

**THE ROLE OF FOXM1 IN GROWTH FACTOR-MEDIATED PANCREATIC
BETA-CELL PROLIFERATION**

By

Jia Zhang

Dissertation

Submitted to the Faculty of the
Graduate School of Vanderbilt University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

in

Cell and Developmental Biology

May, 2010

Nashville, TN

Approved:

Professor David M. Miller

Professor Maureen A. Gannon

Professor Stacey S. Huppert

Professor Patricia A. Labosky

Professor Anna. L Means

DEDICATION

This dissertation is dedicated to my wonderful parents, who made all this possible:

- ◆ the memory of my father, Shaoming Zhang, who has been my role model for hard work, persistence, responsibility, and personal sacrifice and who loved and supported me unconditionally in my entire life.
- ◆ my mother, Ying Ren, who emphasized the importance of education, who instilled in me the inspiration to set high goals and the confidence to achieve them.

ACKNOWLEDGEMENTS

A great many people have contributed to this dissertation. I owe my gratitude to all those people who have made this dissertation possible and because of whom my graduate school experience has been one that I will cherish forever.

First, no words can describe my deep gratitude to my mentor, Dr. Maureen Gannon, who has been an incredible advisor, colleague and friend that everyone wished they had, who believed in me when I was so close to quitting graduate school and convinced me that I should give it another try. Now, almost three years later, I am about to graduate and have set my career path in science. She taught me to think critically and rigorously as a scientist, more importantly, to live honorably and confidently as a human being. She showed me that scientific questions can be discussed and addressed in such a solid, efficient yet enjoyable matter. Being able to work with her is my great motivation to show up in the lab in the morning, especially during my down time. She is always an inspiration to me and many other women in science. I hope that I will be able to pass on her spirits to the people I know in the future.

I truly appreciate my dissertation committee members, my chair, Dr. David Miller and Dr. Patricia Labosky, Dr. Stacey Huppert, and Dr. Anna Means, for serving on all my committee meetings, for their guidance and suggestions to my dissertation work and my career choice, for going out of their way to encourage me and help me whenever I have needed them.

I am grateful to be part of the amazing scientific community here at Vanderbilt University. Thank you to the Department of Cell and Developmental Biology, the Program in Developmental Biology, and the Diabetes Research and Training Center,

and the Beta cell Interest Group for providing cutting-edge training environments and for broadening my scientific horizons.

I owe great appreciation to both the past and present members in the Gannon lab. Thank you all for making the lab not only a wonderful place to work but also my family here in the United States. Hongjie Zhang, Amanda Ackermann-Misfeldt and Renuka Menon, thank you so much for your suggestions and expertise with regard to my dissertation research and for being supportive colleagues and friends. Young Ah Oh and Nikul Patel, thank you for helping me so patiently with countless experiments. Thank you to Shidrokh Ardestani, Obi Umunakwe and Paige Cooper. I am always impressed by the energy and fresh mind you guys brought to my life.

Michelle Guney, Christine Pope Petersen, Kathryn Henley, Maria Golson and Uma Gunasekaran, I will definitely miss the laughter and tears we have shared over the years. Of course, thank you all for being so warm and kind to help me conquer language and cultural barriers on a daily basis. Michelle, thank you for being such a sweet colleague and friend, who supports me both intellectually and emotionally. My hearty appreciation also goes out to Christine, who holds the “Queen” title of so many techniques in the lab. I can not thank you enough for your hard work! Kathryn, I benefit so much from your upbeat personality. Maria, thank you for challenging and refining my scientific thoughts. Uma, I am so glad that I have gotten to know you, thank you for your practical advice on living a healthy and balanced life.

I could never have reached the heights or explored the depths without the help and efforts of a lot of people, including: Dr. Roland Stein and his lab members, Dr. Al Powers and his lab members, Dr. Guoqiang Gu and Dr. Sui Wang, Dr. Richard O'Brien and his lab members, Dr. Mark Magnuson, Dr. Yanyun Gu, Jennifer Plank, Dr. Masa Shiota, Dr. Rob Carnahan, Tracy Triplett, Dr. Garcia-Ocaña and Dr. Rupi Vasavada. I would also like to acknowledge the staff in the Islet Procurement and Analysis Core,

especially Anastasia Golovin, who is absolutely incredible. Many thanks to the staff in the Monoclonal Antibody Core, the Microarray Shared Resource, the Molecular Biology Shared Resource and the Hormone Assay and Analytical Service Core.

I also extend my gratitude to my friends that I feel so fortunate to have. Very special thanks to Pan Fong Chen, Jesse Ward, Yan Hang, Qing Cai, Juan Xing, Joseph Roland, Ziyi Sun, Xiaoming Zhou, Xi Huang, Guanglei Zhuang and Zhibo An for always being by my side to multiply my joy and divide my grief throughout my graduate school years. Good luck with all your future adventures!

TABLE OF CONTENTS

	Page
DEDICATION.....	ii
ACKNOWLEDGEMENTS.....	iii
LIST OF TABLES.....	ix
LIST OF FIGURES.....	x
Chapter	
I. GENERAL INTRODUCTION.....	1
Diabetes Mellitus.....	1
Gestational Diabetes Mellitus.....	2
Definition.....	2
Maternal and Fetal Complications	2
Diagnosis and Treatment.....	3
Pathophysiology.....	4
Genetics.....	6
Animal Models of GDM.....	14
Regulation of Postnatal Pancreatic β -cell Mass.....	16
Neonatal β -cell Mass Expansion.....	18
Adult β -cell Mass Expansion.....	18
Maternal β -cell Mass Expansion During Pregnancy.....	18
Mammalian Cell Cycle Progression.....	21
Regulation of β -cell Proliferation by Cyclins, CDKs and CDKIs.....	24
Regulation of β -cell Proliferation by Growth Factors.....	28
Foxhead Box m1.....	31
Forkhead Transcription Factor Family.....	31
Foxm1 Transcription Factor: Gene and Protein.....	34
Transcriptional Regulation of Foxm1.....	35
Post-translational Regulation of Foxm1.....	37
Cell Proliferative Role of Foxm1: Lessons Learn from Foxm1 Mouse Models.....	40
The Role of Foxm1 in the Pancreas.....	42
Foxm1 Target Genes.....	43
Overview and Aims of Dissertation.....	44
II. MATERIALS AND METHODS.....	52
Mice.....	52
DNA Extraction and Genotyping.....	58
Intraperitoneal Glucose Tolerance Test (IPGTT).....	60
Plasma and Pancreatic Insulin Content.....	60

Islet Isolation.....	61
RNA Isolation and Quantitative Real-Time RT-PCR (qRT-PCR).....	61
Tissue Preparation and Histology.....	62
β-cell Mass Analysis.....	65
β-cell Proliferation Analysis.....	65
β-cell Apoptosis Assay.....	66
Islet Size, β-cell Size Analysis.....	66
<i>In vitro</i> Islet Culture.....	66
Dispersed Islet Cell Culture with HGF.....	67
Western Blotting.....	69
Chromatin Immunoprecipitation (ChIP) Assays.....	69
Statistical Analysis.....	72
III. FOXM1 STIMULATES β-CELL REPLICATION DOWNSTREAM OF PL SIGNALING IN ADULTS.....	73
Introduction.....	73
Results.....	76
Impaired β-cell Proliferation in Pregnant <i>Foxm1^{Δpanc}</i> Mice.....	76
Defective β-cell Mass Expansion in Pregnant <i>Foxm1^{Δpanc}</i> Mice.....	76
<i>Foxm1</i> Islet mRNA Increased During Pregnancy.....	79
Post-partum Islet Changes in <i>Foxm1^{Δpanc}</i> Females.....	82
Increased Expression of Cell Cycle Inhibitors in Pregnant <i>Foxm1^{Δpanc}</i> Islets.....	84
<i>Foxm1</i> Functions Downstream of PL.....	86
Overexpression of PL did not Induce <i>Foxm1</i> or Its Target Gene Expression.....	90
Discussion.....	90
IV. ANALYSIS OF FOXM1 FUNCTION IN HGF AND IGF-1 STIMULATED β-CELL PROLIFERATION IN ADULTS.....	102
Introduction.....	102
Results.....	104
<i>RIP-HGF</i> Transgene Remained Actively Expressed After Genetic Backcrossing.....	104
Overexpression of HGF in the β-cell did not Induce the Expression of <i>Foxm1</i> or its Target Genes.....	105
<i>RIP-IGF-1</i> Transgene was Actively Expressed After Backcro.....	105
Overexpression of IGF-1 in the β-cell did not Induce the Expression of <i>Foxm1</i> or its Target Genes.....	107
Discussion.....	107
V. GENERATING A MOUSE FOXM1 ANTIBODY.....	110
Introduction.....	110
Results.....	112
Mouse A/J-L was Chosen for Fusion in the Generation of Monoclonal Antibody.....	112
Screening of Hybridoma Clones.....	112
Generating <i>Foxm1</i> Polyclonal Antibodies.....	116

Discussion.....	119
VI. SUMMARY AND FUTURE DIRECTIONS.....	121
REFERENCES.....	127

LIST OF TABLES

Table	Page
1. Diagnosis of GDM.....	5
2. Changes in Measures of Metabolism in Normal Pregnancy.....	5
3. Genes associated with GDM.....	7
4. Expression of cell cycle regulators in the β -cell.....	25
5. Direct Foxm1 target genes and Foxm1-regulated genes.....	48
6. Genotyping primers and parameters of PCR programs.....	63
7. Primers for qRT-PCR.....	63
8. Primers for ChIP assays.....	68

LIST OF FIGURES

Figure	Page
1. Glucose Stimulated Insulin Secretion (GSIS).....	10
2. β -cell mass dynamics.....	17
3. β -cell mass dynamics during pregnancy in mice.....	19
4. Maternal β -cell proliferation during gestation in B6D2 mice.....	20
5. The simplified schematic representation of the mammalian cell cycle.....	23
6. Growth factor-stimulated β -cell proliferation.....	33
7. Splicing variants of human FoxM1 gene and structure of protein isoforms.....	38
8. Post-translational modification of human FOXM1.....	38
9. Foxm1 is highly expressed within the endocrine pancreas and is required for normal postnatal β -cell growth and proliferation.....	46
10. β -cell proliferation was unaffected in FoxM1 ^{Δpanc} embryos versus control littermates.....	47
11. Foxm1 directly regulates many genes (yellow ovals) involved in multiple stages of cell cycle regulation.....	51
12. Schematic of Foxm1 ^{fl} gene targeting and generation of Foxm1 ^{Δpanc}	53
13. Pdx1 ^{5.5kb} -Cre transgene.....	54
14. Immunohistochemical localization of PL in the RIP-PL transgenic and normal pancreas.....	56
15. RNase protection analysis of total RNA isolated from pancreas from RIP-HGF transgenic mice (Tg) and normal littermates(NI).....	57

16. Immunohistochemical detection of HGF in the pancreas.....	59
17. Dispersed islet cell clusters immunolabeled with BrdU (red) and insulin (green).....	68
18. The absence of FoxM1 caused glucose intolerance at GD12.5 and GDM at GD15.5.....	77
19. <i>Foxm1</i> ^{Δpanc} females exhibited impaired β -cell proliferation.....	78
20. Decreased β -cell mass in virgin and pregnant <i>Foxm1</i> ^{Δpanc} mice.....	80
21. Elevated <i>Foxm1</i> expression in pregnancy.....	81
22. Lasting post-partum changes in <i>Foxm1</i> ^{Δpanc} female pancreata.....	81
23. Post-partum β -cell mass is restored in <i>Foxm1</i> ^{Δpanc} female mice due to increased islet neogenesis and β -cell hypertrophy.....	83
24. <i>Foxm1</i> ^{Δpanc} female mice are euglycemic after pregnancy.....	85
25. Increased p27 and Menin in <i>Foxm1</i> ^{Δpanc} female during pregnancy.....	87
26. Foxm1 acts downstream of PL to mediate increases in β -cell proliferation and β -cell mass	89
27. Potential Stat5 binding sites in the mouse <i>Foxm1</i> 5' promoter region.....	91
28. Stat5 binds to Foxm1 promoter in PL treated INS-1 cells.....	91
29. Model of PL and Foxm1 regulation of β -cell proliferation during pregnancy..	92
30. PL overexpression did not increase expression of <i>Foxm1</i> or its target genes	93
31. Overexpression of HGF in the β -cell results in increased β -cell proliferation and β -cell mass.....	103
32. Active <i>RIP-HGF</i> transgene in backcrossed <i>RIP-HGF</i> mice.....	106

33. Overexpression of HGF is not able to induce Foxm1 or its target genes....	106
34. Robust IGF-1 expression in backcrossed <i>RIP-IGF-I</i> mice at 9 wks of age..	109
35. Overexpression of IGF-1 did not induce <i>Foxm1</i> or its target gene expression.....	109
36. Available anti-Foxm1 antibodies are not specific.....	113
37. Fragments of mouse Foxm1 for immunization.....	113
38. Generation of a monoclonal antibody.....	114
39. Endogenous mouse Foxm1 was detected by sera from two antigen-injected mice.....	115
40. Endogenous Foxm1 protein was detected by the 6 th bleed antiserum from mouse A/J-L.....	117
41. Representative western blotting of hybridoma producing Foxm1 antibody	117
42. Western blotting examination of first bleed anti-sera for the generation of polyclonal Foxm1 antibody in mice and rats.....	118
43. The central role of Foxm1 in facultative β -cell proliferation.....	124