EPIGENETICS IN CANCER:

BASIC AND TRANSLATIONAL ASPECTS

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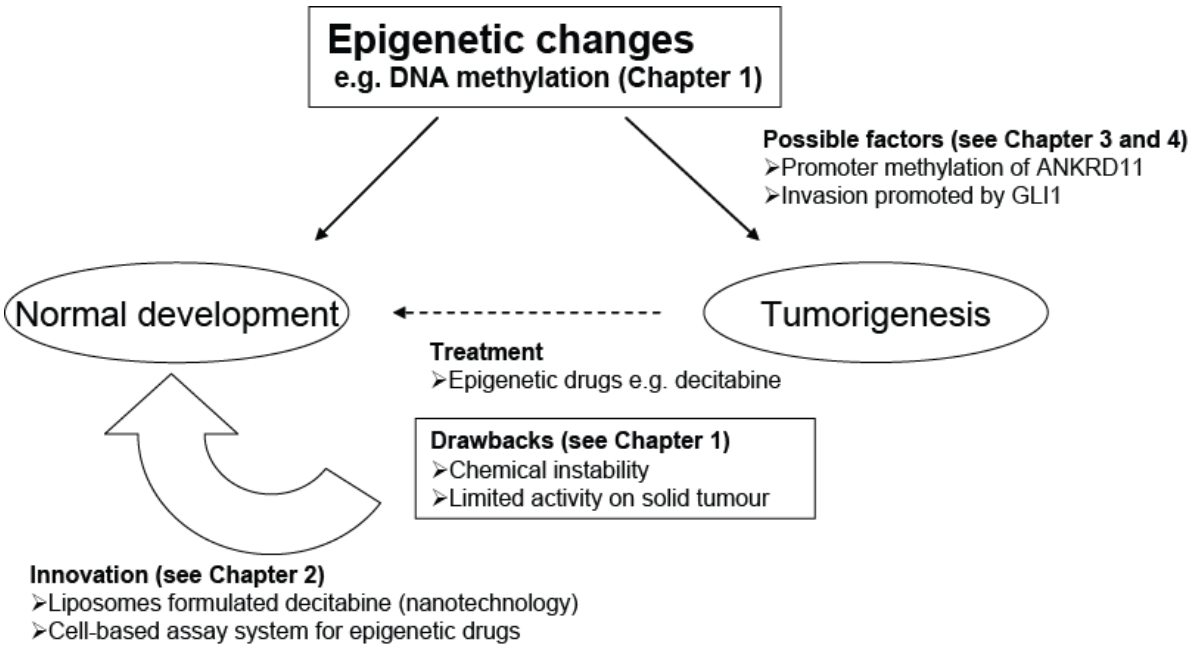
OVERVIEW

Figure: EPIGENETICS IN CANCER: Basic and Translational Aspects



This thesis investigates epigenetics in cancer with particular emphasis on breast cancer. There are two major themes, see Figure above. The first theme relates to the potential for assessing and developing more efficient epigenetic drugs while the second theme investigates mechanism of downregulation of *ANKRD11,* a putative tumour suppressor gene, in human breast cancer. This thesis is in the publication format with Chapters 1 and 3 as published articles, Chapter 2 submitted for publication and Chapter 4 as a manuscript in preparation.

Theme 1: To improve the epigenetic-based therapeutic approach (Chapter 1 and 2)

One of the roles that epigenetics plays in cancer development is the inhibition of transcription of tumour suppressor genes. Chapter 1, published as a review in *Biodrugs*, examines the knowledge of currently available therapeutic approaches related to epigenetic mechanisms such as DNA methylation for cancer treatment. Drug-related issues that could influence the application of therapeutics for clinical use are reviewed and possible developments to improve the clinical use of the drugs explored. Epigenetic-based drugs are emerging as anti-cancer therapies in the clinic. Existing demethylating agents have poor pharmacological properties that limit their clinical use, and the application of nano-based encapsulation to resolve these issues is discussed.

Chapter 2, submitted as an original research article to *Biodrugs*, presents the development and assessment of an assay to allow comparison of epigenetic-related drugs in a high throughput format. Decitabine is encapsulated in a liposomal formulation and the potency of this newly formulated decitabine and existing drugs are effectively compared using the developed assay system. Further development and validation of the assay system and the liposomal formulated decitabine, not included in the submitted manuscript are included as supplementary data.

Theme 2: Investigation of gene silencing mechanism of tumour suppressor ANKRD11 (Chapter 3 and 4)

ANKRD11 is novel gene that was previously characterised in our laboratory, and found to be a putative tumour suppressor gene and a p53-coactivator (Neilsen et al. 2008). Chapter 3, published in *European Journal of Cancer,* investigates the mechanism of downregulation of *ANKRD11* in human breast cancer. This chapter identifies the promoter sequence of *ANKRD11*,

demonstrates the critical region of the *ANKRD11* promoter subjected to DNA methylation, and associates the DNA methylation levels of *ANKRD11* with its gene expression and clinical data. Further analysis of the DNA methylation pattern of this gene revealed a putative GLI1 transcription-factor binding site within the localised region of the promoter that is methylated.

Chapter 4, presented as a manuscript in preparation, further explores the relationship between ANKRD11 and GLI1 in breast cancer. GLI1 is a Hedgehog signalling transcription factor, which has been shown to be involved in breast cancer development. This study analyses the transcriptional activity of *ANKRD11* in the cells overexpressed with GLI1 and quantifies differential expression of these two genes in different stages of breast cancer. Future experiments to confirm and extend these exciting preliminary findings are discussed.

The final chapter of this thesis summarises the findings of these studies and possible future research directions. The impact of these findings for the development of anti-cancer drugs, and the possible role of expression of *ANKRD11* and *GLI1* in breast cancer are highlighted.

DECLARATION

I, Sue Ping Lim, certify that 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 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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Signed…… *Date*……

*LIST OF PUBLICATIONS ARISING FROM THIS THESIS

CHAPTER 1

Lim et al. (2011). The application of delivery systems for DNA methyltransferase inhibitors. *BioDrugs*; 25 (4): 227-242.

CHAPTER 2

Lim et al. (2012).Development of a novel cell-based assay system EPISSAY for screening epigenetic drugs and liposomes formulated decitabine. *Biodrugs*; *Submitted.*

CHAPTER 3

Lim et al. (2012). Specific-site methylation of tumour suppressor ANKRD11 in breast cancer. *European Journal of Cancer*; *In Press*.

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"The only reason for time is so that everything doesn't happen at once."

— Albert Einstein

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LIST OF ABBREVIATIONS

5′UTR	-	5′ untranslated region
7AAD	-	7-amino-actinomycin-D
A_2O_3	-	Arsenic trioxide
AdoHcy	-	S-adenosylhomocysteine
AdoMet	-	S-adenosylmethionine
ANKRD11	-	Homo sapiens ankyrin repeat domain 11
ATCC	-	American type culture collection
Azacitidine	-	5-azacytidine
C	-	Carbon
-C=O	-	Carbonyl group
CB1954	-	5-(azaridin-1-yl)-2,4-dinitro-benzamide
CES1	-	Carboxylesterase 1
ChIP	-	Chromatin immunoprecipitation
CMV	-	Cytomegalovirus
CpG	-	Cytosine-guanine dinucleotide
DCIS	-	Ductal carcinoma *in situ*
dCK	-	Cytidine/deoxycytidine kinase
Decitabine	-	5-aza-2′-deoxycytidine, Dacogen
DHAC	-	5,6-dihydro-5-azacytidine
DIRAS3	-	GTP-binding protein Di-Ras3
DMEM	-	Dulbecco’s modified Eagle’s medium

DLBCL	-	Diffuse large B-cell lymphoma
DMSO	-	Dimethylsulfoxide
DNMT1	-	Homo sapiens DNA (cytosine-5-)-methyltransferase 1
DNMT3B	-	Homo sapiens DNA (cytosine-5-)-methyltransferase 3B
dNTP	-	Deoxyribonucleotide triphosphate
DOPG	-	1,2-dioleoyl-sn-glycero-3-[phospho-rac-(1-glycerol) sodium salt
DSPC	-	1,2 distearoyl-sn-glycero-3-phosphocholine
DSPE-PEG2000	-	1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)-2000] ammonium salt
EGCG	-	(-)epigallocatechin-3-gallate
EMSA	-	Electrophoretic mobility shift assay
EPC	-	Encapsulated papillary carcinoma
EPR	-	Enhanced permeability and retention
ER	-	Estrogen receptor
FCDR	-	5-fluoro-2′-deoxycytidine
FDA	-	Food and Drug Administration
FOXM1	-	Forkhead box protein M1
GLI	-	Glioma-associated oncogene family member
GFP	-	Green fluorescent reporter
H	-	Proton
HA	-	Hyaluronic acid
HDAC	-	Histone deacetylase
HER2	-	Human epidermal growth factor receptor 2

Hh	-	Hedgehog
HPLC	-	High performance liquid chromatography
IBC	-	Invasive breast carcinoma
IC_{50}	-	Half maximal inhibitory concentration
IPC	-	Intracystic papillary carcinomas
LOH	-	Loss of heterozygosity
MALDI-TOF-MS	-	Matrix-assisted laser desorption and ionisation time-of-flight mass spectrometry
mDCIS	-	Micropapillary ductal carcinoma *in situ*
MDS	-	Myelodysplastic syndrome
MIB-1	-	Proliferative index
MLV	-	Multilamellar vesicle
MMP-11	-	Matrix metalloproteinase 11
N	-	Nitrogen
NAD(P)H	-	Nicotinamide adenine (phosphate) oxidase
$-NH_2$	-	Amino group
NKX2.2	-	Homeobox protein Nkx-2.2
NPEOC	-	2'-Deoxy-N4-[2-(4-nitrophenyl) ethoxycarbonyl] group
NTR	-	Nitroreductase
OPN	-	Osteopontin
PAA	-	Poly(acrylic acid)
PAH	-	Poly(allylamine hydrochloride)
PAX6	-	Paired box protein Pax-6

PBS	-	Phosphate buffered saline
PCR	-	Polymerase chain reaction
PEG	-	Poly(ethylene glycol)
PLGA	-	Poly(lactide-co-glycolide)
PR	-	Progesterone receptor
PTCH	-	Transmembrane receptor patched
RT-qPCR	-	Quantitative real-time polymerase chain reaction
RFP	-	Red fluorescent reporter
RG108	-	2-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)-3-(1H-indol-3-yl) propanoic acid
RNR	-	Ribonucleotide reductase
RPS11	-	Homo sapiens ribosomal protein S11
RT-PCR	-	Reverse transcription- polymerase chain reaction
SH	-	Thiolate
SMO	-	Smoothened
SuFu	-	Suppressor of fused
TCEB1	-	Transcription elongation factor B polypeptide 1
THU	-	3,4,5,6-tetrahydrourine
TMnfsB	-	Triple-mutated mammalianised nitroreductase B
TXNIP	-	Thioredoxin interacting protein
VHL	-	Von Hippel-Lindau disease tumour suppressor
Vorinostat	-	Suberoylanilide hydroxamic acid, SAHA
WT1	-	Wilms tumour gene 1

Zebularine	-	1-(beta-D-ribofuranosyl)-1,2-dihydropyrimidin-2-one
β-actin	-	Homo sapiens actin, beta (ACTB)