The Role of HP1α and HP1β in Breast Cancer Progression

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Abstract

Breast cancer is the foremost cause of cancer-related deaths in New Zealand women. Metastasis of breast tumours increases the likelihood of fatality of the disease as treatment becomes more difficult and the tumours may interfere with the function of multiple organ systems. Consequently, the identification of biomarkers that may indicate the potential for a tumour to become metastatic are of great importance and may allow for the selection of more targeted treatment regimes.

Heterochromatin Protein 1 (HP1) is a chromatin associating protein that facilitates heterochromatic spreading through its interaction with trimethylated H3K9. There are three HP1 isoforms found in mammals, HP1α, HP1β and HP1γ, each with differing functions and chromatin localisation patterns. Previous research has demonstrated that deregulation of either HP1α or HP1β expression occurs in several types of cancers. Both increases and decreases in HP1α expression have been reported in breast tumour samples, with a decrease in HP1α associated with breast metastases. However, what role loss of HP1α may have in promoting a metastatic phenotype is unclear, and any contribution of HP1β to this process is also explored.

This thesis examined the roles of HP1α and HP1β in breast cancer progression through the creation of breast cancer cell lines with knock-down of either HP1α or HP1β. These cell lines were characterised for changes in proliferation, cell cycle profile, global chromatin compaction, invasive potential and anchorage independence. Though no changes were observed in the majority of these characteristics, a novel role for HP1β as a potential suppressor of anchorage independence was identified. Additionally, it was found that HP1α may act to enhance anchorage independence. This information could help to further knowledge of how breast cancer cells proceed towards metastasis, and provide new avenues of research into the potential for levels of HP1α or HP1β to be used as biomarkers for breast cancer progression.

Foreword and acknowledgements

The Road goes ever on and on
Down from the door where it began.
Now far ahead the Road has gone,
And I must follow, if I can,
Pursuing it with eager feet,
Until it joins some larger way
Where many paths and errands meet.
And whither then? I cannot say.

-J.R.R. Tolkein, *The Fellowship of the Ring*

Just over two yeas ago I began a new phase of my journey, one that has proved to be both more challenging and more rewarding than I could have imagined. The path leading to this point has traversed many twists, turns, rough patches and the occasional dead end, culminating in what felt like an attempt to scale the slippery slopes of Mount Doom while wearing roller blades. However, I had the great fortune of having many more people to provide help and encouragement throughout my journey than just one extremely loyal gardener. I am eternally grateful to the many people who have supported me over the last couple of years; thank you does not seem like enough. You were all integral in strengthening my resolve to keep going, and it is because of you that I can finally say that I have finished.

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Abbreviations

°C	Degrees celsius
APS	Ammonium persulfate
BCA	Bicinchoninic acid
BRCA1	Breast cancer type 1 susceptibility protein
BrdU	Bromodeoxyuridine
BSA	Bovine serum albumin
c-Myc	Avian myelocytomatosis virus oncogene cellular homolog
CAF-1	Chromatin assembly factor protein 1
CBX	Chromobox homolog
cDNA	Complementary DNA
CI	Cell index
CP	Crossing point (PCR)
DAPI	4',6-diamidino-2-phenylindole
DCIS	Ductal carcinoma in-situ
dH_2O	Distilled water
DMEM	Dulbecco's modified eagle medium
DMSO	Dimethyl sulfoxide
DNA	Deoxyribose nucleic acid
Dnmt	DNA methyltransferase
dNTP	Deoxyribonucleotide triphosphate
E2F5	E2 transcription factor 5
ECM	Extra cellular matrix proteins
EDTA	Ethylenediaminetetraacetic acid
ER	Estrogen receptor
ERK	Extracellular-signal-regulated kinase
FACS	Fluorescence activated cell sorting
FBS	Foetal bovine serum
GDP	Guanosine diphosphate
GTP	Guanosine triphosphate
GTPase	Guanosine triphosphatase
g	grams
GST	Glutathione S-transferase
H3K9	Lysine 9 of histone H3
HC	Hygromycin control cell line
HCl	Hydrochloric acid
HER2/neu	Human epidermal growth factor 2
hMis12	Human MIND kinetochore complex component factor homolog
HP1	Heterochromatin protein 1
HRP	Horse-radish peroxidase

hTERT	Human telomerase reverse trancriptase
IgG	Immunoglobulin G
INCENP	Inner centromere protein
JAK/STAT	Janus kinase/Signal transducer and activator of transcription
Kap1-Tif1β	Kruppel-associated box (KRAB)-associated protein/transcriptional intermediary factor 1β
Kb	Kilobase
KCl	Potassium chloride
KD	Knock-down
kDa	Kilodaltons
KH_2PO_4	Potassium dihydrogen phosphate
Ku70	Ku autoantigen protein 70
L	Litre
LB	Luria-Bertani bacteriological media
M	Moles per litre
mg	Milligram
Mg	Magnesium
µg	Microgram
µL	Microlitre
mL	Millilitre
mM	Millimoles per litre
mRNA	Message RNA
$Na_2HPO_{4-}7H_2O$	Sodium monohydrogen phosphate heptahydrate
NaCl	Sodium hydroxide
$NaHCO_3$	Sodium bicarbonate
NC	Neomycin control cell line
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
ng	Nanograms
nm	Nanometres
NP40	Nonyl phenoxypolyethoxylethanol
NRF-1	Nuclear respiratory factor 1
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
penstrep	Penicillin/streptomycin
PI	Propidium iodide
pmol	Picomole per litre
qRT-PCR	Quantitative reverse transcription polymerase chain reaction
Rac1	Ras-related C3 botulinum toxin substrate 1
RAS	Rat sarcoma protein
RB	Retinoblastoma protein
RIPA	Radioimmunoprecipitation assay buffer
RISC	RNA-induced silencing complex

RLU	Relative light units
RNA	Ribonucleic acid
RNase	Ribonuclease
RT-PCR	Reverse transcription polymerase chain reaction
SDS	Sodium dodecyl sulfate
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electophoresis
shRNA	Short hairpin RNA
siRNA	Small interferring RNA
SUV39HI	Suppressor of variegation 3-9 homolog 1
$TAF_{II}130$	TATA-binding protein associated factor p130
TBE	Tris/borate/EDTA buffer
TBS	Tris-buffered saline
TEMED	Tetramethylethylenediamine
Tris	Tris(hydroxymethyl)aminomethane
U	Units
UV	Ultraviolet
V	Volts
Wnt1	Wingless-integration 1 protein
YY1	Yin-yang 1 transcription factor

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