Publication information

miniannotation	yes
title	High Content and Transcriptome Analysis reveals functional roles of microRNAs in DRG neurite growth and its putative targets.
description	Peripheral neurons regenerate their axons after injury. Transcriptional regulation by microRNAs (miRNAs) is one possible mechanism that controls regeneration. We profiled miRNA expression in dorsal root ganglion (DRG) neurons after a sciatic nerve crush, and identified 49 differentially expressed miRNAs. We were able to evaluate the functional roles of the each miRNA using an High Content Analysis (HCA) approach. In order to predict the targets of the microRNAs we employed RNA-Sequencing to study transcription at the mRNA isoform level. We identified thousands of differentially expressed gene isoforms in the same model and then bioinformatically associated the miRNAs that modulated neurite growth with their putative target isoforms to describe the network of regulatory events underlying peripheral nerve regeneration.
Investigator First Name	Vance
Investigator Last Name	Lemmon
Investigator Email	vlemmon@med.miami.edu
Investigator Street Address	Lois Pope LIFE Center, Room 4-16 1095 NW 14th Terrace
Investigator City	Miami
Investigator State	FL
Investigator ZIP	33136
Investigator Country	United States
Investigator Affiliation	The Miami Project to Cure Paralysis, Miller School of Medicine, University of Miami
Is the submitter a member of the laboratory that performed the research?	Yes
Submitter First Name	Vance
Submitter Mid Initial	P
Submitter Last Name	Lemmon
Submitter Email	vlemmon@med.miami.edu
Submitter Affiliation	The Miami Project to Cure Paralysis, Miller School of Medicine, University of Miami
Publication status	In preparation

Investigator Mid Initial	P
Submitter Street Address	Lois Pope LIFE Center, Room 4-16 1095 NW 14th Terrace
Submitter City	Miami
Submitter State	FL
Submitter ZIP	33136
Submitter Country	United States

Authors

First name	Last name	Identifier	Affiliation
Dario	Motti	0000-0002-7714-4040	The Miami Project to Cure Paralysis, Miller School of Medicine, University of Miami, Miami, Florida, United States of America.
Jessica	Lerch	0000-0002-1210-3184	The Miami Project to Cure Paralysis, Miller School of Medicine, University of Miami, Miami, Florida, United States of America.
Matt	Danzi	0000-0003-1568-5965	The Miami Project to Cure Paralysis, Miller School of Medicine, University of Miami, Miami, Florida, United States of America.
Jared	Gans		The Miami Project to Cure Paralysis, Miller School of Medicine, University of Miami, Miami, Florida, United States of America.

Frank	Kuo	0000-0003-0655-4074	The Miami Project to Cure Paralysis, Miller School of Medicine, University of Miami, Miami, Florida, United States of America.
Richard	Morris		Dept of Genetics, Miller School of Medicine, University of Miami, Miami, Florida, United States of America.
John	Bixby	0000-0003-1633-5318	The Miami Project to Cure Paralysis, Dept of Pharmacology, Miller School of Medicine, University of Miami, Miami, Florida, United States of America.
Vance	Lemmon	0000-0003-3550-7576	The Miami Project to Cure Paralysis, Miller School of Medicine, University of Miami, Miami, Florida, United States of America.

Organism Section

Animals used in a study group	7 Days post surgery
Systematic name	Mus musculus domesticus (western european house mouse)
Provider/Vendor	Jackson Labs
Age at sacrifice	6-8
Age at sacrifice/Unit	Weeks
Sex	male
Geno type	WT
Organism vendor catalogue number	000664
strain	C57BL/6J
Housing type	group
Light/Dark Cycle	12 light / 12 dark
Was light/dark cycle reversed?	no
Enrichment	nestlets
Food	Teklad 7960 irradiated

organism

Animals used in a study group	mice
Systematic name	Mus musculus domesticus (western european house mouse)
Provider/Vendor	Jackson Labs
Age at sacrifice	6-8
Age at sacrifice/Unit	Weeks
Sex	male
Geno type	WT
Organism vendor catalogue number	000664
strain	C57BL/6J
Housing type	group
Light/Dark Cycle	12 light / 12 dark
Was light/dark cycle reversed?	no
Enrichment	nestlets
Food	Teklad 7960 irradiated

organism

Experimental Conditions

Surgery Section

Experimental Condition Type	Surgery
Surgery unique name	Sciatic Nerve 15" Crush
Were anesthetics used?	Yes
Surgery type	Injury
Injury method	Sciatic nerve crush
Compression maintained following impact?	Not described
Age at time of initial Surgery	6-8
Age at time of initial Surgery - Unit	Weeks
Were experimenters blinded to treatments?	No
Does the Phenotype of a transgenic animal make its genetic status obvious?	No

Injury level

Number of surgeons in project	1
Were analgesics used?	No
Was hydration used?	Yes
Post surgery hydration solution	lactated ringers
Post surgery hydration dose volume	2
Post surgery hydration delivery method	i.p.
Post surgery hydration fFrequency	one time
Were antibiotics used?	Yes
Post surgery antibiotic name	gentamicin
Post surgery antibiotic type	Systemic
Post surgery antibiotic dose	5
Post surgery antibiotic dose unit	mg/kg

Post surgery antibiotic solvent	water
Was bladder expression performed?	No
Were exclusion criteria established before the study was intitiated?	No

Anesthetics

Anesthetic name	Anesthetic type	Anesthetic dose	Anesthetic dose unit	Anesthetic source	Anesthetic catalogue number	Anesthetic solvent	Anesthetic delivery method
Ketamine	Systemic	100	mg/kg			water	i.p.
Xylazine	Systemic	10	mg/kg			water	i.p.

surgery

Experimental Condition Type	Surgery
Surgery unique name	Sciatic Nerve Crush - sham
Were anesthetics used?	Yes
Surgery type	Injury
Injury method	Sciatic nerve crush
Compression maintained following impact?	Not described
Age at time of initial Surgery	6-8
Age at time of initial Surgery - Unit	Weeks
Were experimenters blinded to treatments?	No
Does the Phenotype of a transgenic animal make its genetic status obvious?	No

Injury level

Number of surgeons in project	1
Were analgesics used?	No
Was hydration used?	Yes
Post surgery hydration solution	lactated ringers
Post surgery hydration dose volume	2

Post surgery hydration delivery method	i.p.
Post surgery hydration fFrequency	one time
Were antibiotics used?	Yes
Post surgery antibiotic name	gentamicin
Post surgery antibiotic type	Systemic
Post surgery antibiotic dose	5
Post surgery antibiotic dose unit	mg/kg
Post surgery antibiotic solvent	water
Was bladder expression performed?	No
Were exclusion criteria established before the study was intitiated?	No

Anesthetics

Anesthetic name	Anesthetic type	Anesthetic dose	Anesthetic dose unit	Anesthetic source	Anesthetic catalogue number	Anesthetic solvent	Anesthetic delivery method
Ketamine	Systemic	100	mg/kg			water	i.p.
Xylazine	Systemic	10	mg/kg			water	i.p.

surgery

Outcome Measures

Imaging Section

Outcome Measure Type	Imaging
Imaging outcome measure name	Axon growth
Was Live Cell Imaging/ Time lapse performed?	no
Was Immunostaining performed?	no
Were constant image acquisition standards used?	no
Was Pixel binning performed?	no
Was a microscope incubator used?	no
Was the incubator Humidified?	no
Was Temperature of objective controlled?	no
Was Observer blinded to treatment?	no
Was Image analysis performed?	No
Velocity of neurite growth or retraction	No
Varicosities in synapse formation	No
Neurite thickness	No

imaging

Molecular Biology Section

Outcome Measure Type	Molecular Biology
Molecular Biology outcome measure name	miRNA Expression
Was RT-PCR performed?	No
Was DNAse treatment used?	No
Was Semi-quantitative gel based PCR performed?	No

Detection

Was Quantitative Real Time PCR performed?	Yes
Real Time Primers	microRNA Ready-to-Use PCR, Mouse&Rat panels I + II (Exiqon, USA)
Reagent Kit	SYBR Green
Instrument Manufacturer	Roche LightCycler® 480 Instrument
Was Chromatin immunoprecipitation performed?	No
Were Microarrays performed?	No
Was RNA-seq performed?	No
Was in situ hybridization performed?	No

Molecular Biology

Outcome Measure Type	Molecular Biology
Molecular Biology outcome measure name	RNA-seq
Was RT-PCR performed?	No
Was DNAse treatment used?	No
Was Semi-quantitative gel based PCR performed?	No

Detection

Was Quantitative Real Time PCR performed?	No

Was Chromatin immunoprecipitation performed?	No
Were Microarrays performed?	No
Was RNA-seq performed?	Yes
Reference to RNA-seq data in Gene Expression Omnibus at NCBI	GSE59547
Was in situ hybridization performed?	No

Molecular Biology

Outcome Measure Type	Molecular Biology
Molecular Biology outcome measure name	miRNA Expression
Was RT-PCR performed?	No
Was DNAse treatment used?	No
Was Semi-quantitative gel based PCR performed?	No

Detection

Was Quantitative Real Time PCR performed?	Yes
Real Time Primers	microRNA Ready-to-Use PCR, Mouse&Rat panels I + II (Exiqon, USA)
Reagent Kit	SYBR Green
Instrument Manufacturer	Roche LightCycler® 480 Instrument
Was Chromatin immunoprecipitation performed?	No
Were Microarrays performed?	No
Was RNA-seq performed?	No
Was in situ hybridization performed?	No

Molecular Biology

Outcome Measure Type	Molecular Biology

Molecular Biology outcome measure name	RNA-seq
Was RT-PCR performed?	No
Was DNAse treatment used?	No
Was Semi-quantitative gel based PCR performed?	No

Detection

Was Quantitative Real Time PCR performed?	No
Was Chromatin immunoprecipitation performed?	No
Were Microarrays performed?	No
Was RNA-seq performed?	Yes
Reference to RNA-seq data in Gene Expression Omnibus at NCBI	GSE59547
Was in situ hybridization performed?	No

Molecular Biology

Treatments

Crush	7 Days post surgery,	Sciatic Nerve 15" Crush ,
Sham Control	mice,	Sciatic Nerve Crush - sham,

Study Groups

Crush	Crush,	Axon growth, miRNA Expression, RNA-seq,
Sham control	Sham Control,	Axon growth, miRNA Expression, RNA-seq,

Primary findings

Treatment	Surgery - Sciatic Nerve 15" Crush
Outcome measure	Molecular Biology - miRNA Expression
Study group	Crush
Effect	increase
Time of observation	7 days
Baseline control group	Sham surgery control group

Treatment	Surgery - Sciatic Nerve 15" Crush
Outcome measure	Molecular Biology - RNA-seq
Study group	Sham control
Effect	increase
Time of observation	7 days
Baseline control group	Sham surgery control group

Treatment	Surgery - Sciatic Nerve 15" Crush
Outcome measure	Imaging - Axon growth
Study group	Crush
Effect	increase
Time of observation	7 days
Baseline control group	Sham surgery control group