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

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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 | *N'*,*N'*,-methylenebis-acrylamide |
| BSA | bovine serum albumin |
| ºC | 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) |
| $dH_2O$ | 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 | fluorosceine 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 |
| GSK3β | glycogen synthase kinase 3β |
| 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 | *N'*,*N'*,*N'*,*N'*-tetramethylene-diamine |
| TGFα | 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 |