Steroid Receptor Crosstalk in Breast Cancer Cells

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In the middle of difficulty lies opportunity

Albert Einstein

This thesis is dedicated to my Pa, Mum, and Dad

Thank you for this opportunity

CHAPTER 2: MATERIALS AND METHODS

Abstract

Breast cancer is the leading cause of cancer related death in women, and approximately 1 in 11 women will develop breast cancer before the age of 75. In 2003, breast cancer was responsible for 16% of cancer related deaths in Australian women. This demonstrates that throughout the life span of the female, this organ has a high risk of developing cancer. The growth and survival of normal breast epithelial cells and breast cancer cells is promoted by estrogens and progesterone and both estrogen receptor (ER) and progesterone receptor (PR) have been shown to play prominent roles in breast cancer progression. It has also been demonstrated that co-treatment of breast cancer cells with corticosteroids and 17β-estadiol (E2) can have opposing effects on the proliferation of breast cancer cells compared with the single treatment. In addition, glucocorticoid receptor (GR) levels have been shown to have clinical implications for breast cancer cell survival. This suggests a possible role for activated GR in breast cancer development. Forkhead box protein 1 (FoxA1), a member of the forkhead class of DNA-binding proteins, has also been shown to be an important factor in breast cancer development. FoxA1 has been shown to dictate ER binding in breast cancer cells and has been deemed responsible for the rapid reprogramming of ER signalling seen in breast cancers with poor outcomes and treatment resistance. However, the effects of ER on the function of FoxA1 have been controversial. The aim of this thesis is to further investigate and characterise GR, ER, and FoxA1 crosstalk in three estrogenic breast cancer cell lines, MCF-7, ZR-75-1, and T-47D cells.

It has been determined that the combination of dexamethasone (Dex) and E2 have an altered affect on the cell proliferation of breast cancer cells, compared to the single treatment, suggesting GR can modulate the ER response. In an artificial cell model it has been demonstrated by genome-wide investigations, that activated GR and estrogen receptor (ER) can alter the binding of each other at a subset of sites, by a mechanism termed DynaLoad. In addition, it has been shown that Dex and E2 in combination can regulate a unique subset of genes in breast cancer cells. This provides evidence to indicate that Dex can oppose the growth stimulatory effects of E2 signalling, and further, in combination, Dex and E2, can alter the gene transcriptional prolife of MCF-7 breast cancer cells.

To understand how the molecular interplay between GR and ER effect breast cancer progression the genome-wide binding events of activated GR and ER have been investigated. These studies show that a GR and ER DynaLoad mechanism also exists in all three breast cancer cell lines utilised; however, there was very little crossover of binding patterns observed. This suggests that while the mechanisms of DynaLoad are present in all three cell lines, the sites altered are cell specific. Most surprisingly is the discovery of an elevated number of GR sites that are lost upon activation of ER in MCF-7 cells. However, in the other breast cancer cell lines, this finding is not as pronounced. Immunblots show that MCF-7 cells have lower GR protein levels than the other cell lines indicating that steroid receptor (SR) levels play a major role in the effect that the dual hormone treatment has on the cell. This suggests that in a highly estrogenic cell line, ER plays a strong role in modulating GR function, which could have important consequences for disease outcome.

Furthermore, and contrary to previous findings, this thesis establishes that activated ER and GR have the ability to alter the genomic response of the well-established pioneer factor FoxA1. Genome-wide analysis of FoxA1 binding, upon treatment of E2 or Dex, shows that both ER and GR can recruit FoxA1 to specific binding sites within the genome through a DynaLoad mechanism. These results indicate that there is not a specific set of pioneer factors which bind to closed chromatin and establish the binding landscape for other transcription factors (TFs). Instead this data suggests that every factor has the potential to affect the binding landscape of other TFs, depending on the chromatin context.

Overall, the findings from this thesis have provided novel insight into the crosstalk between GR, ER, and FoxA1, further highlighting the ability of activated SRs to alter the response of one another, and other TFs. In addition, it has also been determined that the outcomes of SR crosstalk is cell-specific and that differing estrogenic breast cancer cells can have altered outcomes, which are dependent on SR levels. This can have potential consequences in breast cancer disease outcomes and progression. In addition, the findings in this thesis have begun to shift our classical understanding of pioneer factors in breast cancer, demonstrating that activated GR and ER have the capabilities to recruit and alter the response of FoxA1. This has provided information on a previously unknown complexity to FoxA1 action in breast cancer cells. The studies in this thesis highlight the signalling complexity of TFs in breast cancer cells and provide the basis for further investigations into GR, ER, and FoxA1 mechanisms and the direct consequences of this on breast cancer outcomes.

Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name 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. In addition, I certify that no part of this work will, in the future, be used in a submission in my name for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint award of this degree.

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Erin E. Swinstead

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Abbreviations

17β-HSD	17β-hydroxysteroid dehydrogenase
3β-HSD	3β-hysroxysteroid dehydrogenase
ac	acetylation
AF-1	transcriptional activation function 1
AF-2	transcriptional activation function 2
AP-1	activating protein 1
AR	androgen receptor
Aromatase	aromatase cytochrome P-450 enzyme
bp	base pair
BSA	bovine serum albumin
CTCF	CCCTC-binding factor
ChIP	chromatin immunoprecipitation assay
ChIP-chip	tiled oligonucleotide microarrays
ChIP-seq	chromatin immunoprecipitation sequencing
cHRT	combined hormone replacement therapy
CO_2	carbon dioxide
CSS	charcoal stripped fetal bovine serum
C/EBP	CCAAT/enhancer binding protein
DBD	DNA-binding domain
DCIS	ductal carcinoma *in situ*
Dex	dexamethasone
DHEA	dehydroepiandrosterone
DHEA-S	dehydroepiandrosterone sulphate

DHS	DNase I hypersensitivity
DHT	dihydrotestosterone
DMEM	Dulbecco's Modified Eagle Medium
DNA	deoxyribonucleic acid
DNase I	deoxyribonuclease I
DynaLoad	dynamic assisted loading
E2	17β-estradiol
EDTA	ethylenediaminetetraacetic acid
EGTA	ethylene glycol tetraacetic acid
EMT	epithelial-to-mesenchymal transition
ENCODE	The Encyclopedia of DNA Elements
ER	estrogen receptor
ERE	estrogen receptor response element
ERα	estrogen receptor alpha
ERβ	estrogen receptor beta
FAIRE	formaldehyde-assisted isolation of regulatory elements
FBS	fetal bovine serum
FDR	false discovery rate
FIMO	finding individual motif occurrences
FLIP	fluorescence loss in photobleaching
FoxA1	forkhead box protein 1
FRAP	fluorescence recovery after photobleaching
g	gram
GR	glucocorticoid receptor
GRE	glucocorticoid response element

H	histone
h	hour
H_2O	water
HATs	histone acetyltransferase complexes
HCl	hydrogen chloride
HDACs	histone deacetylases
Helix 1	N-terminal helix
Helix 2	C-terminal helix
Her2	human epidermal growth factor receptor
Homer	Hypergeometric Optimization of Motif Enrichment
HRE	hormone response element
HRT	hormone replacement therapy
IDC	invasive ductal carcinoma
ILC	invasive lobular carcinoma
JunD	jun D proto-oncogene
K	lysine
kb	kilobase
KCl	potassium chloride
L	liter
LBD	ligand-binding domain
LCIS	lobular carcinoma *in situ*
LINE	long interspersed repetitive elements
M	molar
me1	monomethylation

me2	dimethylation
me3	trimethylation
MeV	Multiple Experiment Viewer
min	minute
mL	milliliter
mm	millimetre
mM	millimolar
MMTV array	mouse mammary tumour virus promoter
MMTV LTR	mouse mammary tumour virus long terminal repeat
MMTV-Luc	mouse mammary tumour virus promoter luciferase
MR	mineralocorticoid receptor
mRNA	message ribonucleic acid
MYC	v-myc avian myelocytomatosis viral oncogene homolog
NaCl	sodium chloride
P4	progesterone
PAD2	peptidylarginine deiminase 2
PBS	Dulbecco's phosphate buffered saline
PI	protease inhibitors
PR	progesterone receptor
qPCR	quantitative polymerase chain reaction
R	arginine
RO	reverse osmosis
rpm	revolutions per minute
RNA	ribonucleic acid
RNA PolII	RNA polymerase II

sec	second
SDS	sodium dodecyl sulphate
SR	steroid receptor
STR	short tandem repeats
T	testosterone
TAE	tris-acetate-EDTA
TF	transcription factor
trypsin-EDTA 0.25%	trypsin
TSS	transcription start site
ug	microgram
uL	microlitre
uM	micromolar
UV	ultraviolet
V	volts
WHI	Women's Health Initiative

Other:

ºC	degree Celsius
%	percentage
>	greater than
<	less than
≥	greater than or equal too
≥	less than or equal too