



Universiteit
Leiden
The Netherlands

Inhibition of signaling cascades in osteoblast differentiation and fibrosis

Krause, C.

Citation

Krause, C. (2011, October 5). *Inhibition of signaling cascades in osteoblast differentiation and fibrosis*. Retrieved from <https://hdl.handle.net/1887/17892>

Version: Corrected Publisher's Version

License: [Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden](#)

Downloaded from: <https://hdl.handle.net/1887/17892>

Note: To cite this publication please use the final published version (if applicable).

Inhibition of Signaling Cascades in Osteoblast Differentiation and Fibrosis

Carola Krause

Copyright © 2011 Carola Krause
ISBN/EAN: 978-90-6464-502-0
Printed by Ponsen & Looijen

The work in this thesis has been carried out at the Leiden University Medical Center. The author was funded by the Dutch Organization for Scientific Research (NWO 918.66.606), Centre for Biomedical Genetics and European Union FP7 Program TALOS.

Inhibition of Signaling Cascades in Osteoblast Differentiation and Fibrosis

PROEFSCHRIFT

ter verkrijging van

de graad van Doctor aan de Universiteit Leiden,

op gezag van Rector Magnificus prof. mr. P.F. van der Heijden,

volgens besluit van het College voor Promoties

te verdedigen op woensdag 5 oktober 2011

klokke 11:15 uur

door

Carola Krause

geboren te Neubrandenburg, Duitsland

in 1981

Promotor:

Prof. dr. Peter ten Dijke

Manuscriptcommissie:

Prof. dr. Socrates Papapoulos

Prof. dr. Wim van Hul

Dr. Dorien Peters

Universiteit Antwerpen

CONTENTS

Contents	v
Preface	xi
Scope of investigations	xiii
Outline of this thesis	xv
 I Inhibition of Signaling Cascades in Osteoblast Differentiation	 1
1 Signal transduction cascades controlling osteoblast differentiation	5
1.1 Summary	5
1.2 Introduction	5
1.3 Runx2 and Osterix transcription factors	6
1.4 BMP signaling	7
1.5 TGF- β signaling	8
1.6 Wnt signaling	9
1.7 Hedgehog signaling	9
1.8 PTH signaling	10
1.9 IGF-1 signaling	11
1.10 FGF signaling	12
1.11 Notch signaling	12
1.12 Concluding remarks	12
1.13 Acknowledgments	13
1.14 References	13
 2 Noggin	 23
2.1 Abstract	23
2.2 Introduction	23
2.3 Structure	25
2.4 Expression, activation and turnover	26
2.4.1 Noggin expression in ectoderm derivatives	26
2.4.2 Noggin expression in mesoderm derivatives	26
2.5 Biological Function	28
2.6 Possible Medical and Industrial Applications	28
2.6.1 Noggin's affinity to BMPs	28

2.6.2	Susceptibility of BMPs to Noggin	29
2.6.3	Noggin's bioavailability	29
2.7	Acknowledgments	30
2.8	References	30
3	Identification of a key residue mediating bone morphogenetic protein (BMP)-6 resistance to Noggin inhibition allows for engineered BMPs with superior agonist activity	35
3.1	Abstract	35
3.2	Introduction	35
3.3	Experimental Procedures	37
3.4	Results	41
3.4.1	Comparative Analysis of the Osteogenic Activity of a BMP Panel Revealed a Significant Difference in Activity between BMP-6 and BMP-7	41
3.4.2	BMP-6 and BMP-7 Induce Noggin Expression with Different Potencies	43
3.4.3	BMP-6 is more resistant to Noggin inhibition than BMP-7	45
3.4.4	BMP-6 and BMP-7 have comparable binding characteristics to immobilized Fc- Noggin using a Biosensor assay	45
3.4.5	Noggin inhibition of BMP binding to cell surface BMP receptors is more pronounced in the case of BMP-6 compared to BMP-7	45
3.4.6	A central region of the mature domain of BMP-6 confers Noggin resistance	47
3.4.7	A single amino acid substitution in BMP-7 yields a protein with increased resistance to Noggin	49
3.4.8	Mutation of BMP-2 at a position analogous to BMP-7 E60 yields a BMP-2 variant with increased resistance to Noggin	50
3.5	Discussion	50
3.6	Acknowledgments	54
3.7	Supplementary Data	55
3.8	References	56
4	Osteocyte-derived Sclerostin inhibits bone formation; Its role in BMP and Wnt signaling	63
4.1	Abstract	63
4.2	Identification of the <i>SOST</i> gene encoding Sclerostin	63
4.3	Sclerostin structure and expression	64
4.4	Mechanism of action of Sclerostin	65
4.4.1	Sclerostin as BMP antagonist	66
4.4.2	Sclerostin as Wnt antagonist	66
4.5	Concluding remarks and therapeutic potential	68
4.6	References	68

5	Distinct modes of inhibition by Sclerostin on bone morphogenetic protein and Wnt signaling pathways	75
5.1	Abstract	75
5.2	Introduction	75
5.3	Experimental Procedures	77
5.4	Results	81
5.4.1	Sclerostin inhibits Wnt signaling	81
5.4.2	Sclerostin inhibits BMP signaling	83
5.4.3	Reduced <i>SOST</i> expression leads to elevated BMP signaling	84
5.4.4	Sclerostin sequesters BMP-7 and mediates proteasomal degradation of intracellular BMP-7	86
5.4.5	Sclerostin's effect on BMP signaling is independent of Wnt signaling	87
5.4.6	Elevated Wnt- and BMP-signaling in Sclerostin knock out mice .	88
5.5	Discussion	91
5.6	Acknowledgments	95
5.7	Supplementary Data	95
5.8	References	100
6	Modulating osteoblast differentiation – Concluding Remarks	111
6.1	Noggin – an inhibitor of bone formation	111
6.2	Sclerostin – an inhibitor of bone formation	112
6.3	References	114
II	Inhibition of Signaling Cascades in Fibrosis	117
7	Signal transduction cascades controlling fibrosis in Dupuytren's Disease	121
7.1	Dupuytren's Disease	121
7.1.1	Epidemiology	121
7.1.2	Etiology	121
7.1.3	Pathogenesis	122
7.2	Treatment of Dupuytren's Disease	123
7.3	Growth factor signaling in Dupuytren's Disease	123
7.3.1	TGF- β signaling	123
7.3.2	BMP signaling	125
7.3.3	PDGF signaling	126
7.3.4	bFGF signaling	126
7.3.5	EGF signaling	128
7.3.6	GM-CSF signaling	128
7.3.7	IFN- γ signaling	129
7.4	References	129

8	Elevated TGF-β and MAP kinase pathways mediate fibrotic traits of Dupuytren's disease fibroblasts	137
8.1	Abstract	137
8.1.1	Background	137
8.1.2	Results	137
8.1.3	Conclusion	138
8.2	Background	138
8.3	Methods	139
8.4	Results	145
8.4.1	TGF- β /Smad signaling is upregulated in DD	145
8.4.2	SB-431542 inhibited fibrogenic properties of Dupuytren's fibroblasts	148
8.4.3	BMP-6 attenuated TGF- β signaling in Dupuytren's fibroblasts	150
8.4.4	ERK1/2 MAP kinase signaling elevated in DD	150
8.4.5	Targeting of TGF- β type I receptor and ERK1/2 MAP kinase pathways in Dupuytren's fibroblasts	152
8.5	Discussion	154
8.6	Conclusions	157
8.7	Acknowledgments	157
8.8	Supplementary Data	158
8.8.1	Supplementary Figures	158
8.9	References	161
9	Concurrent inhibition of TGF-β and mitogen driven signaling cascades in Dupuytren's Disease – non-surgical treatment strategies from a signaling point of view	169
9.1	Summary	169
9.2	Introduction	170
9.2.1	TGF- β signaling in Dupuytren's Disease	171
9.2.2	MAPK signaling in Dupuytren's Disease	171
9.3	Hypothesis	171
9.4	Evaluation of the hypothesis	171
9.5	Testing the hypothesis	172
9.6	Consequences of the hypothesis	174
9.7	Acknowledgments	174
9.8	References	174
A	Abbreviations	179
B	Summary	183
C	Samenvatting	185
D	Acknowledgments	189

E Curriculum Vitae (dutch)	191
F Curriculum Vitae (english)	193
G List of Publications	195

