## **Table of contents**

Declaration	i
Abstract	ii
Acknowledgement	iv
Conference	V
List of Figures	xiii
List of Tables	xvi
List of Symbols	xvii
List of Abbreviations	xviii

## Chapter One: Literature Review

1.1 Epithelial development and integrity	1
1.1.1 Epithelial cell adhesion	1
1.1.1.a Cell-ECM interactions	2
1.1.1.b Cell-cell adhesion: The cadherins and the catenins	2
1.1.2 E-cadherin, $\alpha$ - and $\beta$ -catenin and the actin cytoskeleton	6
1.1.2.a Rac1, Cdc42 and IQGAP1	7
1.1.2.b Tyrosine phosphorylation of $\beta$ -catenin	7
1.2 β-catenin	
1.2.1 The $\beta$ -catenin gene and protein	9
1.2.2 β-catenin in the cytoplasm	12
1.2.3 Association of $\beta$ -catenin with LEF/TCF in the nucleus	16
1.2.4 Regulation of $\beta$ -catenin induced transcriptional activity in	
the nucleus	17

1.3 $\beta$ -catenin and its role in pathogenesis	19
1.4 Proposed translocation of $\beta$ -catenin to the nucleus	
1.4.1 The translocation of $\beta$ -catenin to the nucleus by LEF/TCF	20
1.4.2 Importin- $\alpha$ and - $\beta$ and the nuclear pore complex	21
1.4.3 The cytoskeleton	24
1.5 Human oesophageal squamous cell carcinoma	
1.5.1 Invasion and HOSCC	26
1.5.2 β-catenin and HOSCC	26
1.6 Aims and objectives	28

# Chapter Two: Disruption of the actin cytoskeleton and its affect on the distribution of β-catenin in HOSCC cell lines

2.1 Introduction	29
2.2 Materials and Methods	32
2.2.1 Tissue culture	32
2.2.2 Treatment of HOSCC cell lines with cytochalasin D (cytoD)	32
2.2.3 Cytoplasmic/membrane and nuclear extractions	33
2.2.4 Protein estimation of the cytoplasmic/membrane and	
nuclear fractions.	34
2.2.5 Sample preparation for sodium dodecyl sulphate-	
polyacrylamide gel electrophoresis (SDS-PAGE)	35
2.2.6 SDS-PAGE	35
2.2.7 Antibodies	36
2.2.8 Western blot analysis	36
2.2.9 Image analysis	36
2.2.10 Indirect immunofluorescence and confocal microscopy	37
2.3 Results	38
2.3.1 Subcellular localization of $\beta$ -catenin	38
2.3.2 Examination of solvent used for cytoD solubilization	40
2.3.3 $\beta$ -catenin concentration in HOSCC lines following disruption of the	
actin cytoskeleton	42

2.3.4 Localization of $\beta$ -catenin in HOSCC cells upon disruption of the	
actin cytoskeleton.	46
2.4 Discussion	52

# Chapter Three: Preparation of vector containing full length β-catenin cDNA and its transfection into WHCO1 cell line

3.1	Introduction	55
3.2	Materials and Methods	58
	3.2.1 Tissue culture	58
	3.2.2 Plasmids	58
	3.2.3 Preparation of competent cells for electroporation transformation	59
	3.2.4 Electroporation of XL-1 blue <i>E.coli</i> cells with	
	pcDNA-Myc-β-Catenin	60
	3.2.5 Maxi-preparation to obtain adequate amount of purified plasmid	61
	3.2.6 Restriction and isolation of restricted products from the gel	62
	3.2.7 Ligation of $\beta$ -catenin insert and pEGSH	63
	3.2.8 Preparation of competent cells for heat shock transformation	63
	3.2.9 Heat shock transformation of XL-1 blue <i>E.coli</i> cells with ligation	
	product	64
	3.2.10 Primers	64
	3.2.11 Polymerase chain reaction (PCR)	65
	3.2.12 Transfection of WHCO1 cells	65
	3.2.13 Selection of isolated WHCO1-pERV3-pEGSH-β-catenin colonies	66
	3.2.14 Computer analysis programs	67
3.3	Results	68
	3.3.1 Isolation and purification of the pcDNA-Myc-β-Catenin plasmid	68
	3.3.2 The preparation of plasmid and vector for the ligation procedure	70
	3.3.3 Ligation of the $\beta$ -catenin insert into the pEGSH vector	71
	3.3.4 Sequencing of the gene inserted within the MCS region	
	of pEGSH	72
	3.3.5 The 5' end of $\beta$ -catenin insert within pEGSH- $\beta$ -catenin	73

3.3.6 Purification of pEGSH-β-catenin expression vector	75
3.3.7 The tWHCO1: WHCO1 transfected with pERV3 and	
pEGSH-β-catenin.	76
3.4 Discussion	77

Chapter four: Stimulation of exogenous  $\beta$ -catenin expression in WHCO1

cells	
4.1 Introduction	78
4.2 Materials and methods	81
4.2.1 Tissue culture	81
4.2.2 DNA extraction from tWHCO1	81
4.2.3 Primers	82
4.2.4 Polymerase chain reaction (PCR)	83
4.2.5 Transient transfection of WHCO1-pERV3 cells with pEGSH	83
4.2.6 Treatment of tWHCO1 and WHCO1-pERV3-pEGSH cells with	
ponA	83
4.2.7 Whole cell extraction	84
4.2.8 Protein estimation of cell samples	84
4.2.9 SDS-PAGE gel	84
4.2.10 Western blot	85
4.2.11 Treatment of WHCO1 with DMSO	85
4.2.12 Computer analysis programs	85
4.3 Results	86
4.3.1 The presence of pEGSH-β-catenin in tWHCO1	86
4.3.2 The presence of $\beta$ -catenin protein in tWHCO1	89
4.3.3 Stimulation of WHCO1-pERV3-pEGSH with ponA	90
4.3.4 β-catenin expression in total cell population of tWHCO1 i	n
response to ponA stimulation	92
4.3.5 β-catenin expression in tWHCO1 clones	95
4.3.6 Stimulating expression of $\beta$ -catenin in WHCO1 with DMSO	97
4.4 Discussion	99

# **Chapter Five: General Discussion**

5.1 The actin cytoskeleton and its effect on nuclear $\beta$ -catenin	102
5.2 Overexpression of $\beta$ -catenin in WHCO1	111
5.3 How do the results presented impact on HOSCC	112
5.4 Summary and future prospects	114

# Chapter Six: Appendix

5.1 Tissue culture	
6.1.1 Tissue culture medium	116
6.1.1.a DMEM medium solution	116
6.1.1.b Hams F12 medium solution	116
6.1.2 Phosphate buffered saline (1x)	117
6.1.3 Trypsin:Ethylenediaminetetra-acetic acid (EDTA) solution	117
6.1.3.a Trypsin solution	117
6.1.3.b EDTA solution	117
6.2 Cytoplasmic/membrane and nuclear extractions	118
6.2.1 Nuclear preparation buffer	118
6.2.2 Nuclear lysis buffer	118
<ul><li>6.2.2 Nuclear lysis buller</li><li>6.3 Protein estimation</li><li>6.3.1 Coomassie Blue solution (0.25%)</li></ul>	
6.3.1 Coomassie Blue solution (0.25%)	119
6.3.2 Destaining solution	119
6.3.3 Elution solution	119
6.3.4 Example of standard curve (protein estimation)	120
6.4 Sample preparation for SDS-PAGE	120
6.4.1 Double lysis buffer	120
6.5 SDS-PAGE	121
6.5.1 Separating gel	121
6.5.2 Stacking gel	121
6.5.3 Tracking dye	121
6.5.4 Running buffer	122
6.5.5 Destain solution	122

6.5.6 Calibration curve example	122
6.6 Western Blot	123
6.6.1 Transfer buffer	123
6.6.2 Blocking solution	123
6.6.3 SuperSignal West Pico Chemiluminescent Substrate kit	123
6.6.4 Developer	124
6.6.5 Fixer	124
6.7 Indirect immunofluorescence and confocal microscopy	125
6.7.1 Paraformalderhyde (4%)	125
6.7.1.a Solution A	125
6.7.1.b Solution B	125
6.7.2 Elvanol	126
6.7.2.a Solution A	126
6.7.2.b Solution B	126
6.8 Plasmids	127
6.8.1 Elution of pcDNA-Myc-β-Catenin plasmid	127
6.8.1.a Tris buffer	127
6.8.1.b Elution procedure	127
6.8.2 Complete Control <sup>®</sup> Inducible Mammalian Expression	
System plasmids	128
6.8.2.a pERV3	128
6.8.2.b pEGSH	129
6.9 Competent cells preparation for electroporation	130
6.9.1 Luria agar plate	130
6.9.2 Luria broth	130
6.10 Electroporation	131
6.10.1 SOC <sup>E</sup> medium	131
6.10.2 Mini-prep DNA plasmid extraction	132
6.10.2.a Solution A	132
6.10.2.b Solution B	132
6.10.2.c Solution C	132
6.10.2.d Mini-prep procedure	132

6.11 Calculating DNA concentration (example)	133
6.12 Restriction of plasmids	134
6.12.1 Loading buffer	134
6.12.1a Tris-Acetate-EDTA (TAE) 20X	134
6.12.2 Agarose gel (0.8%)	134
6.12.3 Standard curve example of an agarose gel	135
6.12.4 Tris-EDTA (TE)	135
6.12.5 Phenol-chloroform procedure	136
6.12.5.a Phenol	136
6.12.5.b Phenol-chloroform DNA purification	136
6.12.6 Ethanol precipitation	137
6.13 Preparation of competent cells for heat shock transformation	137
6.13.1 SOB <sup>HS</sup>	137
6.13.2 Transformation buffer (TB)	138
6.14 Heat shock transformation of XL-1 blue E.coli cells with ligation	
product	138
6.14.1 SOC <sup>HS</sup> medium	138
6.15 pEGSH-β-catenin plasmid	139
6.15.1 Primers	140
6.16 Polymerase chain reaction	141
6.16.1 Cracking buffer	141
6.17 Sequencing analyses and BLAST search results	142
6.17.1 β-catenin sequence (chromatogram)	142
6.17.2 β-catenin sequence (BLAST result)	146
6.17.3 pEGSH sequence (chromatogram)	147
6.17.4 pEGSH and β-catenin sequence (BLAST result)	151
6.18 Ponasterone A	152
6.19 Whole Cell Extraction	152
6.19.1 Single lysis buffer	152
Chapter Seven: References	153

#### List of Figures

	Figure 1.1:	Cell-cell adhesion mediated by the E-cadherins 5	
	Figure 1.2:	The interaction of $\beta$ -catenin with E-cadherin and $\alpha$ -	
		catenin at the adherens junction	8
	Figure 1.3:	CTNNB1 gene, mRNA and coding sequence	11
<b>Figure 1.4:</b> Negative regulators of $\beta$ -catenin in the		Negative regulators of $\beta$ -catenin in the cytoplasm	14
	Figure 1.5:	Positive regulators of $\beta$ -catenin in the cytoplasm	15
	Figure 1.6:	$\beta$ -catenin in the nucleus	18
	Figure 1.7:	The translocation of nuclear proteins from the cytoplasm	
		to the nucleus via importin- $\alpha$ /importin- $\beta$	22
	Figure 2.1:	SDS-PAGE separation and western blots of $\beta\text{-catenin}$ in	
		nuclear and cytoplasmic/membrane fractions	39
	Figure 2.2:	The effect of $cytoD_{ethanol}$ and $cytoD_{DMSO}$ on $\beta\text{-catenin}$	
		concentration in WHCO5	41
	Figure 2.3:	Western blots of $\beta\text{-catenin}$ in HOSCC lines treated with	43
		cytoD	
	Figure 2.4:	Quantitative analysis of $\beta$ -catenin concentration levels of	
		associated western blots	44
	Figure 2.5:	$\beta$ -catenin concentration in the different cellular fractions	45
	Figure 2.6:	The localization of $\beta$ -catenin in WHCO5	46
	Figure 2.7:	The different sub-cellular levels of WHCO5	48
	Figure 2.8:	Confocal images (z-sections) of HOSCC lines treated with	50
		cytoD	
	Figure 2.8:	Confocal images (z-sections) of HOSCC lines treated with	51
		cytoD (Continued)	
	Figure 3.1:	Schematic diagram of pcDNA-Myc-β-Catenin (415)	58
	Figure 3.2:	Schematic diagram of the pERV3 and pEGSH vectors of	
		the Complete Control <sup>®</sup> Inducible Mammalian Expression	
		System (Stratagene)	59
	Figure 3.3:	Purified pcDNA-Myc-β-Catenin plasmid	69

Figure 3.4:	Restriction of pcDNA-Myc-\beta-catenin plasmid and pEGSH	70
	vector	
Figure 3.5:	Amplification of inserted gene within the MCS of pEGSH	71
Figure 3.6:	Sequencing analysis gene inserted within MCS of pEGSH	72
Figure 3.7:	Amplification of the 5' end surrounding region of $\beta$ -	
	catenin insert within pEGSH-β-catenin	73
Figure 3.8:	Sequencing analysis of the 5' end surrounding region of $\beta$ -	
	catenin insert within pEGSH-β-catenin	74
Figure 3.9:	Purified pEGSH-β-catenin	75
Figure 4.1:	Activation of gene expression via ponA	80
Figure 4.2:	The 3' region of $\beta$ -catenin insert within pEGSH- $\beta$ -catenin	87
Figure 4.3:	Amplification of the GAPDH gene from DNA fraction of	
	tWHCO1	88
Figure 4.4:	SDS-PAGE separation and western blot of $\beta\text{-catenin}$ in the	
	cell extract of tWHCO1	89
Figure 4.5:	Treatment of WHCO1- pERV3-pEGSH with ponA	91
Figure 4.6:	Western blots of $\beta$ -catenin in total cell population of	
	tWHCO1 following ponA treatment	93
Figure 4.7:	$\beta$ -catenin expression in total cell population of tWHCO1	
	treated with ponA (8 hours)	94
Figure 4.8:	$\beta$ -catenin expression levels in tWHCO1 clones treated	
	with ponA	96
Figure 4.9:	$\beta$ -catenin expression in WHCO1 treated with 1% DMSO	98
Figure 5.1:	Downstream events that occur upon disruption of the actin	
	cytoskeleton, which possibly contribute to the decrease of	
	nuclear β-catenin	108
Figure 5.1:	Downstream events that occur upon disruption of the actin	
	cytoskeleton, which possibly contribute to the decrease of	
	nuclear β-catenin (Continued)	109

Figure 5.2:	An increase of LEF/TCF or a decrease of axin and APC		
	expression potentially leads to an increase of nuclear $\beta$ -		
	catenin	110	
Figure 6.1:	Example of a protein estimation standard curve	120	
Figure 6.2:	Example of an SDS-PAGE calibration curve	122	
Figure 6.3:	Example of an agarose gel (DNA) calibration curve	135	
Figure 6.4:	Ponasterone A	152	

## List of Tables

**Table 4.1:**Treatment of total cell population of tWHCO1 with ponAand its effect on  $\beta$ -catenin expression93

#### List of Symbols

- $\alpha$  alpha
- β beta
- γ gamma

#### List of Abbreviations

Abs	the absorbance
activator protein-1	AP-1
ADP	Adenosine diphosphate
ATP-	Adenosine triphosphate
APC	Adenomatous polypsis coli
ARM	Armadillo
BLAST	Basic Local Alignment Search Tool
bp	base pairs
Brg1	Brahma related gene-1
BSA	Bovine serum albumin
Ca <sup>2+</sup>	Calcium
CBP	CREB-binding protein
Cdc42	Cell division cycle 42
СК	Casein Kinase
cm	centimeter
CREB	Camp response element-binding protein 1
CRM-1	Chromosome maintenance region-1
C-terminal	Carboxyl-terminal
cytoD	Cytochalasin D
DEAE	diethylaminoethyl
dH <sub>2</sub> O	distilled water
DKK	Dickkopf
DMSO	Dimethyl sulphoxide
dNTP	nucleotides
dvl	dishevelled
E.coli	Escherichia coli
E/GRE	ecdysone/glucocorticoid responsive element recognition
	sequence
EC	Extracellular
E-cadherin,	Epithilial-cadherin

EcBR	Ecdysone binding receptor
ECM	Extracellular matrix
EDTA	Ethylenediaminetetra-acetic acid
EGF	Epidermal growth factor
EGF-R	EGF-receptor
EGR-1	Early growth response-1
ERK	Extracellular signal-related kinase
F-actin	Filamentous actin
FAK	Focal adhesion kinase
Fer	Fps/fes-related tyrosine kinase
х <i>g</i>	times gravity
G418	G418 sulphate
G-actin	Globular actin
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GDP	Guanosine 5'-diphosphate
GFP	green fluorescence protein
GSK-3β	glycogen synthase kinase-3β
GTP	Guanosine 5'-triphosphate
HCI	Hydrochloric acid
HMG	High mobility group
HOSCC	Human oesophageal squamous cell carcinoma
ICAT	Inhibitor of $\beta$ -catenin and TCF
ILK	Integrin linked kinase
IQGAP1	IQ motif-containing GTPase-activating protein 1
JNK	c-jun N-terminal protein kinase
kb	kilobase-pairs
kDa	kilo-Daltons
L	litre
LA	Luria agar
LB	Luria broth
LEF/TCF	Lymphoid enhancer factor/T-cell factor
mA	milli-Amps

MCS	Multiple cloning site
min	minutes
mM	milli-molar
mm	millimetre
MMP	Matrix metalloproteases
MUC1	MUCIN 1
MWM	Molecular weight markers
N-cadherin	Neuronal-cadherin
NE	Nuclear envelope
NES	Nuclear export signals
NF-ĸB	Nuclear factor-kappa B,
ng	nano-grams
NLS	Nuclear localisation signal
nm	nano-meters
NPB	Nuclear preparation buffer
NPC	Nuclear pore complex
N-terminal	Amino-terminal
ORF	Open reading frame
p53	tumour protein 53
p85	Phosphoprotein 85
PBS	Phosphate Buffer Saline
P-cadherin	Placental-cadherin
PCR	Polymerase chain reaction
Pi	Phosphate
РІЗ-К	Phosphoinositide-3-kinase
Pin1	Propyl isomerase
РКС	Protein kinase C
pmols	pico-mols
ponA	Ponasterone A
PTEN	Phosphatase and tensin homolog
РТР	Tyrosine phosphatase
R2	Linear regression

Rac1	s-related c3 botulinum toxin substrate 1;	
rpm	revolutions per minute	
RxR	retenoid-x-receptors,	
SCC	squamous cell carcinoma	
SDS-PAGE	sodium dodecyl sulphate-polyacrylamide gel	
	electrophoresis	
sec	second	
Siah1	Seven in absentia, drosophila, homolog of, 1	
SIP	Siah interacting protein	
Skp1	S-phase kinase-associated protein 1	
SP1	specificity protein-1	
SV40	Simian Virus 40	
TAE	Tris-Acetate-EDTA	
Taq	Thermus aquaticus	
ТВ	Transformation buffer	
TBP	TATA binding protein	
TCA	trichloroactic acid	
ТЕ	Tris-EDTA	
TEMED	N,N,N'N'-tetramethylenediamine	
β-TrCP	β-transducin repeat-containing protein	
U	units	
uPA	Urokinase type plasminogen activator	
WGA	Wheat germ agglutinin	
wnt	wingless-type	
μg	micro-gram	
μΙ	micro-litres	
μΜ	micro-molar	