# TABLE OF CONTENTS

1 INTRODUCTION

1.1 Human pluripotent stem cell-based neural stem cells as a tool to access key aspects of early human neural development

1.1.1 Human embryonic and induced pluripotent stem cells

1.1.2 Directing human pluripotent stem cells towards the neural lineage

1.1.3 Different types of neural stem cells generated from human pluripotent stem cells

1.1.4 Strategies for the in vitro generation of dopaminergic neurons

1.2 MicroRNAs as important regulators of cell fate

1.2.1 MicroRNA biogenesis and function

1.2.2 The role of miRNAs during pluripotency, neuronal differentiation and neuronal subspecification

1.3 MicroRNA-181 family as potential regulator of neuronal differentiation

1.4 The role of GCNF during embryonic and neural development

1.4.1 Structure and mode of action of the orphan nuclear receptor GCNF

1.4.2 Expression and function of GCNF during embryonic and neural development

1.5 Aims and objectives

2 MATERIAL & METHODS

2.1 Cell culture

2.1.1 Cell lines

2.1.2 Reagents and media for cell culture work

2.1.3 Cryopreservation and thawing of cells

2.1.4 Maintenance of human pluripotent stem cells

2.1.5 Floor plate-based differentiation of hPSCs into dopaminergic neurons

2.1.6 Maintenance of long-term self-renewing neuroepithelial-like stem cells

2.1.7 Neuronal differentiation of It-NES cells

2.1.8 Clonal capacity assay of pre-differentiated It-NES cells

2.2 Lentiviral-based transgenesis of hPSCs and It-NES cells

2.2.1 Cloning and expansion of lentiviral constructs

2.2.2 Production of lentiviral particles

2.2.3 Transduction of hPSCs and It-NES cells with lentiviral particles

2.3 Oligonucleotide transfection experiments

2.3.1 Transfection procedure

2.3.2 Luciferase reporter assays

2.4 Flow cytometry-based assays

2.4.1 Doublecortin-EGFP reporter assay

2.4.2 CaspGLOW fluorescein active caspase assay

2.4.3 Wnt/β-catenin reporter assay

2.5 RNA-based expression analyses

2.5.1 RNA isolation

2.5.2 Northern blot analysis

2.5.3 Quantitative RT-PCR analysis

2.5.4 Semi-quantitative RT-PCR analysis
2.6 Western blot analysis .......................................................................................................................... 37
2.7 Immunocytochemistry .......................................................................................................................... 39
  2.7.1 BrdU incorporation assay .................................................................................................................... 40
  2.7.2 Image analysis and processing ........................................................................................................ 40
2.8 In silico analyses ..................................................................................................................................... 40
  2.8.1 MicroRNA profiling analysis ................................................................................................................ 40
  2.8.2 MicroRNA target gene prediction ...................................................................................................... 41
  2.8.3 GCNF target gene prediction ............................................................................................................ 41
  2.8.4 Software and online tools .................................................................................................................. 42
  2.8.5 Statistical analysis ............................................................................................................................. 42
2.9 Supplementary lists .............................................................................................................................. 43
  2.9.1 Technical equipment .......................................................................................................................... 43
  2.9.2 Primers and oligonucleotides for cloning .......................................................................................... 44
  2.9.3 Primers for RT-PCR .......................................................................................................................... 45
  2.9.4 Antibodies ........................................................................................................................................ 46
3 RESULTS .................................................................................................................................................. 48
  3.1 Identification of miRNA expression patterns associated with human neuronal differentiation .......... 48
    3.1.1 Annotation of miRNA profiles in hESCs, lt-NES cells and neuronal cultures ............................... 48
    3.1.2 Detailed expression analysis of miR-181 family members and processing intermediates .......... 52
  3.2 The role of distinct miRNAs on human neuronal differentiation and subtype specification ............. 54
    3.2.1 Selection of miR-181a, miR-153 and miR-324 as candidates for functional studies .................. 54
    3.2.2 Overexpression of known and candidate neuronal miRNAs shifts lt-NES cells from self-renewal to neuronal differentiation ................................................................................................. 56
    3.2.3 Transfection with miRNA oligonucleotides modulates neuronal differentiation of lt-NES cells .......................................................... 58
    3.2.4 MicroRNA-181a, miR-125b and miR-124 affect neuronal subspecification of lt-NES cells ............ 61
    3.2.5 Opposing functions of miR-181a and miR-124 on dopaminergic differentiation of hPSC-derived floor plate progenitors .......................................................................................................................... 63
    3.2.6 Transfection-based modulation of miR-181a/a*, miR-125b and miR-124 shifts lt-NES cells towards TH-positive neuron differentiation ................................................................................................................. 65
  3.3 Mechanisms underlying miR-181a function on NSC maintenance and dopaminergic subdifferentiation ................................................................................................................................................. 67
    3.3.1 Overexpression of miR-181a induces down-regulation of NSC-associated genes ......................... 68
    3.3.2 MicroRNA-181a promotes neuronal differentiation by targeting GCNF ........................................ 69
    3.3.3 Overexpression of GCNF stabilizes neural rosette morphology and impairs neuronal differentiation ................................................................................................................................. 73
    3.3.4 GCNF represses the expression of pro-neural bHLH transcription factors .................................... 80
    3.3.5 GCNF overexpression inhibits the generation of TH-positive neurons ......................................... 83
    3.3.6 MicroRNA-181a promotes the emergence of TH-positive neurons by potentiating Wnt signaling .... 84
4 DISCUSSION...........................................................................................................................................................87

4.1 MicroRNA expression signatures discriminate distinct stages of hESC-based neuronal differentiation .............................................................................................................................................................................88

4.2 MicroRNA processing intermediates and sister strands show distinct expression during neuronal differentiation .................................................................................................................................................................................................92

4.2.1 Cell type-specific pre-miRNA processing during neuronal differentiation .................................................................93

4.2.2 MicroRNA-181a and miR-181a* show cell type-dependent expression ratios .........................................................94

4.3 Identification of miRNAs promoting differentiation of human NSCs .............................................................................96

4.3.1 Experiments in lt-NES cells underline the role of miR-124 and miR-125b in human neuronal differentiation and process outgrowth .................................................................................................................................96

4.3.2 General function of miR-153 and miR-324 during neuronal differentiation of non-tumorigenic human NSCs .................................................................................................................................................97

4.3.3 MicroRNA-181a acts on several NSC-associated mechanisms to promote neuronal differentiation ..........98

4.3.4 Towards establishing a functional miRNA screening in lt-NES cells .................................................................................101

4.4 The miR-181a target gene GCNF regulates neural stem cell maintenance .................................................................101

4.4.1 GCNF preserves neural stem cell properties and inhibits premature neuronal differentiation ........................................102

4.4.2 GCNF acts in parallel to Notch to repress the expression of pro-neural bhLH genes ...........................................104

4.4.3 Antagonistic roles of GCNF, let-7 and miR-181a as regulators of developmental timing ........................................105

4.5 MicroRNA modulation as a tool to regulate the emergence of dopaminergic neurons ..............................................108

4.5.1 MicroRNA-181a promotes while miR-124 inhibits dopaminergic differentiation .........................................................108

4.5.2 Wnt activity is critical for dopaminergic differentiation and is enhanced by miR-181a ..............................................110

4.5.3 Specific inhibitory effect of the miR-181a target GCNF on dopaminergic differentiation .........................................111

4.6 Implications and future prospects .................................................................................................................................112

5 REFERENCES...........................................................................................................................................................114

6 APPENDIX.............................................................................................................................................................127

6.1 Supplementary figure ..................................................................................................................................................127

6.2 Publication ..............................................................................................................................................................127

6.3 Abbreviations ..........................................................................................................................................................128

6.4 Acknowledgement .....................................................................................................................................................129