Archival Report

Biological Psychiatry

DCC Confers Susceptibility to Depression-like Behaviors in Humans and Mice and Is Regulated by miR-218

Angélica Torres-Berrío, Juan Pablo Lopez, Rosemary C. Bagot, Dominique Nouel, Gregory Dal Bo, Santiago Cuesta, Lei Zhu, Colleen Manitt, Conrad Eng, Helen M. Cooper, Kai-Florian Storch, Gustavo Turecki, Eric J. Nestler, and Cecilia Flores

ABSTRACT

BACKGROUD: Variations in the expression of the Netrin-1 guidance cue receptor *DCC* (deleted in colorectal cancer) appear to confer resilience or susceptibility to psychopathologies involving prefrontal cortex (PFC) dysfunction.

METHODS: With the use of postmortem brain tissue, mouse models of defeat stress, and in vitro analysis, we assessed microRNA (miRNA) regulation of *DCC* and whether changes in *DCC* levels in the PFC lead to vulnerability to depression-like behaviors.

RESULTS: We identified miR-218 as a posttranscriptional repressor of *DCC* and detected coexpression of DCC and miR-218 in pyramidal neurons of human and mouse PFC. We found that exaggerated expression of *DCC* and reduced levels of miR-218 in the PFC are consistent traits of mice susceptible to chronic stress and of major depressive disorder in humans. Remarkably, upregulation of *Dcc* in mouse PFC pyramidal neurons causes vulnerability to stress-induced social avoidance and anhedonia.

CONCLUSIONS: These data are the first demonstration of microRNA regulation of *DCC* and suggest that, by regulating *DCC*, miR-218 may be a switch of susceptibility versus resilience to stress-related disorders.

Keywords: Chronic social defeat stress, Guidance cue, Major depressive disorder, microRNA, Neurodevelopment, Resilience

http://dx.doi.org/10.1016/j.biopsych.2016.08.017

The netrin-1 guidance cue receptor gene, deleted in colorectal cancer (*DCC*), directs growing axons toward appropriate targets and organizes fine neuronal connectivity across the life span by controlling target recognition, axon arborization, and synapse formation (1–3). Variations in *DCC* expression appear to bring about resilience or susceptibility to psychiatric disorders involving prefrontal cortex (PFC) dysfunction (4,5). *Dcc* haploinsufficiency in mice protects against the development of adult phenotypes that resemble traits observed in PFC-related psychopathologies, including deficits in cognitive flexibility and behavioral inhibition (4–6). In contrast, DCC expression in the PFC is increased in putative rodent models of these disorders (5,7). Most notably, *DCC* messenger RNA (mRNA) expression is upregulated (~50%) in the PFC of nonmedicated depressed individuals who died by suicide in comparison with psychiatrically healthy sudden death control subjects (4). *DCC* and its molecular regulators may be targets of protective or risk factors and serve as biomarkers of vulnerability. Currently, however, there is a lack of information about the mechanisms controlling *DCC* gene expression in the brain and about how environmental challenges modulate these processes.

MicroRNAs (miRNAs) are noncoding RNAs (~22 nucleotides) that regulate gene expression at a posttranscriptional level. By binding to the 3′ untranslated region (UTR) of a target mRNA, miRNAs induce RNA transcript degradation or prevent mRNA translation (8). Altered expression of miRNAs in the brain has been associated with major psychiatric conditions, including major depressive disorder (MDD) (9–11). Indeed, the control that miRNAs exert on gene expression is emerging as an important molecular link between environmental risk factors and psychopathology (11–14). Here, we combined postmortem human brain tissue analysis and mouse models of stress-induced psychopathologies to investigate whether 1) *DCC* expression is under miRNA regulation and 2) variations in *DCC* expression in the PFC confer vulnerability to depression-like behaviors.

METHODS AND MATERIALS

Detailed description of procedures is provided in Supplemental Methods and Materials.

Animals

Experimental procedures were performed in accordance with the guidelines of the Canadian Council of Animal Care and approved by the McGill University and Douglas Hospital Animal Care Committee.

 ISSN: 0006-3223

Adult male C57BL/6 wild-type mice (postnatal day 75 ± 15) and male CD-1 retired breeder mice (<4 months) were obtained from Charles River Laboratories (Saint-Constant, QC, Canada). $Dcc^{lox/lox}$ mice (postnatal day 75 ± 15) were obtained from Dr. Berns (University of Amsterdam) (15) and are bred at the Neurophenotyping Center of the Douglas Mental Health University Institute. See Supplemental Methods and Materials for further details.

Chronic Social Defeat Stress Paradigm

We used the chronic social defeat stress (CSDS) protocol described previously (16,17). It consisted of 10 daily sessions in which an adult wild-type C57BL/6 experimental mouse was exposed to 5 minutes of physical aggression by a novel CD-1 mouse, previously screened for aggressive behavior. Control C57BL/6 mice were housed with a different littermate every day, but no physical contact was permitted. Twenty-four hours after the last CSDS session, C57BL/6 mice were assessed in the social interaction test (17). Briefly, mice were allowed to explore an open field in the absence (session 1) or presence (session 2) of a novel CD-1 mouse for a period of 2.5 minutes each session. The social interaction ratio was calculated (time spent in the interaction zone with CD-1 present/time spent in the interaction zone with CD-1 absent), and defeated mice were classified as susceptible (ratio <1) or resilient (ratio ≥1) (16). Complete description of the CSDS paradigm is available in Supplemental Methods.

Behavioral Testing

Tests for elevated plus maze, sucrose preference, and fear conditioning were conducted as described in Supplemental Methods and Materials.

Antibodies

All the antibodies used in this study and their specificity are described in detail in Supplemental Table S1.

Tissue Dissection

Mice were euthanized by decapitation 24 hours after the social interaction test. Bilateral punches of the pregenual medial PFC (mPFC) were taken from 1-mm coronal sections corresponding to plates 15–18 of the Paxinos and Franklin mouse atlas (18) as previously described (6,7). Ventral tegmental area (VTA) punches from 1-mm coronal sections were obtained as described previously (19). See Supplemental Methods and Materials for further details.

Western Blot

mPFC tissue punches were processed for Western immunoblot as before (6,19). Protein samples (25 μg) were separated on a 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis and were transferred to a nitrocellulose membrane that was incubated with antibodies against DCC (BD Pharmingen, Mississauga, ON, Canada) and α-tubulin (Sigma-Aldrich, Oakville, ON, Canada) (Supplemental Table S1). See Supplemental Methods and Materials for details.

RNA Extraction and Quantitative Real-Time Polymerase Chain Reaction for Mouse Tissue

Total RNA, including miRNA fraction, was isolated with the miRNeasy Micro-Kit protocol (Qiagen, Toronto, ON, Canada). Reverse transcription was performed using iScript (Bio-Rad, Saint-Laurent, QC, Canada). Quantitative real-time polymerase chain reaction (qRT-PCR) was performed with an Applied Biosystems (Toronto, ON, Canada) 7900HT RT-PCR system. Complete description of the procedure is available in Supplemental Methods and Materials.

Neuroanatomical Experiments With Mouse Brain Tissue

Mice were anesthetized with overdose via intraperitoneal injection of ketamine 50 mg/kg, xylazine 5 mg/kg, and acepromazine 1 mg/kg and perfused transcardially with 0.9% saline and 4% paraformaldehyde in phosphate-buffered saline (PBS). Double-labeled immunofluorescence was performed on coronal sections of the pregenual mPFC (Supplemental Table S1). Immunostaining was visualized with either Alexa 488-, Alexa Fluor 555-, or Alexa Fluor 633-conjugated secondary antibodies (Life Technologies, Toronto, ON, Canada). See Supplemental Table S1 and Supplemental Methods and Materials for details.

In Situ Hybridization

Frozen coronal sections of the pregenual mPFC were treated with sense and antisense 5′ digoxigenin-labeled locked nucleic acid probes against miR-218 (Supplemental Table S2). PFC tissue was incubated with anti-digoxigenin antibody coupled to horseradish peroxidase (Roche, Mississauga, ON, Canada) and anti-DCC antibody No. 2473 (20). miRNA expression and DCC immunofluorescence were revealed with tyramide-coupled to cyanine 3 (PerkinElmer, Montréal, QC, Canada) and Alexa 488-coupled secondary antibody (Life Technologies), respectively. Complete description is available in Supplemental Methods and Materials.

In Silico Analysis and miRNA Identification

Candidate miRNAs to regulate DCC expression were predicted using five miRNA target prediction databases: miRWalk (21), miRanda (22), miRDB (23), Diana-microT (24), and TargetScan (25). Only miRNAs that were predicted by at least three of the five databases and were confirmed to be expressed in human and mouse brain were selected for downstream experiments. miRNAs were ranked according to their mirSVR predicting score, which is a prediction system that determines the potential of a miRNA to regulate the expression of specific target genes (26), and we selected the miRNA with the highest mirSVR score.

Quantification of miR-218 and *DCC* Expression in Human Brain Samples

The expression of miR-218 and *DCC* was quantified in PFC (Brodmann area 44) tissue samples obtained from two different cohorts, using qRT-PCR. Complete description is available in Supplemental Methods and Materials.