

Cellular Dedifferentiation and Regenerative Medicine

Xiaobing Fu
Andong Zhao
Tian Hu



Springer

Cellular Dedifferentiation and Regenerative Medicine

Xiaobing Fu • Andong Zhao • Tian Hu

Cellular Dedifferentiation and Regenerative Medicine

 Springer

Xiaobing Fu
Key Laboratory of Wound Repair and
Regeneration of PLA
The First Hospital Affiliated to the PLA
General Hospital
Beijing, China

Andong Zhao
Tianjin Medical University
Tianjin, China

Tian Hu
School of Medicine
Nankai University
Tianjin, China

ISBN 978-3-662-56177-5 ISBN 978-3-662-56179-9 (eBook)
<https://doi.org/10.1007/978-3-662-56179-9>

Library of Congress Control Number: 2017964385

© Springer-Verlag GmbH Germany 2018

This work is subject to copyright. All rights are reserved by the Publisher, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broadcasting, reproduction on microfilms or in any other physical way, and transmission or information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed.

The use of general descriptive names, registered names, trademarks, service marks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use.

The publisher, the authors and the editors are safe to assume that the advice and information in this book are believed to be true and accurate at the date of publication. Neither the publisher nor the authors or the editors give a warranty, express or implied, with respect to the material contained herein or for any errors or omissions that may have been made. The publisher remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Printed on acid-free paper

This Springer imprint is published by Springer Nature
The registered company is Springer-Verlag GmbH Germany
The registered company address is: Heidelberger Platz 3, 14197 Berlin, Germany

Preface

Since we have published our discovery of the dedifferentiation of epithelial cells in *The Lancet* in 2001 [Xiaobing Fu et al. Dedifferentiation of epidermal cells to stem cells *in vivo*. *Lancet* 2001; 358: 1067–1068], this phenomenon aroused a great deal of our interest. As researchers of trauma and regenerative medicine, we realized cellular dedifferentiation has been deeply investigated by generations of scientists ranging from botany to zoology, which further kindled our interest in unveiling the relation between dedifferentiation and regeneration.

Through our intensive investigation and discovery, dedifferentiation is found to be an irreplaceable process in biological development and regeneration. The evidence is witnessed by scientists in numerous fields, such as plant, invertebrate, amphibian, and mammal. Scientometric and bibliometric analyses have demonstrated that cellular dedifferentiation attracts researchers all over the world, with accent on those in the USA and Western Europe. Several universities and organizations were quite productive in academic achievements on this issue, such as University College London; the University of California, Irvine; the University of Michigan; etc. Prolific scientists, for example, Prof. David M. Gardiner, Prof. Panagiotis A. Tsonis, Prof. Satoh Akira, etc., have represented their works on many publications.

Our group has made remarkable achievements on skin repair and sweat gland regeneration via the process of dedifferentiation. These achievements of sweat gland *in vivo* and *in vitro* regeneration appeared in the international field of regenerative medicine for the first time and have earned worldwide commendation as a milestone research. Besides, regeneration of other tissues by dedifferentiation has also obtained exceptional results. For example, Jopling Chris et al. discovered zebrafish heart regeneration by cardiomyocyte dedifferentiation [Jopling Chris et al. Zebrafish heart regeneration occurs by cardiomyocyte dedifferentiation and proliferation. *Nature*. 2010 Mar 25; 464(7288): 606–609], and Odelberg SJ et al. induced mammalian myotube dedifferentiation by *Msx1* [Odelberg SJ, et al. *Cell*. 2000 Dec 22; 103(7):1099–1109].

In the light of these, I planned to sketch out the spectrum of cellular dedifferentiation to scientists, researchers, and physicians in 2015. We mapped out the theories,

concepts, discoveries, achievements, practices, and perspectives of this issue in the form of a monograph. First, I would like to acknowledge two of my PhD students, Andong Zhao and Tian Hu, who have accomplished significant work in data collection and compiling. I am also grateful for all the authors who have contributed their articles and reviews in the appendixes. Additionally, I would like to express my great gratitude toward all the scientists and researchers, for their permission, whose figures and tables in their publications were cited in this book. Although we have tried our best to get contact with them and ask for permission, there is still imperfection. Finally, I am also grateful for the research foundation support from the National Key Research Projects offered by Ministry of Science and Technology, China, and the publication aid and support by the press of Springer. The accomplishment of this monograph could never be carried out without the effects of all the people and units I appreciated.

Owing to the busy schedule and limits in knowledge, the book might be incomprehensive with flaws and limitations. We hope readers could point these out and would feel free to contact us, in order to make more contribution to the field of dedifferentiation in the future.

Beijing, China

Xiaobing Fu

Contents

1	Central Nervous System and Dedifferentiation	1
1	Central Nervous Stem Regeneration, Stem Cell, and Dedifferentiation	1
1.1	Central Nervous System Injury and Regeneration.	1
2	Astrocyte Dedifferentiation In Vivo and In Vitro	5
2.1	Astrocyte Dedifferentiation In Vivo.	5
2.2	Astrocyte Dedifferentiation In Vitro	7
3	Direct Reprogramming of Astrocytes into Mature Neurons or Neural Precursors In Vitro and In Vivo	10
3.1	Reprogramming of Astrocytes into Mature Neurons In Vitro.	10
3.2	Reprogramming of Astrocytes into Mature Neurons In Vivo	12
3.3	Reprogramming of Astrocytes into Neural Precursor In Vitro and In Vivo	12
4	Dedifferentiation of Other Cells in CNS	13
5	Conclusions and Perspectives	14
	References.	15
2	Peripheral Nerve Regeneration and Dedifferentiation	19
1	Peripheral Nerve Regeneration	19
2	Overview of the Regeneration Process of Peripheral Nerve	20
3	Schwann Cell Dedifferentiation After Nerve Injury	21
4	Molecular Mechanisms Underlying Schwann Cell Dedifferentiation	23
4.1	Transcription Factors	23
4.2	Signaling Pathway Responsible for Schwann Cell Dedifferentiation (Fig. 2.3)	24
4.3	Other Factors Involved in Schwann Cell Dedifferentiation	31
5	MiRNA in Schwann Cell Dedifferentiation.	31
6	Potentiating Schwann Cells to Promote Nerve Regeneration	34
	References.	35

3	Dedifferentiation and the Heart	39
1	Heart Disease	39
2	Historical and Current Perspectives on Human Heart Regeneration	40
3	Heart Regeneration Models	42
3.1	Heart Regeneration in Lower Vertebrates	42
3.2	Limited Regeneration in Rodent Hearts	42
4	Cardiomyocyte Dedifferentiation and Proliferation Contribute to Heart Regeneration	43
4.1	Zebrafish Cardiomyocyte Dedifferentiation	44
4.2	Murine Cardiomyocyte Dedifferentiation	46
4.3	Human Cardiomyocyte Dedifferentiation	46
5	The Barrier for Mammalian Heart Regeneration	47
5.1	Small-Sized, Mononucleated, and Diploid Cardiomyocytes with Ease of Proliferation	47
5.2	Cell Cycle Regulators	49
5.3	Epigenetic Barrier for Cardiomyocyte Proliferation	50
6	Harnessing the Power of Cardiac Regeneration	52
6.1	Promoting Heart Regeneration by Cell Cycle Regulation	52
6.2	Regulating Signaling Pathway Involved in Cardiomyocyte Proliferation	53
6.3	Increasing Cardiomyocyte Proliferation by miRNA	56
7	Other Cell Sources Involved in Heart Regeneration	57
7.1	Cardiac Progenitor Cells	57
7.2	Heart Repair by Direct Reprogramming	58
	References	59
4	Dedifferentiation and Kidney System	65
1	Introduction of Kidney Development	66
2	Proximal Renal Tubular Cell Dedifferentiation and Kidney Regeneration	67
2.1	Introduction of Acute Kidney Injury	67
2.2	Epidemiology of Acute Kidney Injury	67
2.3	Tubular Cell Injuries Are Involved in the Pathogenesis of Acute Kidney Injury	67
2.4	Repair of Kidney Injury and Regeneration	68
2.5	Renal Proximal Tubular Cells Dedifferentiation	69
2.6	Changes of Proximal Tubular Epithelial Cells During Dedifferentiation	69
2.7	Redifferentiation of Dedifferentiated Tubular Cells	71
2.8	Molecular Mechanisms of Renal Proximal Tubular Cell Renewal	72
2.9	Other Stem Cells Associated with Kidney Regeneration and Their Contribution to Regeneration	75
2.10	Future Work	79

- 3 Podocyte Dedifferentiation and Kidney Diseases 79
 - 3.1 HIV-Associated Nephropathy (HIVAN) and Podocyte Dedifferentiation 79
 - 3.2 Diabetic Nephropathy and Podocyte Dedifferentiation 83
- References..... 86
- 5 Dedifferentiation and Musculoskeletal Repair and Regeneration 91**
 - 1 Articular Cartilage Repair and Chondrocyte Dedifferentiation 92
 - 1.1 Introduction of Articular Cartilage Injury and Repair and Chondrocyte Dedifferentiation 92
 - 1.2 Chondrocyte Dedifferentiation in Monolayer Culture. 93
 - 1.3 Chondrocyte Dedifferentiation-Induced Mediators Associated with OA 94
 - 1.4 Mechanisms Responsible for Chondrocyte Dedifferentiation. 94
 - 2 Bone Regeneration and Dedifferentiation 98
 - 2.1 Zebrafish Bone Regeneration and Osteoblast Dedifferentiation 98
 - 2.2 Bone Regeneration in Mammal and Dedifferentiation 99
 - 3 Skeletal Muscle Regeneration and Dedifferentiation 100
 - 3.1 Mammalian Skeletal Muscle Regeneration 100
 - 3.2 The Amphibian Muscle Regeneration 101
 - 3.3 Mammalian Myotube Dedifferentiation 102
- References..... 111
- 6 Dedifferentiation and Skin Regeneration 117**
 - 1 Skin, Homeostasis, and Epidermal Stem Cells 117
 - 2 Epidermal Cell Dedifferentiation into Epidermal Stem Cells or Precursors 118
 - 3 Molecular Mechanisms Underlying Epidermal Keratinocyte Dedifferentiation 120
 - 4 Reprogramming Keratinocytes to Pluripotent Cells 122
 - 5 Patient-Specific iPSCs from Keratinocytes 124
 - 6 Epidermal Melanocyte and Dedifferentiation 127
 - 6.1 Melanocyte Development and Pigment 127
 - 6.2 Melanocyte and Dedifferentiation 127
- References..... 129
- 7 Dedifferentiation and Vision System. 133**
 - 1 Dedifferentiation and Retinal Regeneration 133
 - 1.1 Introduction of Retinal Regeneration. 133
 - 1.2 Retinal Development and Structure. 135
 - 1.3 Retinal Pigmented Epithelial Cell-Dependent Retinal Regeneration 136
 - 1.4 Müller Glia-Dependent Retinal Regeneration 141

2	Dedifferentiation and Lens Regeneration	150
2.1	Introduction of Lens Regeneration	150
2.2	Lens Development and Structures	150
2.3	Lens Regeneration Models	151
2.4	Molecular Mechanisms of Lens Regeneration	152
2.5	Prospective	157
	References	158
8	Blood Vessel Repair, Atherosclerosis, and Dedifferentiation	163
1	Introduction	163
2	Differentiation Process of SMCs	164
3	Transcriptional Control of SMC Differentiation	164
4	SMC: Phenotypic Modulation, Switching, or Dedifferentiation	165
5	SMC Phenotypic Modulation, Vascular Repair, and Atherosclerosis	166
6	Molecular Mechanisms Underlying the SMC Phenotypic Modulation	167
6.1	Factors Responsible for SMC Phenotypic Modulation	167
6.2	Transcription Factor KLF4	168
6.3	Epigenetic Mechanisms Underlying the SMC Phenotypic Switching	169
6.4	MiRNAs	170
	References	172
9	Dedifferentiation and Adipose Tissue	175
1	Introduction of Adipose Tissue and Adipocyte Dedifferentiation	175
2	The Dedifferentiation Methods of Mature Adipocytes	176
3	Gene Expression Changes During Adipocyte Dedifferentiation	179
4	The Advantages of DFAT Cells as Sources for Cell-Based Therapy	180
5	The Signaling Mechanism Underlying Dedifferentiation of Adipocytes	181
6	Multilineage Differentiation Potential of DFAT Cells and Application	182
6.1	Adipogenesis	182
6.2	Osteogenesis and Chondrogenesis	182
6.3	Myogenesis	184
6.4	Angiogenesis	185
6.5	Neurogenesis	188
7	Comparison Between DFAT Cells, ASCs, and MSCs	189
8	Conclusions and Perspectives	190
	References	190
10	Dedifferentiation and Organ Regeneration	195
1	Introduction	195
2	Model Systems for Regeneration Study	196
2.1	Planarian Regeneration	197
2.2	Hydra Regeneration	198

- 2.3 Salamander Limb Regeneration. 198
- 2.4 Xenopus Tadpole Tail Regeneration 199
- 2.5 Zebrafish Heart Regeneration
and Fin Regeneration 199
- 2.6 Mammalian Liver Regeneration 200
- 3 The Cellular Basis for Regeneration 201
 - 3.1 Pluripotent “Neoblasts” and Planarian Regeneration 202
 - 3.2 Multipotent Adult Stem Cells
and Hydra Regeneration 203
 - 3.3 Lineage-Restricted Progenitors and Xenopus
Tadpole Tail Regeneration 203
 - 3.4 Cardiomyocyte Dedifferentiation
and Zebrafish Regeneration 204
 - 3.5 Cell Dedifferentiation, Stem Cell, and Regeneration
of Salamander Limb or Zebrafish Fin 204
 - 3.6 Mature Hepatocytes, Liver Stem Cells,
and Mammalian Liver Regeneration 205
- 4 Growth Factors for Regeneration. 207
 - 4.1 Epidermal Growth Factors (EGFs) 207
 - 4.2 Fibroblast Growth Factors (FGFs) 208
 - 4.3 Insulin-Like Growth Factors (IGFs) 211
 - 4.4 Vascular Endothelial Growth Factors (VEGFs) 211
 - 4.5 Platelet-Derived Growth Factors (PDGFs) 212
 - 4.6 Bone Morphogenetic Proteins (BMPs) 212
- 5 The Molecular Basis for Regeneration 213
 - 5.1 Signaling Pathways (Fig. 10.2) 213
 - 5.2 Epigenetic Mechanism Underlying Regeneration 222
- 6 What Controls the Difference in Regenerative Ability 225
 - 6.1 Resident Stem/Progenitor Cells 225
 - 6.2 Dedifferentiation Potential. 226
 - 6.3 Transdifferentiation Potential. 227
 - 6.4 Specific Regeneration Genes 228
 - 6.5 Epigenetic Modification Difference. 228
 - 6.6 Immune Response and Inflammation. 230
- 7 Regenerative Medicine. 232
 - 7.1 Stem Cell-Based Therapy 232
 - 7.2 Tissue Engineering. 233
 - 7.3 Proteins and Small Molecules 233
- 8 Perspectives 234
- References. 236

11 Dedifferentiation and Regenerative Medicine:

- The Past and the Future. 247**
 - 1 Dedifferentiation and Cellular Plasticity 247
 - 1.1 General Understanding of Differentiation 248
 - 1.2 Cellular Identity and Plasticity. 249
 - 1.3 Dedifferentiation in Broad and Narrow Sense. 253

2 Present Situation and Emerging Trends in Dedifferentiation and Regenerative Medicine 256

2.1 Bibliometric Analysis of Regenerative Medicine 256

2.2 Bibliometric Analysis of Cellular Dedifferentiation 257

3 Dedifferentiation Studies in Botany. 259

4 Perspectives of Cellular Dedifferentiation. 263

4.1 Dedifferentiation and Noncoding RNAs 263

4.2 Dedifferentiation and Extracellular Vesicles 265

4.3 Vistas of Dedifferentiation in Regenerative Medicine. 266

References. 266

12 Authors’ Related Publications 273

1 Authors’ Related Publication 1: Dedifferentiation of Epidermal Cells to Stem Cells In Vivo 273

2 Authors’ Related Publication 2: Dedifferentiation: A New Approach in Stem Cell Research. 274

3 Authors’ Related Publication 3: Cutaneous Stem Cells: Something New and Something Borrowed 274

4 Authors’ Related Publication 4: Can Hematopoietic Stem Cells Be an Alternative Source for Skin Regeneration? 275

5 Authors’ Related Publication 5: Acclimatized Induction Reveals the Multipotency of Adult Human Undifferentiated Keratinocytes 276

6 Authors’ Related Publication 6: Induced Pluripotent Stem Cells: The Dragon Awakens 276

7 Authors’ Related Publication 7: How Far Are Induced Pluripotent Stem Cells from the Clinic?. 277

8 Authors’ Related Publication 8: Can Controlled Cellular Reprogramming Be Achieved Using MicroRNAs?. 277

9 Authors’ Related Publication 9: Epidermal Stem Cells: An Update on Their Potential in Regenerative Medicine 278

10 Authors’ Related Publication 10: Oriented Cell Division: New Roles in Guiding Skin Wound Repair and Regeneration 279

11 Authors’ Related Publication 11: Epigenetic Control of Reprogramming and Transdifferentiation by Histone Modifications. 279

12 Authors’ Related Publication 12: What Determines the Regenerative Capacity in Animals? 280

Index. 281

Chapter 1

Central Nervous System and Dedifferentiation

Abstract Central nervous system serves as the leading organ controlling, manipulating, and involving into almost every aspects of human body's functions. Researches and neuroscientists have been trying to find out varieties of approaches to repair and restore the damaged or degenerated central nervous system. It is generally believed that there are hundreds of billions of neurons in our brain, and the quantity would not change after birth. The olfactory bulb and hippocampus are the only two regions that could undergo self-renewal during our lifetime. Neural stem cells could differentiate into neuronal restricted progenitors and glial restricted progenitors. Glial restricted progenitors could produce type I astrocytes, type II astrocytes, and oligodendrocytes. But the regenerative capacity of these stem cells is far insufficient. Dedifferentiation of certain types of cells that resided in the central nervous system has provided the opportunity for neural regeneration, since other approaches, such as transplantation or drugs, could hardly take effects. Specifically, astrocyte dedifferentiation was observed successfully both in vivo and vitro. Injury triggers the dedifferentiation in vivo, while astrocytes could be reprogrammed to dedifferentiated types in vitro. This review summarized the current understandings and researches on central nervous regeneration, astrocyte differentiation, and direct reprogramming of astrocytes. In order to achieve the goal of CNS regeneration, clarifying the molecular mechanisms of regulating dedifferentiation and redifferentiation in situ would lay the solid foundation for further researches.

Keywords Central nervous system • Neural stem cell • Astrocytes
Dedifferentiation • Regeneration • Brain injury • Spinal cord injury

1 Central Nervous Stem Regeneration, Stem Cell, and Dedifferentiation

1.1 Central Nervous System Injury and Regeneration

Central nervous system (CNS) comprises of the brain and spinal cord. Injuries and diseases of CNS, such as Parkinson's disease, multiple sclerosis, stroke, traumatic brain injury, and spinal cord injury (SCI), result in various functional deficits and

abnormalities. At the cellular level, all of them lead to apoptotic and necrotic death of neurons. Therefore, replacing the lost neurons with new neurons is essential for CNS repair and regeneration. Unlike non-mammals that exhibit tremendous capacity to regenerate neurons from damaged CNS [1, 2], mammals have limited capacity to spontaneously regenerate the lost neurons. Mammalian CNS was considered as a tissue where new neurons could not be generated once development finished. However, this dogma has been challenged by studies showing that newborn neurons can be generated throughout life in a process called “adult neurogenesis.”

1.1.1 Neural Stem Cells

Grown neurogenesis is the process of putting out novel neurons integrating into the existent circuits after early postnatal and fetal development. In mammalian brain, this action predominantly occurs in two portions of the forebrain, subventricular zone (SVZ) of lateral ventricles in telencephalon and subgranular zone (SGZ) of the dentate gyrus in the hippocampus [3]. As neural stem cells (NSCs) dwell in both zones, SGZ and SVZ are called neurogenic area. NSCs are cells of self-renewing multipotent in the adult and developing mammalian CNS. Throughout development, assorted specified precursors dividing a restricted number of times are produced by NSCs before they differentiate into glial cells or neurons terminally, such as oligodendrocytes and astrocytes. In adult mammalian CNS, resident NSCs in SVZ and SGZ maintain neurogenesis throughout adult life: adult NSCs bring about neuroblasts further differentiating into matured neurons integrating into local circuitry within the olfactory bulbs or dentate gyrus. Unlike developing NSCs, fully grown NSCs originate from radial glia or so-called radial glial cells converting into astrocytic-like NSCs in the postnatal brain. Radial glia stem from neuroepithelial cells at the early stage of neurogenesis and are the principal cell type in the underdeveloped brain, where they both serve as scaffolds and neural progenitors for newborn neurons migration. Thus, NSCs in the SVZ share many characteristics with astrocytes. The finding of NSCs and neurogenesis in the grown mammalian CNS modifies our apprehension of the plasticity and role of brain and stimulates passion for harnessing their regenerative possibility in novel treatments for disorders like depression, stroke, SCI, and Parkinson’s disease. Native NSCs’ therapeutic potentiality is unfortunately limited by the confinement of sturdy neurogenesis to SVZ and the grown SGZ. Furthermore, novel neurons could be generated by adult NSCs only under normal physiological circumstances in SGZ region and SVZ. For example, while NSCs that are separated from SGZ or SVZ are transplanted into the adult brain’s ectopic areas, they differentiate into astrocytes and oligodendrocytes mostly [4]. It may be owing to adult NSCs that share some features of glial lineage or neurogenic local microenvironment that is favorable to neuronal differentiation of NSCs. This is further supported by NSCs from SGZ region of the dentate gyrus that can differentiate into olfactory bulb neurons when grafted to SVZ and NSCs isolated from a non-neurogenic region, such as the spinal cord, that can differentiate into neurons when transplanted into dentate gyrus [3]. Therefore, the fact that the

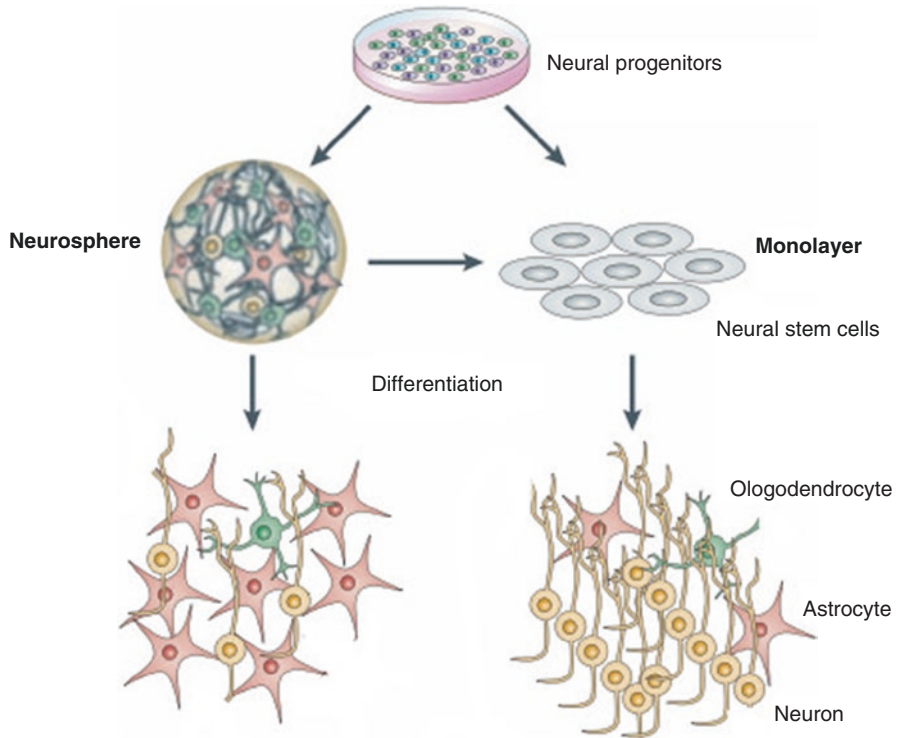


Fig. 1.1 Schematic diagram of neural progenitor differentiation

origin of adult NSCs and its neuronal differentiation are both restricted to the neurogenic niches *in vivo* largely limits the application of NSCs in various CNS diseases and injuries (Fig. 1.1).

1.1.2 Radial Glia

Radial glia, derived from neuroepithelium, is a ubiquitous glial cell type during the development of all vertebrate brains; they act as stem and progenitor cells that give rise to all neurons of mammalian CNS. However, radial glia represent more fate-restricted progenitors than NSCs, because radial glia are inclined to the generation of a single cell type, either astrocytes, oligodendrocytes, or neurons, rather than all of them like NSCs [5]. Stem cell properties not only are interestingly possessed by radial glia, astroglial properties are also exhibited. They express stem cell markers like the intermediate filament protein nestin and keep significant qualities of apical-basal polarity. They also have an ultrastructural distinctive of astroglial glycogen granules and express assorted molecules that are typical of astrocytes, such as astrocyte-specific glutamate transporter (GLAST), Ca^{2+} -binding protein S100 β , glial fibrillary acidic protein (GFAP), vimentin, and brain lipid-binding protein (BLBP). More fate