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The investigation of the integrative function of focal adhesion kinase (FAK) in human oesophagal squamous cell carcinoma cell lines

Worsley, Catherine Mary

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**The investigation of the integrative function of focal
adhesion kinase (FAK) in human oesophageal
squamous cell carcinoma cell lines**

Catherine Mary Worsley



A dissertation submitted to the Faculty of Science, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of Master of Science.

Johannesburg, 2008

DECLARATION

I 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 other degree or examination at any other University.

Catherine Worsley

Date: _____ day of _____ 20____

ABSTRACT

The expression of focal adhesion kinase (FAK), a non-receptor cytoplasmic tyrosine kinase, is often upregulated in many cancer types. FAK influences cellular adhesion and migration, as well as significantly mediating downstream signalling to components involved in cellular proliferation and survival. Many of these cellular pathways are facilitated by the interaction of FAK with epidermal growth factor receptor (EGFR), which is overexpressed in human squamous cell carcinoma. This cancer type is highly prevalent in South Africa and is characterized by extremely aggressive clinical behaviour and very poor patient prognosis. The aim of this study was to investigate FAK expression, localization, and the effects of EGFR activation on the expression and tyrosine phosphorylation status of FAK in order to shed light on the migratory behaviour of human oesophageal squamous cell carcinoma. This is the first study that semi-quantifiably details FAK expression in 5 South African human oesophageal squamous cell carcinoma cell lines as demonstrated by western blot analysis. Furthermore, as shown by indirect immunofluorescence, FAK is localized to focal contacts within migratory structures as well as being abundantly present within the cytoplasm of the oesophageal squamous cell carcinoma cell lines. Localization of FAK to the migratory front of these cells may promote focal adhesion turnover and stimulate cell migration in these cell lines. This study is also the first demonstration in this cancer type that illustrates the modulation of the expression, cellular localization, proteolytic cleavage and tyrosine phosphorylation status of FAK by active EGFR. These findings may uncover some of the molecular mechanisms by which upregulated cell movement influences the metastatic behaviour of this cancer. Furthermore, the results presented in this study identify FAK as a key candidate for anti-cancer therapy in squamous cell carcinoma of the oesophagus.

LIST OF ASSOCIATED PUBLICATIONS AND PRESENTATIONS

Worsley CM; Driver G; and Veale RB: **ILK phosphorylation of PTEN in human oesophageal squamous cell carcinoma (HOSCC)**. *SASBMB XX Conference, Pietermaritzburg*. 3 - 6 July 2006

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LIST OF ABBREVIATIONS AND SYMBOLS

A	adenine
α	alpha
APC	adenomatous polyposis coli
APS	ammonium persulphate
ATP	adenine triphosphate
β	beta
Bad	Bcl-2 associated death protein
Bis	<i>N,N'</i> , -methylenebis-acrylamide
BSA	bovine serum albumin
°C	degrees Celsius
CAM	cell adhesion molecule
CAS	Crk-associated substrate
caspase	cysteine-dependent aspartate-directed protease
CDK	cyclin-dependent kinase
cDNA	complementary deoxyribonucleic acid
CO ₂	carbon dioxide
c-Src	cellular-Src
C-terminal	carboxy-terminal
Da	Dalton(s)
dH ₂ O	distilled water
DMEM	Dulbecco's Modified Eagle's Medium
DNA	deoxyribonucleic acid
E-cadherin	epithelial-cadherin
ECM	extracellular matrix
EDTA	ethylenediaminetetra-acetic acid
EGF	epidermal growth factor
EGFR	epidermal growth factor receptor
ERK	extracellular signal-regulated kinase
FAK	focal adhesion kinase
FAT	focal adhesion targeting

FCS	foetal calf serum
FERM	band 4.1, ezrin, radixin, moesin
FITC	fluoresceine isothiocyanate
FRNK	FAK related non-kinase
γ	gamma
g	gram(s)
x g	gravitational units
G	guanine
Grb2/7	growth factor receptor binding protein 2/7
GSK3 β	glycogen synthase kinase 3 β
GTP	guanine triphosphate
GTPase	guanine triphosphatase
HASM	human airway smooth muscle
HGF/SF	hepatocyte growth factor/scatter factor
HOSCC	human oesophageal squamous cell carcinoma
HPV	human papilloma virus
hr(s)	hour(s)
HRP	horseradish peroxidase
IAP	inhibitor-of-apoptosis protein
IB	immunoblot
Ig	immunoglobulin
IgCAMs	immunoglobulin superfamily of cell adhesion molecules
ILK	integrin-linked kinase
IP	immunoprecipitation
JNK	Jun N-terminal kinase
κ	kappa
k	kilo
kDa	kilodalton(s)
l	litre(s)
LD2	leucine aspartate repeat 2
Lef	lymphoid enhancing factor
Lys	lysine

μ	micro
μg	microgram(s)
μl	microlitre(s)
M	molar
mA	milliampere(s)
MAPK	mitogen-activated protein kinase
MDM2	murine double minute 2
min	minute(s)
ml	millilitre(s)
mm	millimetre(s)
mM	millimolar
MMP	matrix metalloprotease
mRNA	messenger ribonucleic acid
MW	molecular weight
NF-κB	nuclear factor kappa B
ng	nanogram(s)
nm	nanometre(s)
N-terminal	amino-terminal
PAGE	polyacrylamide gel electrophoresis
PAK	p21-activated kinase
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PDGF	platelet-derived growth factor
PDGFR	platelet-derived growth factor receptor
PI3K	phosphatidylinositol 3'-kinase
PIP ₃	phosphatidylinositol-(3,4,5) triphosphate
PKB/Akt	protein kinase B
PLCγ	phospholipase Cγ
PMSF	phenyl-methyl-sulphonyl fluoride
pRb	retinoblastoma protein
PTB	phosphotyrosine binding
PTEN	phosphatase and tensin homologue deleted on chromosome 10

PTK	protein tyrosine kinase
PTP	protein tyrosine phosphatase
Rho	Ras homologue
RNA	ribonucleic acid
RNAi	ribonucleic acid interference
RTK	receptor tyrosine kinase
SDS	sodium dodecyl sulphate
SDS-PAGE	sodium dodecyl sulphate – polyacrylamide gel electrophoresis
sec	second(s)
SH2/3	Src homology 2/3
Shc	Src homology containing protein
SHIP-2	Src-homology-domain 2-containing tyrosine phosphatase-2
SFK	Src family kinase(s)
siRNA	small interfering ribonucleic acid
SOS	son of sevenless
STAT	signal transducer and activator of transcription
TBS	tris-buffered saline
TCA	tri-chloroacetic acid
Tcf	T-cell factor
TEMED	<i>N,N',N',N'</i> -tetramethylene-diamine
TGF α	transforming growth factor alpha
Tris	tris(hydroxymethyl)aminomethane
Tyr	tyrosine
uPA	urokinase-type plasminogen activator
uPAR	urokinase-type plasminogen activator receptor
UV	ultraviolet
V	volt(s)
VEGF	vascular endothelial growth factor
v-Src	viral Src
WHCO	Witwatersrand human carcinoma of the oesophagus
Y	tyrosine

