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á-Catenin expression in oesophageal squamous cell carcinoma

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i DECLARATION

I (Desmond Jo Schnugh), declare that this dissertation is my own, unaided work. It is being submitted for the Degree of Master of Science in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination in any other University.

_____

(Signature of candidate)

_____ day of _____ 2005

ii ABSTRACT

α-catenin plays a crucial role in cell adhesion. Expression levels of α-catenin have been shown to be decreased in almost all tumours studied. The levels of the epidermal growth factor receptor (EGFR) were shown to be increased in oesophageal squamous cell carcinoma (OSCC) cell lines. α-catenin therefore, may play a part in linking the EGF pathway or other signal transduction pathways, bringing about some of the changes in the OSCC cell lines. The α-catenin gene from five OSCC cell lines was sequenced. Three out of five OSCC cell lines studied were found to harbour mutations. One of the mutations resulted in a change in the amino acid sequence of α-catenin. It was concluded that this alteration may not have affected the functioning of α-catenin. α-catenin was largely expressed at the plasma membrane with some weaker cytoplasm/nuclear expression occurring in all of the OSCC cell lines. Treatment of the OSCC cells with EGF for a 12 hour period resulted in no noticeable change in the expression levels of α-catenin. The results obtained from this study indicated that α-catenin could play a role in signal transduction pathways in the OSCC cell lines.

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vii ABBREVIATIONS

A	A	Absorbance
	A	Adanine
	Ala	Alanine
	APC	Adenomatous Polyposis Coli
	ATP	Adenosine triphosphate
B	BLAST	Basic Local Search Alignment Tool
	Bis	NN-methylene Bisacrylamide
	bp	Base pair
	BSA	Bovine serum albumin
	βTrCP	Ubiquitin ligase
C	C	Cystine
	^{o}C	Degrees celcius
	Ca^{2+}	Calcium ion
	CC	Colon cancer
	Cdc	Cyclin dependent kinase
	cDNA	Complementary deoxy riobonucleic acid
	CE	Concentration of estimated sample in μg/μl
	CF	Concentration factor
	$CH_3COONa.\ 3H_2O$	Sodium acetate
	CKIε	Casein Kinase Iε
	CIP	Calf intestine alkaline phosphatase
	CL	Cell line
	Clu 1	Ubiquitin conjugating enzyme
	CO_2	Carbon dioxide
	CS	Concentration of samples in μg/μl
D	dH_2O	Distilled water
	DLD	D. L. Dexter
	DNA	Deoxy ribonucleic acid

	dNTP	Deoxynucleotide triphosphate
	DTT	Di thio threatol
	Dvl	Dishevelled
E	EDTA	Ethylene diamine tetra-acetic acid sodium salt
	EGF	Epidermal growth factor
	EGFP	Enhanced green fluorescent protein
	EGFR	Epidermal growth factor receptor
	E/GRE	ecdysone/glococorticoid responsive elements
	Elk 1	elk 1 transcription factor
	EtBr	Ethidium bromide
	Erk	Extracellular signal-regulated kinase
F	F	Fraction
	Fer	Src like protein kinase
	FITC	Fluorescine isothiocyanate
	5’ term	Amplification of α-catenin’s 5’ terminus
	f1 ori	f1 origin of ss-DNA replication
	Fyn	p59 src like protein tyrosine kinase
G	G 1	Gap 1 phase
	G 2	Gap phase 2
	GrB	Adaptor molecule
	GSK-3β	Glycogen synthase kinase 3β
H	HCl	Hydrochloric acid
	H_2O	Water
I	IgG	Immunoglobulin G
	IRES	internal ribosome entry site
J	JNK	c-Jun N-terminal kinase
	JUNKK	Jun kinase kinase
K	KCl	Potassium chloride
	KH_2PO_4	Potassium dihydrogen orthophosphate
L	L	Litre
	LA	Luria burtani medium containing ampicillin

	LB	Luria burtani
	Lef	Lymphoid enhancer factor
M	MAP	Mitogen-activated protein
	MCS	Multiple cloning site
	Mek	Met-enkephalin
	Mekk 1	MAP kinase kinase 1
	$MgCl_2$	Magnessium chloride
	$MgSO_4$	Magnessium sulphate
	m HSP	minimal heat shock promoter
	µl	Micro leter
	µg	Micro grams
	min	Minutes
	ml	Milli liters
	mM	Milli molar
	MMLV-RT	Moloney Murine Virus Reverse Transcriptase
	mRNA	Mesenger RNA
	mV	Mili volts
N	N	Normal
	$NaCl_2$	Sodium chloride
	$Na_2HPO_4.12H_2O$	Sodium dihydrogen orthophosphate dihydrate
	NaOH	Sodium hydroxide
	neo/kan	neomycin/kanamycin resistance ORF
O	ORF	Open reading frame
	OSCC	Oesophageal squamous cell carcinoma
	OV	Origional sample
P	P	Phosphate group
	$p90^{rsk}$	p90 ribosomal S6 kinase
	PBS	Phosphate buffered saline
	P bla	bla promoter
	p CMV	CMV promoter
	PCR	Polymerase chain reaction.

	pEGSH	Not supplied by manufacturer
	pERV 3	Not supplied by manufacturer
	PMSF	Phenylmetha-sulfonyl fluoride
	P SV40	SV40 promoter
	P TK	HSV-thymidine kinase promoter
	P UC ori	pUC origin of replication
R	R^2	Degree of fit of the line or regression
	Ras	Ras tyrosine kinase
	Raf	Raf tyrosine kinase
	RNA	Ribonucliec acid
	RNAsin®	RNase inhibitor (Promega)
	RT-PCR	Reverse transcriptase polymerase chain reaction
	RXR	retinoid-x-receptor
S	S	Synthesis phase
	SAPK	Stress activated protein kinase
	SDS	Sodium dodecyl sulphate
	SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
	Sek	Adaptor molecule
	SHC	Src homology 2
	SH2	Smad-interacting, multi-zinc finger protein I
	SIP 1	Ubiquitin activating enzyme
	Skp 1	Guanine nucleotide releasing factor
	Sos	Son of Sevenless
	SP1	Sp1 binding sites
	SV40 pA	SV40 poly a signal
T	TAE	Tris-acetate/EDTA eletrophoresis buffer
	TC	Tissue culture medium
	TCA	Tri-chloroacetic acid
	Tcf	T cell factor
	TE	Tris-EDTA
	TEMED	NNNN-tetramethylene-diamine

	TkpA	HSV-thymidine kinase (TK) poly A signal
	3’ term	Amplification of the 3’ terminus of α-catenin
	Tris.Cl	Tris(hydroxymethyl)-aminomethan chloride
U	Ub	Ubiquitin
V	V	Volts
	VgEcR	ecdysone-receptor element
	VL	Volume lyophilized
	VR	Volume required to obtain 5µg of protein
	VZA	Amplification of α-catenin’s vinculin, ZO-1 and actin binding domains
W	WHCO	Wits human carcinoma of the oesophagus
	Wnt	Wnt-wingless pathway
X	x	multiply
	x g	Times gravity
Z	$ZnCl_2$	Zinc chloride
	ZO-1	Zonular occludens 1