Functional Analysis of ANKRD11 and FBXO31:

Two Candidate Tumour Suppressor Genes from the 16q24.3

Breast Cancer Loss of Heterozygosity Region

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Thesis submitted for the Degree of Doctor of Philosophy

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January 2008

This thesis is dedicated to my brother;

Although you are no longer with us, your undying determination and passion for life remains with us all.

You have always be my guiding light in times of dark, my guardian angel in the presence of evil.

I know you will always be with me, and together we will walk, hand in hand, along the road towards a cure.

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Erratum

Erratum to the doctoral thesis entitled "Functional Analysis of *ANKRD11* and *FBXO31*: Two Candidate Tumour Suppressor Genes From the 16q24.3 Breast Cancer Loss of Heterozygosity Region" by Paul Neilsen.

Abstract – Page VII – Second Paragraph – Line 3:

Replace the comma with "which"

Introduction – Page 2 – Third Paragraph – Line 7:

Change "single nucleotide polymorphism" to "tumour-restricted single nucleotide polymorphism"

Chapter 3 – Page 54 – First Paragraph – Line 4:

Insert the sentence "Transiently-expressed ANKRD11 protein accumulated in nuclear foci that were more numerous and larger in size than that of endogenous ANKRD11."

Abstract

Loss of heterozygosity (LOH) on the long arm of chromosome 16 is frequently observed during the onset of breast cancer. Our laboratory has recently identified both *ANKRD11* and *FBXO31* as candidate tumour suppressor genes in the chromosome band 16q24.3, which is the smallest region of overlap for breast cancer LOH. This thesis focuses on the functional analysis of these two novel genes and implicates a role for them as breast cancer tumour suppressors.

ANKRD11: a novel p53 coactivator involved in the rescue of mutant p53

The ability of p53 to act as a transcription factor is critical for its function as a tumour suppressor. Ankyrin repeat domain 11 (ANKRD11) was found to be a novel p53-interacting protein which enhanced the transcriptional activity of p53. ANKRD11 expression in breast cancer cell lines was shown to be down-regulated when compared to ANKRD11 expression in finite life-span HMECs and non-malignant immortalized breast epithelial cells. Restoration of ANKRD11 expression in MCF-7 (p53 wild-type) and MDA-MB-468 ($p53^{R273H}$ mutant) cells suppressed the oncogenic properties of these breast cancer cell lines through enhancement of $p21^{waf1}$ expression. ShRNA-mediated silencing of ANKRD11 reduced the ability of p53 to activate $p21^{waf1}$ expression in response to DNA damage. ANKRD11 was shown to associate with the p53 acetyltransferase, P/CAF, and exogenous ANKRD11 expression increased the levels of acetylated p53. Exogenous ANKRD11 expression enhanced the DNA-binding properties of the $p53^{R273H}$ mutant to the *CDKN1A* promoter, implicating a role for ANKRD11 in the restoration of mutant $p53^{R273H}$ function. These findings demonstrate a role for ANKRD11 as a p53 coactivator and illustrate the potential of ANKRD11 in the restoration of mutant $p53^{R273H}$ function.

ANKRD11 has roles beyond that of p53 coactivation. This thesis also presents preliminary findings to suggest that ANKRD11 may be involved in the regulation of eukaryotic cell division. Furthermore, ANKRD11 was shown to function as an estrogen receptor coactivator. Taken together, these finding suggest that ANKRD11 is a multi-functional cancer-related protein.

FBXO31: the 16q24.3 senescence gene

A BAC located in the 16q24.3 breast cancer loss of heterozygosity region was previously shown to restore cellular senescence when transferred into breast tumour cell lines. We have shown that *FBXO31*, although located just distal to this BAC, can induce cellular senescence in the breast cancer cell line MCF-7 and is the likely candidate senescence gene. Exogenous FBXO31 expression inhibited the oncogenic properties of the MCF-7 breast cancer cell line. In addition, compared to the relative expression in normal breast, levels of FBXO31 were down-regulated in breast tumour cell lines and primary tumours. FBXO31 protein levels were cell cycle regulated, with maximal expression from late G_2 to early G_1 phase. Ectopic expression of FBXO31 in the breast cancer cell line MDA-MB-468 resulted in the accumulation of cells at the G_1 phase of the cell cycle. FBXO31 was also shown to be a component of a SCF ubiquitination complex. We propose that FBXO31 functions as a tumour suppressor by generating SCF^{FBXO31} complexes that target particular substrates, critical for the normal execution of the cell cycle, for ubiquitination and subsequent degradation.

Declaration

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university and that, to the best of my knowledge and belief, the thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

I consent to the thesis being made available for photocopying and loan if accepted for the award of the degree.

Signed: Date:

List of Publications

Kumar R, **Neilsen PM**, Crawford J, McKirdy R, Lee J, Powell JA, Saif Z, Martin JM, Lombaerts M, Cornelisse CJ, Cleton-Jansen A-M, Callen DF. (2005). FBXO31 is the chromosome 16q24.3 senescence gene, a candidate breast tumor suppressor, and a component of an SCF complex. *Cancer Res* 65:11304-11313. *Impact Factor = 7.7*

Neilsen PM, Cheney KM, Li CW, Chen JD, Cawrse JE, Schulz RB, Powell JA, Kumar R, Callen DF. (2007). Identification of ANKRD11 as a novel p53 coactivator involved in the rescue of mutant p53. *J Cell Sci.* Submitted November 2007. *Impact Factor = 6.4*

Kumar R, Cheney KM, McKirdy R, **Neilsen PM**, Schulz RB, Lee J, Cohen J, Booker GW, Callen DF (2007). CBFA2T3-ZNF652 corepressor complex regulates transcription of the E-box gene *HEB*. *J Biol Chem.* Submitted November 2007. *Impact Factor = 5.8*

Abbreviations

α-X – Anti-X antibody (e.g. α-FLAG)

ACTR – Acetyltransferase

AD – Activator domain

ADH – Atypical ductal hyperplasia

AIB1 – Amplified in breast cancer 1

AML – Acute myeloid leukaemia

ANK domain – Ankyrin repeat domain

ANOVA – Analysis of variance

AR – Androgen receptor

ASPP – Ankyrin-repeats, SH3 domain and proline-rich region containing protein

ATCC – American type culture collection

BAC – Bacterial artificial chromosome

bHLH – Basic helix-loop-helix

CBP – CREB binding protein

Cdk – Cyclin dependent kinase

cDNA – Complementary DNA

ChIP – Chromatin immunoprecipitation

CKI – Cyclin dependent kinase inhibitor

CREB – cAMP response element-binding

DAPI – 4',6-diamidino-2-phenylindole

DBD – DNA-binding domain

D-box – Destruction Box

DCIS – Ductal carcinoma *in situ*

DNA – Deoxyribonucleic acid

DSB – Double-strand breaks

DTT – Ditheothiol

E1 – Ubiquitin activating enzyme

E2 – Ubiquitin conjugating enzyme

E3 – Ubiquitin ligase

EBI – European Bioinformatics Institute

EBV – Ebstein-Barr virus

EGF – Epidermal growth factor

EGFP – Enhanced green fluorescent protein

ER – Estrogen receptor

ERα – Estrogen receptor alpha

ERβ – Estrogen receptor beta

ERE – Estrogen response element

FCS – Fetal calf serum

GFP – Green fluorescent protein

GR – Glucocorticoid receptor

GRIP-1 – Glucocorticoid receptor interacting protein 1

GST – Glutathione S-transferase

H2A – Histone 2A

HA – Hemagglutinin

HAT – Histone acetyltransferase

HDAC – Histone deacetyltransferase

HMEC – Human mammary epithelial cell

IDC – Invasive ductal carcinoma

IF – Immunofluorescence

IHC – Immunohistochemistry

ILC – Invasive lobular carcinoma

IP – Immunoprecipitation

KLH – Keyhole limpet hemocyanin

LBD – Ligand-binding domain

LCIS – Lobular carcinoma *in situ*

LOH – Loss of heterozygozity

LRES – Long-range epigenetic silencing

MAPK – Microtubule-associated protein kinase

MBP – Maltose-binding protein

MEK2 – MAPK kinase 2

miRNA – Micro RNA

MMC – Mitomycin C

MR – Mineralocorticoid receptor

mRNA – Messenger RNA

NCBI – National center for biotechnology information

NCoA – Nuclear receptor coactivator

NLS – Nuclear localisation signal

NPC – Nasopharengeal carcinoma

ONPG – *O*-nitrophenyl-β-galactopyranoside

ORC2 – Origin recognition complex subunit 2

p53-RE – p53 response element

p/CIP – p300/CBP interacting protein

PAC – P1 artificial chromosome

PAS domain – Per-Arnt-Sim domain

PCR – Polymerase chain reaction

PEST sequence – Proline, glutamic acid, serine and threonine rich sequence

PR – Progesterone receptor

RAC3 – Receptor-associated coactivator 3

RD – Repressor domain

Real-time RT-PCR – Reverse transcription real time-PCR

RNA – Ribonucleic acid

ROS – Reactive oxygen species

RT – Room temperature

SAC – Spindle assembly checkpoint

SAHA – Suberoylanilide hydroxamic acid

SDS – Sodium dodecylsulphate

SDS-PAGE – SDS Polyacrylamide gel electrophoresis

SEM – Standard error of the mean

SERM – Selective estrogen receptor modulator

shRNA – Short hairpin RNA

siRNA – Small interfering RNA

SNP – Single nucleotide polymorphism

SRC – Steroid receptor coactivator

SRO – Smallest region of overlap

SSCP – Single-strand conformation polymorphism

TIF2 – Transcriptional intermediary factor 2

TK – Thymidine kinase

TRAM-1 – Thyroid hormone receptor activator molecule 1

Ub – Ubiquitin

UDH – Usual ductal hyperplasia

WB – Western blot

Y2H – Yeast-2-hybrid

Acknowledgements

As I reflect on the work that has culminated in this thesis, I wish to acknowledge the contributions made by the following people.

Firstly, I sincerely wish to thank my supervisors, Associate Professor David Callen and Dr Raman Sharma. Without their daily guidance, encouragement and support, I certainly would have struggled throughout my candidature. Their generosity and friendship are invaluable to me and will be remembered throughout the rest of my career. One could not have asked for better mentors.

I would like to thank the dynamic team at the Breast Cancer Genetics Laboratory. The friendships that I have developed over the years certainly made my candidature most enjoyable. I also wish to thank those work colleagues who have made direct contributions to my PhD research, including Associate Professor David Callen, Dr Raman Sharma, Dr Kelly Cheney, Renée Schulz, Jacqueline Cawrse, Jaclyn Lee, Zarqa Saif and Professor J. Don Chen. I would also like to thank Dr Ghafar Sarvestani for his expertise on the confocal microscope.

Finally, I sincerely wish to thank my family. The emotional and financial support has provided me with the necessary foundations to build my PhD upon, and without it I surely would not have made it through my candidature. I thank you from the bottom of my heart.