

Table of contents

Acknowledgements.....vi

Abbreviationsvii

Chapter 1

Introduction1

Inner ear development3

FGF signalling9

Human embryonic stem cell state maintenance13

Therapeutic approaches for hearing impairment17

Gene therapy in the inner ear17

Adult stem cells for hearing impairment20

Embryonic stem cells: progress and challenges.....22

Our hESC differentiation protocol25

Aims26

Particular Aims27

Chapter 2

Methods28

Cell culture29

RNA extraction and cDNA synthesis31

RT-PCR, Q-PCR and gel electrophoresis31

Immunofluorescence	35
Flow cytometry	37
Transfection	39
Protein extraction and western blot	41
Cloning	42
Statistical analysis	45

Results

Chapter 3

FGF3 and FGF10 induce the expression of otic markers in human pluripotent stem cells	46
---	-----------

<i>Introduction</i>	47
---------------------------	-----------

FGFs during inner ear induction	47
--	-----------

Our differentiation protocol: a developmental biology approach	49
---	-----------

Results

Otic induction by FGF3 and FGF10	51
---	-----------

Are hES and hiPS cells the same?	58
---	-----------

Differentiation propensity in pluripotent stem cells	62
---	-----------

The effect of cell density	65
---	-----------

<i>Discussion</i>	69
-------------------------	-----------

Variation between cell lines in their response to FGFs	70
---	-----------

PAX2, PAX8 and FOXG1 are part of the same otic differentiation route	71
---	-----------

Chapter 4

Otic differentiation propensity in human pluripotent stem cells	75
<i>Introduction</i>	76
<i>Results</i>	77
FGF inhibition impedes otic differentiation in hESC	77
Endogenous FGF secretion induces differentiation in control medium	81
FGF10 induces higher expression of otic genes than FGF3	83
<i>Discussion</i>	91
FGF signalling is required for otic differentiation	91
Otic differentiation is affected by cell density	92
FGF3 and FGF10 are not equivalent	93
Cell line heterogeneity can be caused by the endogenous production of FGFs	94

Chapter 5

Expression time-course of otic transcription factors during differentiation	97
<i>Introduction</i>	98
<i>Results</i>	101
The time course of otic differentiation	101
PAX8 and PAX2 proteins confirm early otic differentiation	103
<i>Discussion</i>	114
Otic progenitors express otic markers in a similar pattern to inner ear development <i>in vivo</i>	114

Chapter 6

The role of FGFR2IIIb during otic induction <i>in vitro</i>	117
<i>Introduction</i>	118
<i>Results</i>	121
FGFR2IIIb is upregulated by FGF3 and FGF10	121
hESCs express FGFR2 at the protein level	123
Robust expression of FGFR2IIIb can be achieved with the CAG promoter	130
FGFR2IIIb overexpression in hESCs increases otic differentiation	138
<i>Discussion</i>	148
FGFRs in human embryonic stem cells	148
Establishing an overexpression system	149
FGFR2IIIb induces the expression of otic markers in hESCs	151

Chapter 7

Knockdown of FGFR2IIIb	153
<i>Introduction</i>	154
RNA-mediated silencing	154
A competitive specific inhibitor of FGFR2IIIb	157
<i>Results</i>	159
The Dox inducible system	159
Establishing a dox-inducible FGFR2 knockdown	164
Differentiation of clones in the presence of Dox	170
An alternative approach: a competitive inhibitor of FGFR2IIIb	175

<i>Discussion</i>	180
Inducible knockdown is inefficient	180
Differentiation of dox-inducible sh clones	181
Concentration-dependent role of FGFs	183

Chapter 8

Conclusions	186
Variation between pluripotent stem cell lines	186
Endogenous production of FGFs	187
Time course of otic gene expression	189
The role of FGFR2IIIb	190
References	191