

II. Material

2.1 Chemikalien, Mediumzusätze

basischer Fibroblasten-Wachstumsfaktor (bFGF)	Invitrogen
Butyliertes-Hydroxy-Anisol (BHA)	Sigma
B-27 Supplement ohne Antioxidantien, 50x (B-27)	Invitrogen
4',6'-Diamidin-2'-phenylindol-dihydrochlorid (DAPI)	Serva
Diazobicyclo[2.2.2]octan (DABCO)	Sigma
Dimethylsulfoxid (DMSO)	Sigma
Eserserum	Dianova
Forskolin	Sigma
Glutamax I, 100x	Gibco
L-Glutamin, 200 mM	Cambrex
Hydrocortison	Sigma
Insulin (4 mg/ml)	Gibco
Nerven-Wachstumsfaktor 2.5S (NGF)	Invitrogen
Penicillin, 10000 U/ml; Streptomycin, 10000 µg/ml (PEN-STREP)	Cambrex
TritonX-100	Sigma
0,25% Trypsin/1 mM EDTA (Trypsin)	Invitrogen
Valproinsäure (VPA)	Sigma

2.2 Verwendeter Mausstamm

CD1-Mäuse, weiblich, Alter 6-8 Wochen	Charles River Laboratories
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2.3 Zellkulturmaterialien

Dulbecco's Modified Eagle Medium (DMEM)	Invitrogen, Cambrex
Dulbecco's Phosphate Buffered Saline (DPBS)	Cambrex
Minimum Essential Medium Eagle, Alpha-Modification (α -MEM)	Sigma
Neurobasal Medium	Invitrogen
Fötale Rinderserum (FBS)	BioWhittaker
Zellkultur-Plastikflaschen, 25 cm ² , 75 cm ² und 150 cm ²	TPP
Zellkultur-Plastikplatten, Ø 9,5 cm ² je Probenvertiefung	TPP
Zellkultur-Plastikplatten, Ø 2 cm ² je Probenvertiefung	Greiner Bio-one

2.4 cDNS-Synthese und RT-PCR

Trizol TM Reagenz	Invitrogen
Deoxynucleoside Triphosphate Set PCR Grade (dATP, dCTP, dGTP, dTTP, je 100 mM)	Roche
DNase	Promega
DNase 10x Buffer	Promega
Superscript II	Invitrogen
5x Reaction Buffer	Invitrogen
DTT	Invitrogen
Oligo(dT)	Amersham Pharmacia
Maus CoT-DNS	Invitrogen
Poly(dA)	Amersham
Cy3-/Cy5-dUTP	Amersham
Random Primer (500 µg/ml)	Promega
Taq-Polymerase	Perkin-Elmer
M-MLV Reverse Transkriptase (200 U/µl)	Promega
M-MLV 5x reaction buffer	Promega
RedTaq DNA Polymerase (1U/µl)	Sigma
RedTaq PCR Reaction buffer (10x)	Sigma
RNAguard, Rnase Inhibitor (32,970 U/ml)	Amersham
Molekulargewichtsstandard für die Agarose-Gelelektrophorese:	
pUC Mix (0,5 mg DNA/ml)	Fermentas

2.5 Antikörper

primäre Antikörper:

alpha-Smooth Muscle Actin, Klon IA4	Sigma
Desmin	Progen
GalC	Chemicon
GFAP, C-19, polyklonal	Santa Cruz Biotechnology
GFAP, monoklonal	Chemicon
HuC/HuD	Molecular Probes
Ki67	Dako

L1-CAM	P. Pesheva, Uni-Bonn
Nestin	BD Bioscience
NF-M	Chemicon
NSE	Polysciences
O4	Chemicon
Pleiotrophin	R&D Systems
RC2	Hybridoma Bank
Slug	Santa Cruz Biotechnology
TrkA	Santa Cruz Biotechnology
VE-Cadherin	Alexis
Vimentin	Chemicon

sekundäre Antikörper:

Esel anti-Kaninchen, Cy3 gekoppelt	Dianova
Esel anti-Maus, Cy3 gekoppelt	Dianova
Esel anti-Ratte, Cy3 gekoppelt	Dianova
Esel anti-Ziege, Cy3 und FITC gekoppelt	Dianova

2.6 Primer RT-PCRs

Genname (engl.)	Primername	Sequenz	Größe des PCR-Prod.
Amyliod beta precursor protein	AbPP for	AACGGATATGAGAATCCAAC	311
	AbPP rev	CAGTACACAAAACCCATGAA	311
Adipocyte protein P4	Adipo for	TGGAAGCTTGTCTCCAGTGA	356
	Adipo rev	CTTGTGGAAGTCACGCCTTT	356
Beta-actin cytoplasmatic	B-ACT for	TTCTACAATGAGCTGCGTGTG	603
	B-ACT rev	ACGGATGTCAACGTCACACTT	603
Calmodulin	CALMOD for	TGGTGCCGTTACTCGAAGTC	612
	CALMOD rev	GGTCTTCATTTTGCAGTCATCA	612
CD81-antigen	CD81 for	CTCCAGGCTATCTGCCAGTC	454
	CD81 rev	CTTGGCGATCTGGTCTTTGT	454
Cofilin 2, muscle isoform	Cofi for	CGGAGCTATTTATGGCAAACA	509
	Cofi rev	TGGTGAAAAAGTCCTTTGGTG	509
Cathepsin B	CTSB for	ATGACAAGCCTTCCTCCAC	597
	CTSB rev	GGGAGTAGCCAGCTTCACAG	597
Ferritin heavy chain	FHC for	AGCAGATGTTTTGGTGCAACT	393
	FHC rev	AAAGAAACCAGACCGTGATGA	393
Gamma-2-Actin	G2ACT for	ATACTCTGGCTTGCTGATCCA	350
	G2ACT rev	AGGTCATCACTATTGGCAACG	350
Macrophage migration inhibitory factor	MMIF for	GTGCCAGAGGGGTTTCTGTC	350
	MMIF rev	GAGCGAGGCTCAAAGAACAG	350
Neural cell adhesion molecule	NCAM for	CAGTGAACCGTATTGGACAGG	600
	NCAM rev	TTCTTGCTGGTTTTTCAGCTA	600
Neural precursor cell expressed developmentally downregul. 4	NPG4 for	AGACAGGGAAGCACACATGAC	291
	NPG4 rev	GATTCTAGGTGGTTCCGTCT	291
Nerupilin	NRP for	AAGGAAACCTTGGTGAATTG	598
	NRP rev	GCTCATTGAAGCTCCTGAGAA	598
Neuron specific gene family member 1	NSGF1 for	TCGGATCCCTCTACAGACAAG	277
	NSGF1 rev	AGAAGTCTCGGCCTCCTTTAG	277
Pigment epithelium derived Factor	PEDF for	CTTCAAGGTCCTGTGAACAA	635
	PEDF rev	CAGAGTCCAAGCCGTATCGTA	635
Prion Protein	Pripro for	TGATCTTTAGCCTTGCTTGA	491
	Pripro rev	GGGTCACACGGTAAGCATTTA	491
Reticulon 3	Reti 3 for	GTGCTCATTTGGATCGTGTTT	494
	Reti 3 rev	ACGAGGTCCTTGTTAGGAGA	494
Syntaxin Binding Protein 1	SBP1 for	CACGGTTGTGCTTCCATACTT	254
	SBP1 rev	GGTGAACCTTTTCCCTTAGGA	254
Stem cell growth factor	SCGF1 for	GGGGTGGGAAATGAGGATAA	606
	SCGF1 rev	GCCGTTCTCGAAAAGGTAGA	606
Stromal cell derived factor 1	SDF1for	AAACTTTAGGGAGCCGGATTT	600
	SDF1rev	GACTGACTTCTGGGAGGAAGC	600
Synapsin 1	SYN1 for	CAACAACGCTGAGAAGAGAGG	248
	SYN1 rev	GTGAAGGGAGCAGGTTGTTCT	248
Syntenin	SYNT for	CTAAAACGACCCCAACCTAGC	304
	SYNT rev	ATTGCTGCCTTCTGAACTTG	304
Tyrosin beta-4	TYB4 for	AGCAACCATGTCTGACAAACC	132
	TYB4 rev	CCAGCTTGCTTCTCTTGTTCA	132
Voltage dependent anion Channel 1	VDAC1 for	AGGTACAGCAGAAACCCATT	503
	VDAC1 rev	GGAGAACCAGGAAGCTATTTCA	503
Vimentin	VIM for	CCAAGTTTGCTGACCTCTCTG	697
	VIM rev	TTTTCTACGCTTTTGGGGTGT	697

2.7 Primer sq-RT-PCRs

Genname	Primername	Sequenz
Agrin	Agrin_for	ACTTATGAGTGCCTGTGTCC
	Agrin_rev	TAAGCTCAGCTCAAAGTGGT
BMP4	BMP4_for	GCTACCAGGCCTTCTACTG
	BMP4_rev	CACCTTGTCACTCATCC
CIR	CIR2_for	CACAGTCCAGAGAAGAAAGG
	CIR2_rev	GCTAAAGATGTCCTTGATGC
EF-1b	EF-1b_for	GATCTGATGATGAGGAGGAA
	EF-1b_rev	AGTTTTGTGTCATGTCCGTCTC
Ephrin B1	EphrinB1_for	AGCAAGGAGTCAGACAACAC
	EphrinB1_rev	ATGATGAGCAGGAAGATGAC
Ezrin	Ezrin_for	GGAGGACGAGGTAGAAGAGT
	Ezrin_rev	GTTCTCGTTGTGGATGATGT
Jun-B	Jun-B_for	ACCTCAGCAGTTACTCTCCA
	Jun-B_rev	TTCTCAGCCTTGAGTGTCTT
Lim Kinase 1	LimK1_for	TACCCTCACCAGATTTCTCA
	LimK1_rev	CACTTCTAAAGCTGCCTTCT
MAP 1 A/B	MAP1A/B_for	TGTGGTTGTCAAGTGGTAGA
	MAP1A/B_rev	ACATTGAGGGTTAGCAAAGA
Neurogenin-2	Neurogenin-2_for	CCAGCTCTGGTTTATTGAAG
	Neurogenin-2_rev	GAAGGTGAAAAGATCACAGG
Noelin-1	Noelin-1_for	ATGGTGTCTCTCTGTGTTT
	Noelin-1_rev	CAATCCTAACAGTGGGTTGT
Pax-3	Pax-3_for	TAATGGGACTTCTGACCAAC
	Pax-3_rev	TTGTCCATACTGCCATACT
Pax-6	Pax-6_for	GGAAGCTGCAAAGAAATAGA
	Pax-6_rev	TGTGACTAGGAGTGTGCTG
rb-like p107	p107_for	TTTGTATCACCTTTGTTCC
	p107_rev	CTAGACTGCCAAGCCAATAC
rb-like p130	p130_for	CCTATCTACATTTCCCCACA
	p130_rev	ACGACGTAACAGAGCAGAGT
Reticulon 4	Reticulon4_for	TTGTAAGCTGCTGTGTATGG
	Reticulon4_rev	CACTGTGAAAAGGGTTGTTT
Snail 1	Snail_for	AGAACTAAAGCCAACCCACT
	Snail_rev	CATTATTCATGGTCCCTTCT
Sox-10	Sox-10_for	CTGCTCCTGTTATCTCCTTG
	Sox-10_rev	GTTATTGACACGGAAGTGA
Sox-8	Sox-8_for	AACTACTACAGCCCCTACCC
	Sox-8_rev	GTACACTCCGGAAGAACTCA
Sox-9	Sox-9_for	AAGTTGATCTGAAGCGAGAG
	Sox-9_rev	GTAAGTCCAGTGTAGGTGA
Twist	Twist_for	AAGCTGAGCAAGATTCAGAC
	Twist_rev	CCTTCTCTGGAAACAATGAC
Wnt-1	Wnt-1_for	CAGTTACCGTAGGTCAGCAC
	Wnt-1_rev	GGGAGATTCCCAAGTAGAAC
Wnt-3a	Wnt-3a_for	CTTGGAGGGGTCTCTTACTT
	Wnt-3a_rev	TTGAGGGAGTAGCTTACCTG

Die Produktlänge für alle Primerpaare lag bei ca. 200 Basenpaaren

2.8 Puffer und Lösungen

FACS-Puffer	1x PBS 0,2% NaAzid 2% FBS 2mM EDTA	1x PBS	8 mM Na ₂ HPO ₄ 1,5 mM KH ₂ PO ₄ 140 mM NaCl 3 mM KCl
MACS-Puffer	1x PBS 0,5% FBS 2 mM EDTA	Waschlösung X	0,2x SSC, 0,01%SDS
Einbettmedium	90% Glycerin 0,1 M Tris-Hcl,pH 8 2,3% DABCO 0,5 µg/ml DAPI	Waschlösung Y	0,2 x SSC

2.9 Geräte und sonstige Materialien

Axiovert 100	Zeiss
Axioskop 2	Zeiss
428 Array Scanner	Affymetrix
Casy1 Zellzähl- und Analyse System Model TT	Schärfe System
CO ₂ -Inkubator	Thermo Lifescience
Concentrator 5301	Eppendorf
GAPSII coated Slides (für cDNS-Mikroarrays)	Corning
GenePix 4000B Array-Scanner	Axon Instruments
Fluoreszenzmikroskop Leica DM IRE 2	Leica Microsystem
MACS LD Depletion Columns	Miltenyi Biotech
MACS Microbeads	Miltenyi Biotech
MidiMACS Separation Unit	Miltenyi Biotech
SDDC-2 MicroArrayer	ESI, Eurogentec

2.10 Software

Microsoft Word	Microsoft
Microsoft Excel	Microsoft
Photoshop 5.0	Adobe
IPLab	Scanalytics
428 Reader 1.1	Affymetrix
Microarray Suite 2.1.4	Scanalytics
Simple PCI Imaging Software 5.3	Compix