Characterisation of an oocyte specific knockout model of Cdh1

Suzanne My-Trinh Tran

PhD,

BSc (Hons) Chemistry,

BSc (Hons) Human Physiology

A thesis submitted in fulfilment

of the requirements for the degree of

Master of Philosophy

April 2012

School of Biomedical Sciences and Pharmacy

University of Newcastle

Callaghan NSW 2308

Australia

Statement of Originality

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. I give consent to this copy of my thesis, when deposited in the University Library**, being made available for loan and photocopying subject to the provisions of the Copyright Act 1968.

****Unless an Embargo has been approved for a determined period.**

Statement of Collaboration

I hereby certify that the work embodied in this thesis has been done in collaboration with other researchers, or carried out in other institutions. I have included as part of the thesis a statement clearly outlining the extent of collaboration, with whom and under what auspices.

Acknowledgements

I would like to acknowledge the following people involved in this research:

- My supervisor, Prof Keith Jones.
- Dr Phoebe Jennings for performing the majority of the kinetochore counts for not only me, but also other members of our research group.
- Dr Janet Holt for her part in the Western blots of cyclin B1 and securin and the primers for chromosomes 15-19.
- Prof Eileen McLaughlin for the ZP3-Cre male mice and the Cre primers.
- Dr Sergio Moreno (Instituto de Biología Molecular y Celular del Cáncer, CSIC / Salamanca University, Campus Miguel de Unamuno, 37007 Salamanca, Spain) for the supplying the initial six male and six female mice used to set up the colony of loxFZR mice.

I would also like to thank The University of Newcastle, Australia for UNIPRS and UNRSC scholarships and the faculty for funding this project.

I would like to extend my thanks to the University of Newcastle's Security for escorting me home safely on countless late nights from the lab, and other staff and students of the university who have been kind to me throughout my time in Australia.

Last but not least, I would like to convey my gratitude for the love and support from my family and friends, both old and new.

Contents

CHAPTER 2 Materials and Methods

CHAPTER 3 Studies on Cdh1 using a ZP3-Cre driven oocyte specific knockout model

List of Figures

CHAPTER 1

CHAPTER 2

CHAPTER 3

List of Tables

Abbreviations

Approx.	approximately
BSA	bovine serum albumin
cAMP	cyclic adenosine 3’-5 monophosphate
CCD	charge coupled device
CEO	cumulus enclosed complex
Cntl	control
dbcAMP	dibutyrl cAMP
dNTPs	Deoxyribonucleotide triphosphate
EDTA	Disodium Salt Dihydrate or Ethylenediaminetetraacetic acid
EGTA	Ethylene glycol-bis(2-Amino ethyl ether)-N,N, N’,N’-tetra acetic acid
EtBr	Ethidium bromide
F	female
FCS	foetal calf serum
fl	floxed
FSH	follicle stimulating hormone
GV	germinal vesicle
GVB	germinal vesicle breakdown
H&E	Hematoxylin & Eosin
h	hour
hCG	human chorionic gonadotrophin
HEPES	N-(2-Hydroxyethyl) piperazine-N'-(2-ethanesulfonic acid)
Het	heterozygote
IVM	*in vitro* maturation
KO	knockout
LH	luteinizing hormone
MEM	minimal essential medium

mg	milligrams
MgCl	magnesium chloride
MI	meiosis I
MII	meiosis II
Mil	milrinone
Milli-Q	Distilled water filtered through 0.22 um Millipak®Express Millipore
Min	minutes
PBE	polar body extrusion
PBS	Phosphate buffered saline
PCR	polymerase chain reaction
PDE	phosphodiesterase
PFA	paraformaldehyde
Pgcs	primordial germ cells
PIPES	piperazine-N,N'-bis(ethanesulfonic acid)
PKA	protein kinase A
PMSG	pregnant mare's serum gonadotrophin
PVP	polyvinylpyrrolidone
Rpm	revs per minute
Rt	room temperature
SDS	sodium dodecyl sulfate
sec	seconds
TAE	Tris-Acetate-EDTA
µm	micrometer
µM	micromolar
V	volts
Wt	wildtype
Zona	zona pellucida

Abstract

Cdh1, a co-activator of the Anaphase Promoting Complex (APC) has recently been shown to be important in germinal vesicle stage arrest and in the prevention of aneuploidy during the first meiotic division of mouse oocytes. However, this was through antisense knockdown approaches done *in-vitro*. Therefore, here I generated an oocyte-specific knockout of Cdh1 (Δ/Δ) to explore this further. In this way, Cdh1 protein was specifically deleted only in germinal vesicle (GV) stage oocytes from growing follicles. Fewer cumulus enclosed oocytes were observed from Δ/Δ mice, of which significant numbers had undergone GVB. Furthermore, significantly more meiotically advanced, fragmented or degenerate oocytes were observed in knockouts. Denuded Δ/Δ GV oocytes also displayed a propensity for spontaneous GV breakdown (GVB) which could be partially rescued by maintaining an intact cumulus mass. Δ/Δ oocytes also underwent accelerated GVB on release from arresting media. Western Blots revealed a 5 fold increase in cyclin B1 levels following loss of Cdh1, whereas other substrates of APC^{Cdh1}, securin and Cdc25B, remained unchanged.

In-vivo and *in-vitro* matured metaphase II (MII) oocytes were analysed for aneuploidy rates. *In-vivo* matured knockout oocytes had higher, but not statistically significant, rates of aneuploidy than controls. Denuded oocytes that underwent IVM also had a

higher incidence of aneuploidy in knockouts and in this group this was highly significant. In summary, data from the *in-vivo* knockout model supports those of the *in-vitro* antisense approach and provide further evidence for the role of Cdh1 in both GV arrest and the prevention of aneuploidy, at least in the situation where oocytes are cultured *in-vitro*.