Analysis of the function and regulation of the centrosomal protein NEDD1 during cell division and development

A thesis submitted for the

degree of Doctor of Philosophy

Jantina Manning

B.Biotech. (Hons.)

Enrolled through the Department of Medicine, Faculty of Health Sciences, University of Adelaide

Research conducted in the Department of Haematology, Institute of Medical and Veterinary Science, Adelaide

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Amendments

Abstract

- Line 5: "neural precursor cell expressed, developmentally down-regulated gene 1" should follow Nedd1

Abbreviations

- The following abbreviations should be included: SCL, stern cell leukemia; Plk, polo-like kinase; γ-TuRC, γ-tubulin ring complex; MOPS, 3-{N-morpholino] propanesulfonic acid; FA, formamide; TMR, tetramethyl-rhodamine

Chapter 1

- p11, line 7: "was" should be deleted
- p15, line 13: results should read "can result"
- p16, line 12: beidl should read "biedl"

Chapter 2

- Throughout: manufacturers' should read manufacturer's
- p28, line 15: "at 260 nm" should be added after (OD)
- p30, line 18: "KOAc" should read "potassium acetate", KCL should read "KCl"
- p34, line 11: $\Delta\Delta C_T$ method is C_t value for any sample normalised to GAPDH minus the C_t value for the calibrator also normalised to GAPDH
- p41, line 1: "laminar flow" should be deleted
- p47, line 19: "mAmps" should read "mAMPs"
- p53, line 5: "dioxygen" should read "digoxigenin"
- p80, line 1 and 2: "Fig. 7C-E" and "Fig. 7C" should read "Fig. 4.7C-E" and "Fig. 4.7C"

Chapter 4

- p88, line 6/7: should read " This chapter has confirmed that NEDD1 can be regulated by its phosphorylation state."

Chapter 5

- p93, line 9: "sufficient" should read "required"
- p96, line 21: "flag-conjugated" should read "flag antibody-conjugated"
- p104, line 25: "Fig. 5.10" should read "Fig. 5.11"
- p105, line 25: "caused" should read "may cause"

Chapter 6

- Figure 6.7D: the final lane (1-676) should also be labelled "no immunoprecipitation"
- p118, line 13: "other in" should read "in other"
- p118, line 23: "(human neuronal protein C)" should be inserted after HuC

Chapter 7

- p130, line 25: "the primary" should read "one"

References

- Zhang 2009 reference should include "122, 2240-2251"

Abstract

The centrosome is the major microtubule organising centre of cells and serves as a centralised location for controlling many cellular processes. A critical component of the centrosome is the γ-tubulin ring complex (γ-TuRC) which is required for the nucleation of microtubules, correct formation of the mitotic spindle and hence progression of the cell cycle. NEDD1 (mouse: Nedd1), was recently discovered as a centrosomal protein which functions primarily in targeting the γ-TuRC to the centrosome and spindle.

Given the fundamental role of the centrosome in mitosis and other processes, it is no surprise that this organelle is essential during mouse development. To examine the precise role of the centrosome during development, this study analysed the expression and localisation of Nedd1 during mouse embryogenesis. This revealed a dynamic localisation of Nedd1 and the centrosome during development, and provides further evidence for their critical role in development.

To investigate the regulation of NEDD1, its expression during the cell cycle was analysed. It was found that phosphorylation is the primary method of NEDD1 regulation. Additionally, it was observed that Nedd1 levels decreased upon the entry of mouse embryonic fibroblasts into cell culture-induced senescence (an irreversible state of cell cycle arrest). This correlated with a loss of centrosomal integrity. Ablation of Nedd1 in healthy cells caused premature senescence and centrosome abnormalities, suggesting that Nedd1 and the centrosome may contribute to this senescence.

NEDD1 is also important in the recruitment of the γ-TuRC to the centrosome, which is essential for correct centrosome biogenesis and function. This study identified a 62 amino acid region of NEDD1 that interacts with γ-tubulin and can abrogate its function. Key

residues important for this interaction were also revealed. Additional interacting proteins of NEDD1 were also identified, and the chaperone TCP-1α was characterised in more detail and shown to regulate NEDD1.

Given the currently known functions of NEDD1, it was expected to be important in development. Zebrafish were chosen as a model to study this because of their many advantages for developmental studies. A zebrafish homologue of NEDD1 was identified that displayed a similar localisation and function to mammalian NEDD1. Depletion of this protein caused lethality or phenotypic abnormalities which were most obvious in the central nervous system, depending on the extent of knockdown. This demonstrates that NEDD1 is critical for development, particularly in the nervous system.

The results presented in this thesis contribute to the understanding of the function and regulation of NEDD1, and thus also the centrosome, and highlights the importance of this protein during development. Additionally, this study forms the foundation for further work on centrosomes, using NEDD1 as a marker for centrosomal dynamics and function.

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Jantina Manning 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.

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* **Manning, J.**, Colussi, P., Koblar, S., and Kumar S. (2008). Nedd1 expression as a marker of dynamic centrosomal localization during mouse embryonic development. *Histochem Cell Biol, 129*(6),751-64.

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Publications

The following publications have resulted from work performed by the candidate during the period of this candidature:

Manning, J., Colussi, P., Koblar, S., and Kumar S. (2008). Nedd1 expression as a marker of dynamic centrosomal localization during mouse embryonic development. *Histochem Cell Biol, 129*(6), 751-64.

Manning, J. and Kumar, S. (2007). NEDD1: function in microtubule nucleation, spindle assembly and beyond. *Int J Biochem Cell Biol, 39*(1), 7-11.

Ekberg, J., Schuetz, F., Boase, NA., Conroy, SJ., **Manning, J.**, Kumar, S., Poronnik, P., Adams, DJ. (2007). Regulation of the voltage-gated K(+) channels KCNQ2/3 and KCNQ3/5 by ubiquitination. Novel role for Nedd4-2. *J Biol Chem*, *282*(16),12135-42.

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Abbreviations

γTuRC	γ-tubulin ring complex
aa	amino acid
AP	alkaline phosphatase
BCIP	5-bromo-4-chloro-indolyl-phosphatase
bp	base pair
BSA	bovine serum albumin
C	carboxyl
Cdk	cyclin-dependent kinase
cDNA	complementary deoxyribonucleic acid
CNS	central nervous system
DAB	3, 3’ diaminobenzidine
DEPC	diethyl pyrocarbonate
DIG	digoxigenin
DMF	dimethylformamide
DNA	deoxyribonucleic acid
DOC	deoxycholate
DRG	dorsal root ganglia
DTT	1,4-dithiothreitol
E	embryonic day
E. coli	*Escherichia coli*
ECL	enhanced chemiluminescence
EDTA	ethylenediaminetetra-acetic acid
FBS	fetal bovine serum
FL	full length
g	gravity
GENSAT	gene expression nervous system atlas
GFP	green fluorescent protein
GRP	glucose-related protein
GST	glutathione-S-transferase
h	hour(s)
HAUS	human augmin complex
HEPES	N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid
His	histidine

hpf	hours post fertilisation
HRP	horse radish peroxidase
HSP	heat shock protein
IB	immunoblot
INL	inner neuroblastic layer
IP	immunoprecipitation
IPTG	isopropyl-β-D-thiogalactopyranoside
JUP	junction plakoglobin
kb	kilobase
kDa	kiloDalton
L	leucine
LB	Luria-Bertani
LV	lens vesicle
MBT	midblastula transition
MEF	mouse embryonic fibroblast
min	minute(s)
MO	morpholino
MOPS	3-(n-morpholino) propanesulfonic acid
MQ	milli-Q
mRNA	messenger ribonucleic acid
MS	mass spectrometry
MTOC	microtubule organising centre
NBT	nitroblue tetrazolium
NCBI	National Center for Biotechnology Information
NEDD1/Nedd1	*neural precursor cell expressed, developmentally down-regulated gene 1*
Ni-NTA	nickel-nitrilotriacetic acid
NP-40	nonidet P40
OD	optical density
ONL	outer neuroblastic layer
ORF	open reading frame
p	passage
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate buffered saline
PCM	pericentriolar matrix
PCR	polymerase chain reaction
PEG	polyethylene glycol
PFA	paraformaldehyde

pH3	phosphorylated histone H3
PI	propidium iodide
PLB	protein loading buffer
PVDF	polyvinylidene difluoride
Q	glutamate
qPCR	quantitative polymerase chain reaction
RNA	ribonucleic acid
RNAse	ribonuclease
RS	replicative senescence
RT	room temperature
SA-β-gal	senescence associated β-galactosidase
SDS	sodium dodecyl sulfate
sec	second(s)
SEM	standard error of the mean
siRNA	small interfering RNA
SSC	sodium chloride-sodium citrate
TAE	tris-acetate-EDTA
TCP-1	t-complex protein 1
TEMED	tetramethylethylenediamine
WT	wild type
X-gal	5-bromo-4-chloro-3-indolyl-β-D-galactosidase