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Micro RNA 181c-5p: a promising target for post-stroke recovery in socially isolated Mice

Maria Antony^{1,2}, Victoria Scranton¹, Pranay Srivastava¹, Rajkumar Verma, PhD^{1,*}

¹Department of Neuroscience, UConn Health, Farmington, CT 06032, USA

²Department of Molecular and Cell Biology, University of Connecticut, Storrs, CT 06268, USA

Abstract

Dysregulation of microRNAs (miRNAs) has been tied to several neurological disorders, including ischemic stroke. It has also been established that social environments can modulate miRNA profiles. We have previously shown that post-stroke social isolation (SI) is linked to poor stroke outcomes and that miR-181c-5p emerged as one of few lead miRNAs that was downregulated in both stroke and SI. Therefore, in this study we examined the potential role of miR-181c-5p mimic in reversing the detrimental effects of post-stroke SI. Two to three-month-old C57BL/6 male mice were pair-housed (PH) for at least two weeks. After two weeks, mice underwent stroke survival surgery using middle cerebral artery occlusion (MCAO) and were randomly assigned to one of two housing conditions: stroke isolation (ST-ISO) or stroke pair-housing with a healthy partner (ST-PH). ST-ISO mice were randomized to receive either miR-181c-5p mimic or a scrambled RNA (7 mg/kg i.v./day x drug) control at 24 h and 48 h after stroke. The effects of miR-181c-5p mimic treatment were evaluated at 1, 3, and 7 days after stroke at histological, behavioral, and biochemical levels. Target genes of miR-181c-5p were then analyzed by qPCR using an RT² Profiler qPCR Array of pre-coated miR-181c gene targets. Temporal profile expression data suggested that miR-181c-5p was significantly downregulated ($p < 0.05$ vs ST-PH) up to 7 days after post-stroke SI. MiR-181c-5p mimic treatment significantly increased miR-181c-5p expression in brain tissue and showed partial swift recovery in sensorimotor deficit. Target gene analysis identified downregulation of several calcium signaling-related genes, e.g., *Cpne2* and *Gria 1 & 2* after miR-181c-5p mimic treatment. In summary, present data suggests that miR-181c-5p is a potential target for post-stroke SI. Data also suggests that genes related to calcium and glutamate signaling might be involved in the beneficial effect of the miR-181c-5p mimic.

Keywords

miRNA analysis; Post-stroke social isolation; miR-181c-5p; inflammation; target validation

*Correspondence: Rajkumar Verma, Department of Neurosciences, UConn Health, Farmington, CT 06032, USA. raverma@uchc.edu, Phone: +1 8606794552.

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Conflict of interest:

The authors declare no financial interests.

1. INTRODUCTION

Examining major contributing and modifiable lifestyle factors could provide greater insight into coordinated cerebrovascular disease management. Social isolation (SI) or loneliness is a well-established risk factor for delayed or poor recovery after stroke that has been reported by multiple clinical studies (Haun et al., 2008; Perissinotto et al., 2012). In addition to behavioral changes resulting from isolation following stroke, survivors also experience neuropsychiatric complications and functional decline (Craft et al., 2005; Perissinotto et al., 2012). Isolation may also contribute to further limited social integration and prolonged disability (Craft et al., 2005). Poor functional social support is associated with higher incidences of post-stroke depression, impaired physical recovery, and low quality of life after stroke (Northcott et al., 2016). These findings suggest that there is a unique relationship between biological response and social behavior that could underlie the clinical manifestations associated with poor outcomes after post-stroke SI. We and others have previously characterized molecular events following post-stroke SI, which may provide some insight into the effect of post-stroke SI at RNA as well as protein interaction levels (O'Keefe et al., 2014; Venna et al., 2014; Verma et al., 2016).

MicroRNAs (miRNAs) are small non-coding RNAs that serve a critical regulatory role in the body's stress response to several types of injuries including ischemic stroke. Because of their short seed region of 6–8 nucleotides, a single miRNA lead can have multiple downstream gene targets, thereby regulating multiple pathways implicated in disease and health (Kehl et al., 2017). miRNA functionality is evolutionarily conserved in nature, suggesting that animal studies could be closely tied to the etiological factors implicated in ischemic stroke injury and recovery in the human disease (Bentwich et al., 2005). miRNA profiles conducted on blood samples of stroke survivors have also shown dysregulation of several miRNAs that were closely and uniquely linked to cerebral ischemia (Tan et al., 2009). miRNA dysregulation according to transcriptome studies temporally persists in both early and late phases of injury onset (Ouyang et al., 2013).

Modulation of in vivo candidate miRNAs using microRNA mimics or inhibitors can serve as a powerful tool for discovering key regulatory pathways of post-stroke SI. We have previously shown that several miRNAs exacerbate ischemic injury after SI (Verma et al., 2018), and among those miRNAs, miR-181c-5p has been previously linked to psychiatric conditions (Ghahramani et al., 2011). miR-181c-5p negatively regulates the inflammatory response and apoptosis in oxygen-glucose-deprived microglial culture (Zhang et al., 2012, 2015). Interestingly, miR-181c-5p and other members of miR-181 family miRNAs have also been associated with signs of social withdrawal in autism (Hicks and Middleton, 2016). The miR-181 family miRNAs also affect a number of ion channel coding genes that are activated during the early events of ischemia, particularly those involved in mitochondrial homeostasis (Indrieri et al., 2019). Based on these observations and findings, we hypothesize that miR-181c-5p is a potential candidate miRNA that can influence post-stroke SI. This work aims to validate and generate proof of concept data on miRNA modulation using a miR-181c-5p mimic in an in vivo model of post-stroke SI.

2. MATERIALS AND METHODS

1.1 Experimental animals and design:

A total of 52 C57BL/6 mice (2–3-month-old) were purchased from The Jackson Laboratory (Bar Harbor, ME). Animals were maintained at ambient temperature and humidity. Mice had access to food and water ad libitum. All animal care protocols were performed in accordance with the Institutional Animal Care and Use Committee at UConn Health. All mice were pair-housed (PH) for at least two weeks prior to middle cerebral artery occlusion (MCAO) surgery or sham surgery. Mice were examined for signs of aggression or incompatibility between pairs, including scratches, bites, and scars. They were also weighed on a daily basis to ensure compatibility and normal eating behavior. No mice were excluded due to incompatibility prior to surgery. Mice were randomly assigned to one of two housing conditions: isolation (single-housed denoted: ST-ISO) or continued paired-housing (PH) immediately after stroke or sham surgery (Figure 2). In the paired house group, two mice were PH, in which one underwent MCAO (denoted ST-PH) and the other underwent sham surgery without ischemia (denoted SH-PH). The post-surgery assigned housing conditions were maintained until animals were euthanized. There were a total of 27 ST-ISO mice, 8 ST-PH mice, and 8 SH-PH surviving mice used for these experiments.

1.2 Middle cerebral artery occlusion (MCAO) surgery:

Ischemic stroke was done by temporarily blocking the origin of middle cerebral artery as described in previous studies (Verma et al., 2018). To induce a focal transient cerebral ischemia, a ventral, midline incision was made under isoflurane anesthesia. Thereafter, the right external carotid artery was incised before inserting a 6.0 mm silicone-coated nylon filament (Docol Corporation, Sharon, MA) from the internal carotid artery bifurcation via the external carotid artery stump. The sham-operated mice underwent an identical surgical procedure without inserting monofilament into the internal carotid artery. Throughout the procedure, rectal temperatures were maintained at $\approx 37^{\circ}\text{C}$ with the help of heating pads. A laser-Doppler probe (DRT 4, Moor Instruments, Devon, United Kingdom) was placed on the skull to monitor cerebral blood flow after MCAO to determine if occlusion was successful, defined by a 15% reduction of baseline cerebral blood flow. After one hour of occlusion, mice were reperused for either 3 or 7 days. Both groups were fed with wet mash following stroke to encourage hydration and ample nutrition after MCAO. In the event of gross weight loss, a subcutaneous injection of normal saline was injected to ensure hydration.

1.3 MiRNA-181c-5p mimic treatment:

Commercially available miR-181c-5p mimics were procured and prepared according manufacturer's protocol of "In Vivo Ready miRNA mimic with InvivoFectamine 2.0 Reagent" for in vivo delivery (Ambion, Life Technologies; Camarillo, CA). In the treatment study group, ST-ISO mice were randomly assigned to receive either exogenous miR-181c-5p mimic or a scrambled miRNA (control) (7 mg/kg/day) both administered through lateral tail vein at 24 and 48 hours after stroke. The successful delivery and efficacy of the treatment were determined by measuring miRNA-181c-5p expression in brain tissue using qPCR.

1.4 Total RNA isolation and cDNA synthesis:

Total RNA was extracted from perilesional ipsilateral cerebral cortex of stroked mice using the mirVana miRNA Isolation Kit (Life technology Waltham, MA). For miRNA expression analyses, 50–250 ng of cDNA was made with corresponding miRNA primer (Life Technologies; Camarillo, CA) and the TaqMan Reverse miRNA Transcription Kit (Applied Biosystems; Thermofisher; Foster City, CA). For gene expression analyses, 5 µg of total RNA for 96-well plate formats was isolated using TRIzol reagent (Life Technology, Waltham, MA). Both RNA extraction protocols were conducted according to the manufacturer's protocols (Life Technology Waltham, MA). RNA was stored at –80°C before cDNA synthesis.

1.5 Real-Time qPCR for miRNA analysis:

All of the following real-time qPCR protocols were conducted using the CFX Connect™ Real-Time PCR Detection System (Bio-Rad, Hercules, CA). Using cDNA samples of given miRNAs, qPCR reactions were prepared according to manufacturer's protocols using TaqMan universal PCR master mix (Applied Biosystems; ThermoFisher; Foster City, CA). miRNA 181c-5p, miRNA 200c-3p, miRNA-429, and miR-124–5p primers were used for amplification (Ambion; Life Technologies; Camarillo, CA). Sno135 was used as the housekeeping gene for normalization (Ambion; Life Technologies; Camarillo, CA).

1.6 miR-181c-5p gene targets analysis using RT2 Profile PCR Array:

Mice sacrificed at 7 days after stroke were used for gene expression analysis by qPCR. Total RNA isolated as above was used to analyze target gene expression. An equal amount of RNA was converted into cDNA using SA Biosciences's RT² First Strand Kit (Cat # 330401) as per manufacturer's protocol. Gene expression analysis was done a 96-well format RT2 Profile PCR Array mmu-mir-181 target gene array (SABiosciences, Cat # PAMM-6011ZD-12) with a Bio-Rad CFX 96 qRT-PCR instrument. This array analyzed 84 target mRNAs of the miRNA-181 family. Out of these, 54 mRNA were the direct targets of miR-181c-5p. The relative abundance of each mRNA species was assessed following the manufacturer's recommendations. The data were analyzed using data analysis software provided by the manufacturer. In the expression studies, a gene was considered differentially regulated if the difference was 1.5 fold with $P < 0.05$ compared to control. A total of 9 animals were utilized for this experiment ($n = 4-5/\text{group}$).

1.7 Immunohistochemistry:

Mice were sacrificed with overdose of avertin (250 mg/kg) and perfused transcardially with phosphate-buffered saline (PBS) and followed by 4% paraformaldehyde in cold PBS. Brains were then post-fixed in 4% paraformaldehyde and dehydrated in 30% sucrose solution prior to sectioning. They were sliced into 30 µm sections on a freezing microtome before mounting and staining. Immunohistochemistry was used to visualize differences in the quantity and morphology of astrocytes and microglia between miR-181c-5p mimic treatment and control groups. The primary antibodies and their dilutions used in this study were as follows: Glial fibrillary acidic protein (GFAP; 1:1000; Abcam; Cambridge, MA) and Ionized calcium binding adaptor molecule 1 (IBA-1; 1:1000; Abcam; Cambridge, MA). Two

coronal brain sections per mouse ($n = 3$ per group), taken 0.45 and 0.98 mm from bregma, were stained and visualized for quantification at 10x magnification at the junction core and penumbra region. A blinded observer quantified IBA-1 and 4',6-diamidino-2-phenylindole (DAPI) or GFAP and DAPI-positive cells using Image J software (NIH). DAPI staining was used to determine the number of nuclei and to assess gross cell morphology. The average numbers of cells visualized from 3 separate regions at the junction core and penumbra were recorded for each mouse. IBA1-positive and GFAPpositive cells were quantified (separately) in the 10x field at the penumbra for each mouse ($n=3$ /group).

1.8 Behavioral Analysis:

Stroke outcome was assessed by multiple behavior tests including open field test (OFT) and rotarod to measure spontaneous locomotor activity/anxiety-like behavior and motor balance coordination, respectively. The OFT is a common measure of exploratory behavior and general activity in rodents, which can be used both qualitatively and quantitatively. Briefly, we placed mice in a corner of a clear acrylic box ($16'' \times 16''$) and allowed them to explore the box for ten minutes. We quantified locomotor activity as the total number of beam breaks by a computer-operated, open-field photo beam activity system (San Diego Instruments, San Diego, CA). We calculated the percentage of beam breaks in the center zone ($16/3'' \times 16/3''$) compared to the total as a measure of anxiety-like behavior. Importantly, OFT can be administered at several time points to view trends without hindrance by habituation (Moy et al., 2012). Anxiety-like behavior was characterized by diminished exploration and a preference towards the outer walls of the field.

The rotarod test examines motor coordination in mice. We placed mice on a rotating cylindrical rod accelerating from 4–40 rotations per minute, over a span of five minutes. Each subject performed two trials with a 20 min break between the two trials. We recorded the latency to fall from the rotating rod for each trial (in seconds), and used the mean latency for comparison between groups (Truong et al., 2012). Mice were assessed at several time points after stroke onset (Figure 2). Mean latency to fall returned to baseline levels after seven days of stroke onset, presumably as a result of rapid stroke recovery in young mice.

1.9 Ingenuity Pathway Analysis (IPA):

A commercial software package (IPA; from QIAGEN, Redwood City, CA) was used to categorize the mRNA hits based on the signaling network, biological function, and disease involvement. All the significantly downregulated mRNAs were uploaded to IPA and core analysis was performed. The software identifies the biological functions most relevant to the dataset and calculates p values using the right-tailed Fisher Exact Test. $P < 0.05$ indicate statistically significant, non-random associations (www.ingenuity.com).

1.10 Statistical Analysis:

We present data from individual experiments as mean $\pm$ SD. We statistically evaluated the data by the Student *t* test (for comparison between 2 experimental groups) or by 1-way ANOVA with a Bonferroni post hoc test to correct for multiple comparisons (GraphPad Prism Software Inc, San Diego, CA). A probability value of $P < 0.05$ was considered

statistically significant. An investigator blinded to the experimental groups performed the data analyses.

3. Results:

3.1 Endogenous miRNA expression is affected by post-stroke social isolation (SI) in both young and aged mice.

Post-stroke SI has previously been shown to upregulate several miRNAs like miR-181c-5p, miR-200c-3p, miR-429, and miR-124-5p in aged mice (Verma, 2018). In this study, we examined the expression of these miRNAs in young mice to confirm their expression levels at this age. We determined their temporal expression profiles using qPCR at 3 and 7 days post-stroke SI in young mice (Figure 3A and B). Among all miRNAs, three miRNAs (miR-200c-3p, miR-429, and miR-124-5p) showed an upward trend while miR-181c-5p showed a downward trend as compared to ST-PH. We observed significant upregulation (* $p < 0.05$ vs ST-PH) of miR-124-5p at 3 days post-stroke (Figure 3A), while miR-181c-5p expression was reduced significantly (* $p < 0.05$ vs ST-PH) at 7 days post-stroke (Figure 3B).

3.2 Validation of in vivo efficacy of exogenous miR-181c-5p mimic treatment.

In order to confirm that the miR-181c-5p mimic crosses the blood-brain-barrier and elicits its effect, we subjected total RNA isolated from the ipsilateral cortex of various treatment groups for qPCR analysis. miR-181c-5p mimic-treated mice showed increased miR-181c-5p expression (* $p < 0.05$ vs control) at 7 days after stroke, confirming its efficacy in the brain after intravenous injection (Figure 3C).

3.3 miR-181c-5p mimic treatment reduces astrocyte/microglial reactivity.

We next examined the effects of miR-181c-5p mimic treatment on gliosis, a nonspecific reactive change of glial cells in response to post-stroke SI. Increased/reduced astrocytic and microglial reactivity during the acute phase of stroke could serve as another indicator of CNS damage or recovery, respectively (O'Keefe et al., 2014). Qualitative analysis suggested diminished astrocyte and microglial swelling in the miR-181c-5p mimic-treated group after post-stroke SI (Figure 4). Further, quantitative analysis suggested significantly reduced number of GFAP+ astrocytes in the miR-181c-5p mimic-treated group ($p > 0.05$ vs control). These data suggest that miR-181c-5p might influence stroke outcomes through its effect on glial cell activity in ipsilateral brain tissue.

3.4 miR-181c-5p mimic reduces anxiety-like behavior and promote swift recovery in motor coordination task in post-stroke SI.

We found no change in total locomotion, measured by total beam breaks in the OFT, between any groups at any time point (Figure 5A). Anxiety-like behavior is characterized by diminished exploration and a preference towards the outer walls of the field in the OFT apparatus. miR-181c-5p mimic-treated mice showed significantly reduced anxiety-like behavior at day 7 as compared to both control or ST-PH groups (Figure 5B). Similarly, miR-181c-5p mimic-treated mice also displayed swift recovery on sensori-motor deficit (* $P < 0.05$ vs control) at Day 3 (Figure 5C) however, changes were not significantly different on Day 7 after stroke probably due to spontaneous recovery in control treated mice. We

observed an approximately 15% reduction in mortality at 7 days post-stroke after miR-181c-5p mimic treatment (Figure 5D).

3.5 mRNA target analysis of miR-181c-5p mimic treatment:

To provide further insight into the transcription-level changes resulting from miR-181c-5p mimic treatment, we used an RT² profiler qPCR Array of pre-coated mRNA target of the miR-181 family. Out of 54 direct target mRNAs of miR-181c-5p, we found several significantly downregulated mRNAs after treatment with miR-181c-5p mimic in post-stroke SI mice (Table 1). Importantly mRNAs such as *Cpne2* and *Gria 1* and *2* belong to calcium signaling pathways, which play central roles in the ischemic cascade (Kristián and Siesjö, 1998). Pathway analysis by IPA (Ingenuity Pathway Analysis) suggest that many of the mRNAs belong to glutamatergic, neuropathic pain, CD27 signaling, inflammatory, and synaptogenesis signaling pathways (Figure 6).

4. Discussion:

SI is a well-established risk factor for increased mortality and morbidity after stroke. We have previously shown that post-stroke SI increases ischemic injury in both young and aged mice populations (Verma et al., 2014, 2016). SI mediates its detrimental effects by several mechanisms; therefore, in this study we sought to examine the contribution of miRNAs in post-stroke SI and identify their downstream mRNA targets to delineate its mechanism of action. Several miRNAs including miR-181c-5p, miR-200c3p, miR-141-3p, and miR124-5p were modulated by post-stroke SI in aged mice (Verma et al., 2018). Among several dysregulated miRNAs in post-stroke SI, miRNA-181c-5p was found to be progressively downregulated up to seven days post-stroke in young mice, which is consistent with previously reported downregulation of miR-181c-5p in aged mice after post-stroke SI (Verma et al., 2018). These data suggest that miR-181c-5p is a valid target for therapeutic exploitation across the lifespan. Using pharmacological tools, we showed miR-181c-5p restoration can reduce post-stroke SI-mediated exacerbation of ischemic injury as supported by swift recovery in a sensorimotor coordination task and moderate improvement in anxiety-like behavior in miR-181c-5p mimic-treated mice.

These beneficial effects might occur through several downstream target mRNAs of miR-181c-5p. Of these targets, mRNA *Cpne2* and *Gria 1* and *2* belong to calcium signaling pathways and regulate Ca⁺⁺ influx in the cells (Cross et al., 2010; Nakayama et al., 1999). Excessive influx of Ca⁺⁺ plays detrimental roles during ischemic injury via upregulating or activating several calcium-dependent enzymes which degrade cellular content after overactivation (Kristián and Siesjö, 1998). Excessive calcium influx occurs via NMDA receptors, a subclass of glutamate receptors present during stroke (Pivovarov and Andrews, 2010). *Cpne2* also affects tumor necrosis factor-alpha (*Tnf-α*) signaling in a calcium-dependent manner (Tomsig et al., 2004). *Tnf-α* is a well-established inflammatory cytokine (Liu et al., 2005) and has also been implicated in blood-brain-barrier permeability and integrity after stroke (Pan and Kastin, 2007). It is possible that miR-181c-5p might have brought its beneficial effects by diminishing *Cpne2*-mediated *Tnf-α* response after stroke. Our *in vivo* data further validates previous *in vitro* findings of oxygen glucose deprivation

model of ischemic injury where increased miR-181c-5p expression protected neuronal cell death by regulating *Tnf-α* or *Tlr4* expression-mediated NF-κB activation in activated microglia. (Zhang et al., 2012, 2015).

Despite these translationally relevant findings of our data, we acknowledge some limitations such as small sample size and absence of infarct data after miR-181c-5p mimic treatment. However, the latter outcome was not considered to be important as the primary focus of this study was to see if delayed treatment with miR-181c-5p mimic can reduce SI-mediated damage during stroke recovery. Indeed, our data show despite delayed treatment (24 hours after stroke), miR-181c-5p mimic can mitigate some of the SI-mediated detrimental effects on recovery after stroke.

In summary, whole miRNAome analysis of total RNA revealed miR-181-5p as one of the most differentially expressed miRNAs in the brain tissue of post-stroke SI mice. Temporal profile expression data suggests that, miR-181c-5p was significantly down-regulated ($p < 0.05$ vs ST-PH) up to 7 days after post-stroke SI. Treatment with miR-181c-5p mimic successfully reinstated miR-181c-5p miRNA expression and partially restored motor deficit and reduced anxiety-like behavior in post-stroke SI mice. miR-181c-5p mimic-treated mice also showed reduced number of GFAP+ astrocytes and microglia swelling. Overall, our data suggest that miR-181c-5p is a potential target for therapeutic exploitation in post-stroke SI.

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Highlights

- Post-stroke social isolation downregulates miR-181c-5p expression in mouse brain.
- Treatment with miR-181c-5p mimic restore miR-181c-5p levels and partially improved acute sensori-motor deficit in post stroke social isolation.
- miR-181c-5p works by modulating mRNA involved in inflammation and calcium signaling response.

Figure 1:

Schematic of the mechanism of action of miR-181c-5p mimic in ischemic stroke. miRNAs respond to physiological stress by modulating downstream gene targets. During post-stroke social isolation, miR-181c-5p, which may suppress stress-induced inflammatory responses, is downregulated after stroke. Supplementation of miR-181c-5p mimic post-stroke restores miR-181c-5p expression in post-stroke SI. This increases RNA-induced silencing complex (RISCs) interactions with gene targets resulting in suppression of inflammatory gene expression (e.g. CPNE2, Gria1&2) after post-stroke SI.

Figure 2:
Schematic of experimental design, including stroke pair-housing (ST-PH) conditioning, stroke isolation (ST-ISO), survival surgery, behavioral assessment, and treatment dosage/schedule.

Figure 3:

Temporal profile of endogenous miR-124-5p, miR-429, miR-200-3p, and miR-181c-5p expression in mice subjected to post-stroke SI (ST-ISO), as compared to the respective miRNA expression of pair-housed controls (ST-PH) (A) at 3 days after stroke, we observed significantly (* $p < 0.05$ vs ST-PH; $n = 4$ per group/time point) increased expression of miR-124-5p and a downward trend in miR-181c-5p expression. (B) At 7 days after stroke, miR-181c-5p expression was reduced significantly (* $p < 0.05$ vs ST-PH, $n = 4$ per group/time point). (C) Exogenous miR-181c-5p mimic treatment significantly restored (# $p < 0.05$ vs control, $n = 5$ per group/time point) the isolation-mediated reduction (* $p < 0.05$ vs ST-PH, $n = 4$ per group/time point) of miR-181c-5p expression in brain tissue at day 7 after stroke. Data in all the panel are expressed as mean $\pm$ SD and values on the Y axis are presented as fold change in gene expression of ST-ISO mice against their respective ST-PH control, whose value were kept constant at 1 in determining fold change in ST-ISO.

Figure 4:

Glial cell activation in control (Control) and miR-181c-5p mimic (mimic)-treated post-stroke SI mice. **(A)** Immunostaining with IBA1 (green) and DAPI (blue) in upper panel shows stroke-induced microglial activation (loss of processes and swollen and round soma) was reduced by miR-181c-5p mimic treatment as determined by normal microglial soma, shape, and processes (micrographs, left panels). Quantitative analysis of IBA1+ cells (graph, right panel). **(B)** Immunostaining with GFAP (green) and DAPI (blue) show that GFAP + cells were less prominent in the ipsilateral cortex of the miR-181c-5p mimic-treated group (micrographs, left panels). Quantitative analysis shows significantly reduced number of GFAP+ cells in the miR-181c-5p mimic-treated group (graph, right panel). Data are expressed as mean $\pm$ SD, $n=3$ /group; * $p < 0.05$ compared to Control.

Figure 5:

Effect of miR-181c-5p mimic treatment on functional recovery in post-stroke SI mice. (A) No change was found in total exploratory activity between any groups measured up to day 7 post-stroke SI. (B) miR-181c-5p mimic-treated mice displayed less anxiety-like behavior at day 7 in OFT, (C) showed swift recovery from sensorimotor deficit at 3 days after stroke in rotarod test, and (D) had approximately 15% reduced mortality in post-stroke SI mice compared with control mice. Data are expressed as mean $\pm$ SD, $n=4-5$ per group/time point; * $p < 0.05$ compared to Control.

Figure 6.

Top 5 canonical pathways derived from ingenuity pathway analysis (IPA) gene ontology algorithms for the 9 mRNAs from Table 1. These pathways emerged following IPA “Core Analysis.” Graph shows category scores; “threshold” indicates the minimum significance level [scored as $-\log(p \text{ value})$ from Fisher’s exact test, set here to 1.25]. “Ratio” (differential yellow line and markers) refers to the number of molecules from the dataset that map to the pathway listed divided by the total number of molecules that map to the canonical pathway from within the IPA knowledgebase.

Table 1.

List of mRNAs that were significantly downregulated by miR-181c-5p mimic.

Name of mRNA	Fold change	P-value
BDNF	−3.37	0.0368
CPNE	−2.05	0.00435
FKB1A	−1.88	0.018
Fos	−4.63	0.0272
GIs	−2.09	0.0219
Gria1	−1.75	0.0223
Gria2	−1.68	0.0486
Klhl2	−2.58	0.011788
Map1b	−1.83	0.009131
Map3k10	−1.99	0.030689