	307	380	372	370	0.19145803	0.02666667	-0.277064	0.03847415
rno-miR-129-5p	306	276	214	307	0.35384615	-0.1063465	0.51592086	-0.1535704
rno-miR-129-5p	303	280	212	301	0.35339806	-0.0722892	0.51525353	-0.1043367
rno-miR-181b-5p	302	242	154	204	0.64912281	0.17040359	0.9716182	0.2464379
rno-miR-29c-5p	293	292	229	243	0.24521073	0.18317757	0.35555307	0.26501206
rno-miR-29c-5p	293	292	229	243	0.24521073	0.18317757	0.35555307	0.26501206
rno-miR-3068-3p	293	267	175	230	0.5042735	0.14889336	0.74354574	0.21520588
rno-miR-139-3p	292	289	162	225	0.57268722	0.24902724	0.84997456	0.36114449
rno-miR-23b-5p	290	249	219	312	0.27897839	-0.2245989	0.40512203	-0.3254003
rno-miR-188-5p	279	161	123	206	0.7761194	-0.2452316	1.18160681	-0.3555836
rno-miR-22-5p	273	325	264	244	0.03351955	0.28471002	0.04836302	0.41355857
rno-miR-212-5p	263	420	243	307	0.07905138	0.31086657	0.11410649	0.45215067
rno-miR-674-5p	261	260	169	213	0.42790698	0.1987315	0.62702656	0.28765819
rno-miR-1224	257	298	234	216	0.09368635	0.31906615	0.13525983	0.46428102
rno-miR-488-3p	251	257	200	216	0.22616408	0.17336152	0.32768736	0.25073705
rno-miR-331-3p	251	244	155	213	0.4729064	0.1356674	0.69541915	0.19602772
rno-miR-344b-5p	250	245	157	221	0.45700246	0.10300429	0.67116354	0.14873538
rno-miR-344b-5p	250	245	157	221	0.45700246	0.10300429	0.67116354	0.14873538
rno-miR-344b-5p	250	245	157	221	0.45700246	0.10300429	0.67116354	0.14873538
rno-miR-30e-3p	248	305	201	266	0.20935412	0.13660245	0.30314462	0.197383
rno-miR-190a-5p	246	195	162	145	0.41176471	0.29411765	0.6026645	0.42742122
rno-miR-190a-5p	246	195	162	145	0.41176471	0.29411765	0.6026645	0.42742122
rno-miR-671	245	286	177	253	0.32227488	0.12244898	0.46903239	0.17687776
rno-miR-879-5p	238	201	152	179	0.44102564	0.11578947	0.64689025	0.16723591
rno-miR-1843b-5p	223	197	133	197	0.50561798	0	0.74561746	0
rno-miR-29b-5p	221	196	129	164	0.52571429	0.17777778	0.7766753	0.25715784
rno-miR-29b-5p	221	196	129	164	0.52571429	0.17777778	0.7766753	0.25715784
rno-miR-326-3p	213	261	121	136	0.5508982	0.62972292	0.81584638	0.94044316

rno-miR-3559-3p	212	171	172	185	0.20833333	-0.0786517	0.3016557	-0.1135289
rno-miR-30b-3p	208	205	217	250	0.04235294	-0.1978022	-0.0611115	-0.2863042
rno-miR-301a-3p	204	186	110	151	0.59872611	0.20771513	0.89106563	0.30075407
rno-miR-130b-3p	203	161	108	130	0.61093248	0.21305842	0.91044842	0.30854907
rno-miR-7b	202	225	123	142	0.48615385	0.45231608	0.71569698	0.66403407
rno-miR-93-3p	202	151	118	161	0.525	-0.0641026	0.77556843	-0.0925121
rno-miR-148b-5p	198	199	140	179	0.34319527	0.10582011	0.5000736	0.15280884
rno-miR-9a-3p	188	200	143	171	0.27190332	0.15633423	0.39471751	0.22600367
rno-miR-9a-3p	188	200	143	171	0.27190332	0.15633423	0.39471751	0.22600367
rno-miR-9a-3p	185	191	141	168	0.26993865	0.1281337	0.39183011	0.18511141
rno-miR-324-5p	184	190	139	124	0.27863777	0.42038217	0.40462088	0.6156593
rno-miR-1249	183	206	92	110	0.66181818	0.60759494	0.99213788	0.90514081
rno-miR-298-3p	181	197	111	166	0.47945205	0.1707989	0.70543002	0.24701239
rno-miR-500-3p	175	151	141	140	0.21518987	0.07560137	0.31165976	0.10912172
rno-miR-350	173	213	96	122	0.57249071	0.54328358	0.84966573	0.80397228
rno-miR-350	173	213	96	122	0.57249071	0.54328358	0.84966573	0.80397228
rno-miR-877	171	111	78	103	0.74698795	0.07476636	1.1324503	0.10791534
rno-miR-125b-1-3p	167	202	135	189	0.21192053	0.06649616	0.3068887	0.09596906
rno-miR-194-5p	160	155	154	170	0.03821656	-0.0923077	0.05514155	-0.1332665
rno-miR-3068-5p	160	152	110	150	0.37037037	0.01324503	0.54056838	0.01910882
rno-miR-25-5p	160	103	97	117	0.49027237	-0.1272727	0.72201525	-0.1838642
rno-miR-138-5p	157	138	93	70	0.512	0.65384615	0.75546194	0.97924144
rno-miR-339-3p	153	164	150	128	0.01980198	0.24657534	0.02856915	0.357552
rno-miR-148a-3p	149	150	104	147	0.35573123	0.02020202	0.5187288	0.02914635
rno-miR-18a-5p	148	144	76	79	0.64285714	0.58295964	0.96152585	0.86614425
rno-miR-33-5p	146	213	94	132	0.43333333	0.46956522	0.63523571	0.6903155
rno-miR-3473	143	160	119	150	0.18320611	0.06451613	0.26505357	0.0931094
rno-let-7f-2-3p	142	131	96	130	0.38655462	0.00766284	0.56478462	0.01105519
rno-miR-301b-5p	142	106	64	117	0.75728155	-0.0986547	1.14974712	-0.1424443
rno-miR-338-5p	138	131	106	129	0.26229508	0.01538462	0.380604	0.02219575
rno-let-7b-3p	132	133	95	127	0.32599119	0.04615385	0.47453851	0.06659775
rno-miR-194-5p	126	107	105	116	0.18181818	-0.0807175	0.26303441	-0.116514
rno-miR-138-5p	126	102	77	54	0.48275862	0.61538462	0.71049338	0.91753784
rno-miR-1306-5p	125	124	84	93	0.3923445	0.28571429	0.57346686	0.4150375
rno-miR-503-3p	114	106	116	142	0.0173913	-0.2903226	-0.025091	-0.4218267
rno-miR-503-3p	114	106	116	142	0.0173913	-0.2903226	-0.025091	-0.4218267

rno-miR-6329	106	90	87	90	0.19689119	0	0.28497696	0
rno-let-7e-3p	104	111	71	115	0.37714286	-0.0353982	0.5506926	-0.0510742
rno-miR-19a-3p	104	86	57	80	0.58385093	0.07228916	0.8675497	0.10433666
rno-miR-450b-3p	98	117	96	127	0.02061856	-0.0819672	0.02974734	-0.11832
rno-miR-450b-3p	98	117	96	127	0.02061856	-0.0819672	0.02974734	-0.11832
rno-miR-150-5p	96	104	92	87	0.04255319	0.17801047	0.06140054	0.25749622
rno-miR-1843a-3p	96	105	66	75	0.37037037	0.33333333	0.54056838	0.48542683
rno-miR-218a-2-3p	89	82	57	57	0.43835616	0.35971223	0.64284342	0.52466199
rno-miR-425-3p	84	77	51	66	0.48888889	0.15384615	0.71989208	0.22239242
rno-miR-455-5p	83	121	68	97	0.1986755	0.22018349	0.28757659	0.3189504
rno-miR-126a-5p	83	80	64	61	0.2585034	0.26950355	0.37503943	0.39119076
rno-miR-342-5p	82	75	58	82	0.34285714	-0.089172	0.49957101	-0.1287333
rno-miR-1306-3p	79	61	28	54	0.95327103	0.12173913	1.49642583	0.17584984
rno-miR-3099	78	74	41	65	0.62184874	0.1294964	0.92785021	0.18708555
rno-miR-125a-3p	77	106	93	113	0.18823529	-0.0639269	-0.2723723	-0.0922585
rno-miR-345-3p	75	65	50	57	0.4	0.13114754	0.5849625	0.1894778
rno-miR-331-5p	74	86	79	73	0.06535948	0.16352201	-0.0943274	0.2364402
rno-miR-191a-3p	73	77	39	72	0.60714286	0.06711409	0.90442234	0.09686154
rno-miR-615	71	66	51	68	0.32786885	-0.0298507	0.47732178	-0.0430687
rno-miR-26b-3p	71	56	39	50	0.58181818	0.11320755	0.8643449	0.16349873
rno-miR-137-3p	69	99	54	53	0.24390244	0.60526316	0.35363695	0.90143617
rno-miR-92a-1-5p	68	56	86	74	0.23376623	-0.2769231	-0.3388019	-0.4020984
rno-let-7f-1-3p	68	51	44	62	0.42857143	-0.1946903	0.62803122	-0.281771
rno-miR-210-5p	67	39	38	77	0.55238095	-0.6551724	0.81816168	-0.9813843
rno-miR-219a-1-3p	65	64	52	70	0.22222222	-0.0895522	0.32192809	-0.129283
rno-miR-672-3p	62	58	46	58	0.2962963	0	0.43063435	0
rno-miR-664-3p	51	54	30	38	0.51851852	0.34782609	0.76553475	0.50695999
rno-miR-664-3p	51	54	30	38	0.51851852	0.34782609	0.76553475	0.50695999
rno-miR-293-5p	50	33	36	36	0.3255814	-0.0869565	0.47393119	-0.1255309
rno-miR-212-3p	49	110	52	60	0.05940594	0.58823529	-0.0857299	0.87446912
rno-miR-7a-1-3p	49	71	41	44	0.17777778	0.46956522	0.25715784	0.6903155
rno-miR-17-1-3p	48	51	31	26	0.43037975	0.64935065	0.63076619	0.97198562
rno-miR-200c-3p	48	35	20	21	0.82352941	0.5	1.26303441	0.73696559
rno-miR-34c-5p	46	25	48	38	0.04255319	-0.4126984	-0.0614005	-0.6040713
rno-miR-219a-5p	46	24	29	31	0.45333333	-0.2545455	0.66558096	-0.3692338
rno-miR-153-3p	45	44	39	37	0.14285714	0.17283951	0.20645088	0.24997825

rno-miR-128-1-5p	44	37	35	23	0.2278481	0.46666667	0.3301486	0.68589141
rno-miR-183-3p	43	43	26	37	0.49275362	0.15	0.72582504	0.21681139
rno-miR-676	42	42	27	28	0.43478261	0.4	0.63742992	0.5849625
rno-miR-184	40	81	23	25	0.53968254	1.05660377	0.79836614	1.69599381
rno-miR-1949	39	55	53	63	0.30434783	-0.1355932	-0.4425182	-0.1959202
rno-miR-193a-5p	37	31	30	39	0.20895522	-0.2285714	0.30256277	-0.3312059
rno-miR-127-3p	37	84	24	51	0.42622951	0.48888889	0.62449086	0.71989208
rno-miR-874-3p	36	56	35	39	0.02816901	0.35789474	0.04064198	0.5219527
rno-miR-16-3p	36	30	18	30	0.66666667	0	1	0
rno-miR-196a-5p	35	42	27	33	0.25806452	0.24	0.37439551	0.3479233
rno-miR-1839-3p	35	32	22	19	0.45614035	0.50980392	0.6698514	0.75207249
rno-miR-100-5p	34	48	26	38	0.26666667	0.23255814	0.38702312	0.33703499
rno-miR-29a-5p	33	25	23	16	0.35714286	0.43902439	0.52083216	0.64385619
rno-miR-411-5p	33	59	23	28	0.35714286	0.71264368	0.52083216	1.07528813
rno-miR-341	33	37	5	28	1.47368421	0.27692308	2.72246602	0.40209844
rno-miR-129-1-3p	32	55	21	27	0.41509434	0.68292683	0.60768258	1.02647221
rno-miR-379-5p	32	66	20	26	0.46153846	0.86956522	0.67807191	1.3439544
rno-miR-338-3p	31	23	24	33	0.25454545	-0.3571429	0.36923381	-0.5208322
rno-miR-30c-1-3p	30	24	15	28	0.66666667	-0.1538462	1	-0.2223924
rno-miR-98-3p	29	28	18	26	0.46808511	0.07407407	0.68805599	0.1069152
rno-miR-505-5p	29	20	15	20	0.63636364	0	0.9510904	0
rno-miR-181b-5p	29	20	10	14	0.97435897	0.35294118	1.5360529	0.51457317
rno-miR-18a-3p	28	26	21	22	0.28571429	0.16666667	0.4150375	0.2410081
rno-miR-6319	28	24	15	24	0.60465116	0	0.90046433	0
rno-miR-6319	28	24	15	24	0.60465116	0	0.90046433	0
rno-miR-450a-3p	28	23	13	21	0.73170732	0.09090909	1.1069152	0.13124453
rno-miR-6318	26	5	9	6	0.97142857	-0.1818182	1.53051472	-0.2630344
rno-miR-92b-3p	26	11	5	16	1.35483871	-0.3703704	2.37851162	-0.5405684
rno-miR-345-5p	25	12	22	16	0.12765957	-0.2857143	0.18442457	-0.4150375
rno-miR-541-5p	25	110	20	35	0.22222222	1.03448276	0.32192809	1.6520767
rno-miR-501-5p	25	28	17	36	0.38095238	-0.25	0.55639335	-0.3625701
rno-miR-351-3p	24	30	20	26	0.18181818	0.14285714	0.26303441	0.20645088
rno-miR-351-3p	24	30	20	26	0.18181818	0.14285714	0.26303441	0.20645088
rno-miR-3064-5p	24	16	7	17	1.09677419	-0.0606061	1.77760758	-0.0874628
rno-miR-193b-5p	23	21	21	10	0.09090909	0.70967742	0.13124453	1.07038933
rno-miR-3102	23	24	16	18	0.35897436	0.28571429	0.52356196	0.4150375

rno-miR-664-2-5p	23	20	13	20	0.55555556	0	0.82312224	0
rno-miR-34a-5p	20	13	25	20	0.22222222	-0.4242424	-0.3219281	-0.6214884
rno-miR-145-5p	19	22	17	24	0.11111111	-0.0869565	0.16046467	-0.1255309
rno-miR-6315	18	12	8	13	0.76923077	-0.08	1.169925	-0.1154772
rno-miR-130a-5p	17	31	27	17	0.45454545	0.58333333	-0.6674247	0.86673347
rno-miR-199a-3p	17	25	23	21	0.3	0.17391304	-0.4360991	0.25153877
rno-miR-200a-3p	17	10	20	19	0.16216216	-0.6206897	-0.2344653	-0.9259994
rno-miR-301b-3p	16	20	15	18	0.06451613	0.10526316	0.0931094	0.15200309
rno-miR-129-1-3p	16	19	12	14	0.28571429	0.3030303	0.4150375	0.44057259
rno-miR-129-2-3p	16	19	12	14	0.28571429	0.3030303	0.4150375	0.44057259
rno-miR-146b-3p	16	6	10	9	0.46153846	-0.4	0.67807191	-0.5849625
rno-miR-879-3p	16	23	9	26	0.56	-0.122449	0.830075	-0.1768778
rno-miR-3577	16	12	5	12	1.04761905	0	1.67807191	0
rno-miR-218a-1-3p	15	17	24	26	0.46153846	-0.4186047	-0.6780719	-0.6129769
rno-miR-190b-5p	15	14	10	13	0.4	0.07407407	0.5849625	0.1069152
rno-miR-421-5p	15	13	7	16	0.72727273	-0.2068966	1.09953567	-0.2995603
rno-miR-3074	15	9	6	5	0.85714286	0.57142857	1.32192809	0.84799691
rno-let-7g-3p	15	14	3	6	1.33333333	0.8	2.32192809	1.22239242
rno-miR-702-5p	14	13	19	11	0.3030303	0.16666667	-0.4405726	0.2410081
rno-miR-486	14	10	11	8	0.24	0.22222222	0.3479233	0.32192809
rno-miR-20b-5p	14	10	5	5	0.94736842	0.66666667	1.48542683	1
rno-miR-500-5p	14	14	4	7	1.11111111	0.66666667	1.80735492	1
rno-miR-382-3p	14	42	2	6	1.5	1.5	2.80735492	2.80735492
rno-miR-344i	13	12	8	14	0.47619048	-0.1538462	0.70043972	-0.2223924
rno-miR-145-3p	12	9	8	10	0.4	-0.1052632	0.5849625	-0.1520031
rno-miR-3075	12	14	7	8	0.52631579	0.54545455	0.77760758	0.80735492
rno-miR-370-3p	12	16	6	18	0.66666667	-0.1176471	1	-0.169925
rno-miR-148a-5p	12	13	6	12	0.66666667	0.08	1	0.11547722
rno-miR-434-5p	12	23	4	8	1	0.96774194	1.5849625	1.52356196
rno-miR-434-5p	12	23	4	8	1	0.96774194	1.5849625	1.52356196
rno-miR-1956-5p	11	24	18	17	0.48275862	0.34146341	-0.7104934	0.49749966
rno-miR-3084a-3p	11	7	7	4	0.44444444	0.54545455	0.6520767	0.80735492
rno-miR-3084b-3p	11	7	7	4	0.44444444	0.54545455	0.6520767	0.80735492
rno-miR-3084b-3p	11	7	7	4	0.44444444	0.54545455	0.6520767	0.80735492
rno-miR-3084d	11	7	7	4	0.44444444	0.54545455	0.6520767	0.80735492
rno-miR-433-3p	11	19	7	9	0.44444444	0.71428571	0.6520767	1.07800251

rno-miR-409a-3p	11	34	5	10	0.75	1.09090909	1.13750352	1.76553475
rno-miR-32-3p	11	4	4	10	0.93333333	-0.8571429	1.45943162	-1.3219281
rno-miR-455-3p	11	10	3	7	1.14285714	0.35294118	1.87446912	0.51457317
rno-miR-542-5p	10	23	15	23	0.4	0	-0.5849625	0
rno-miR-542-5p	10	23	15	23	0.4	0	-0.5849625	0
rno-miR-542-5p	10	23	15	23	0.4	0	-0.5849625	0
rno-miR-434-3p	10	12	6	9	0.5	0.28571429	0.73696559	0.4150375
rno-miR-434-3p	10	12	6	9	0.5	0.28571429	0.73696559	0.4150375
rno-miR-99a-5p	9	7	12	10	0.28571429	-0.3529412	-0.4150375	-0.5145732
rno-miR-200b-3p	9	4	9	13	0	-1.0588235	0	-1.7004397
rno-miR-221-3p	9	63	7	12	0.25	1.36	0.36257008	2.39231742
rno-miR-1956-3p	9	10	4	11	0.76923077	-0.0952381	1.169925	-0.1375035
rno-miR-196c-5p	9	11	2	2	1.27272727	1.38461538	2.169925	2.45943162
rno-miR-741-3p	8	5	11	10	0.31578947	-0.6666667	-0.4594316	-1
rno-miR-190a-3p	8	6	11	6	0.31578947	0	-0.4594316	0
rno-miR-190a-3p	8	6	11	6	0.31578947	0	-0.4594316	0
rno-miR-494-3p	7	14	10	5	0.35294118	0.94736842	-0.5145732	1.48542683
rno-miR-328b-3p	7	14	7	14	0	0	0	0
rno-miR-300-3p	7	37	5	9	0.33333333	1.2173913	0.48542683	2.03952836
rno-miR-323-3p	7	18	3	8	0.8	0.76923077	1.22239242	1.169925
rno-miR-5132-5p	6	9	15	7	0.85714286	0.25	-1.3219281	0.36257008
rno-miR-382-5p	6	34	9	9	0.4	1.1627907	-0.5849625	1.91753784
rno-miR-487b-3p	6	19	5	4	0.18181818	1.30434783	0.26303441	2.24792751
rno-miR-134-5p	6	29	3	8	0.66666667	1.13513514	1	1.857981
rno-miR-409a-5p	6	17	2	10	1	0.51851852	1.5849625	0.76553475
rno-miR-543-3p	6	11	0	7	2	0.44444444	0	0.6520767
rno-miR-146a-5p	5	9	11	14	0.75	-0.4347826	-1.1375035	-0.6374299
rno-miR-369-5p	5	23	6	12	0.18181818	0.62857143	-0.2630344	0.93859946
rno-miR-336-5p	4	9	13	9	1.05882353	0	-1.7004397	0
rno-miR-211-5p	4	10	10	12	0.85714286	-0.1818182	-1.3219281	-0.2630344
rno-miR-122-5p	3	2	25	13	1.57142857	-1.4666667	-3.0588937	-2.7004397
rno-miR-222-3p	3	39	4	4	0.28571429	1.62790698	-0.4150375	3.28540222
rno-miR-411-3p	1	12	1	6	0	0.66666667	0	1