FLJ22318: A Novel Binding Partner of the NKX3-1 Homeodomain Protein in Prostate Cancer Cells

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I declare that this thesis is my own account of my research and contains as its main content work which has not previously been submitted for a degree at any tertiary education institution.

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Linda Fiona Dawson

Thesis Summary

Prostate cancer is a frequently diagnosed malignancy which ranges from an indolent asymptomatic tumour to an aggressive, rapidly lethal systemic disease. Determination of chromosomal, genetic and epigenetic alterations associated with prostate carcinogenesis have led to the characterisation of functional consequences of these alterations, thereby elucidating pathways contributing to malignant growth that can be utilised clinically and therapeutically.

FLJ22318 is a novel hypothetical protein that was identified by yeast two-hybrid analysis to interact with the prostatic homeodomain protein, NKX3-1. Expression of NKX3-1 is largely restricted to epithelial cells of the adult prostate where it is involved in maintaining the prostatic phenotype, while NKX3-1 expression is reduced or absent in prostate tumours. In contrast, FLJ22318 expression is documented in cDNA libraries derived from a variety of human adult and foetal tissues including the prostate, suggesting that it may be ubiquitously expressed and that it potentially interacts with a number of proteins in addition to NKX3-1. FLJ22318 expression is undocumented in human prostate tumours.

Bioinformatic analyses have postulated multiple FLJ22318 mRNA transcripts however the proposed open reading frames encoded by these transcripts predict the FLJ22318 protein to contain three strong protein-protein interaction domains, a Lissencephaly type-1-like homology (LisH), a C-terminal to LisH (CTLH) and a CT11-RanBPM (CRA) domain. Of the 44 single nucleotide polymorphisms identified within the *FLJ22318* gene, none are located within the protein coding region suggesting that FLJ22318 may be critical for cell survival and/or function. Comparison of the amino acid sequence between human FLJ22318 and its orthologues in a diverse range of mammalian species identified >97% sequence homology, providing further strong evidence of the critical cellular function of FLJ22318.

To characterise the biological activity of FLJ22318 in prostate cancer cells, the FLJ22318 coding region was amplified by polymerase chain reaction (PCR) and ligated into mammalian and bacterial expression vectors to encode V5-, myc-, GFP-, HA-, and

GST-FLJ22318 fusion proteins. Interaction between FLJ22318 and NKX3-1 was confirmed using (reverse) yeast two-hybrid, GST pull-down and co-immunoprecipitation assays. These data were supported by confocal microscopy which demonstrated the perinuclear and nuclear co-localisation of FLJ22318 and NKX3-1 in prostate cancer cells. Northern blotting identified expression of ~2Kb and ~4Kb FLJ22318 mRNA's in prostate cancer cell lines, which was consistent with bioinformatic analyses of mRNA species. Transfection of prostate cancer cells to overexpress FLJ22318 did not alter endogenous NKX3-1 levels, however FLJ22318 exhibited transcriptional repressor function on an NKX3-1 responsive element and increased NKX3-1 transcriptional repressor activity on this element.

To further investigate FLJ22318 function, additional yeast two-hybrid analyses were performed in prostate cancer cells to identify potential FLJ22318 binding proteins. These studies isolated cDNA's encoding 33 different proteins involved in cell metabolism and apoptosis as well as transcriptional regulators associated with control of cellular proliferation. One of the candidate FLJ22318 interactors, protein kinase, interferon-inducible double stranded RNA dependent activator (PRKRA/PACT) was shown using confocal microscopy to extensively co-localise with FLJ22318 in the cytoplasm and perinuclear regions of prostate cancer cells. Preliminary co-immunoprecipitation and GST pull-down assays have provided additional evidence of the interaction of PRKRA and FLJ22318.

Results of this thesis have generated important information characterising the structure of the FLJ22318 gene and protein, the interaction between FLJ22318 and NKX3-1 and the potential functions of FLJ22318 in prostate cancer cells. As the FLJ22318 gene is located on 5q35, a chromosomal region frequently disrupted in a variety of tumours, future studies of the biological activity of FLJ22318 will clarify its normal cellular functions and its contribution to tumorigenesis or malignant progression in the prostate and in other tissues.

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Awards

During my PhD studies I have been the recipient of a Murdoch University Research Studentship and an Australian Postgraduate Award.

I was the recipient of the Scientific Section – Encouragement Award for my presentation "FLJ22318: A Novel Co-factor of NKX3.1" at The Merck Sharp & Dohme Young Investigators Meeting in September, 2004.

Publications

Abstracts

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Abbreviations

5αR	*5α-reductase inhibitor*
aa	*amino acids*
AD	*activation domain*
Ade	*adenine*
AES	*amino enhancer of split*
AF2	*activation function 2*
AI	*androgen independence*
Ald	*aldolase*
AMV	*avian myeloblastosis virus*
APS	*ammonium persulphate*
AR	*androgen receptor*
ARE	*androgen response element*
Arg	*arginine*
AS	*antisense*
Asn	*asparagine*
ATP	a*denosine 5'-triphosphate disodium salt*
BAC	*β-actin*
BD	*binding domain*
BLAST	*Basic Local Alignment Search Tool*
bp	*base pairs*
BPH	*benign prostatic hyperplasia*
BSA	*bovine serum albumin*
C	*carboxy*
CAB	*combined androgen blockade*
cDNA	*complementary deoxyribonucleic acid*
CDS	*coding domain sequence*
CIAP	*calf intestinal alkaline phosphatase*
CK	*cytokeratin*
CK2	*casein kinase 2*
CMV	*cytomegalovirus*
CNS	*central nervous system*

CO_2	*carbon dioxide*
CRA	*CT11-RanBPM*
CS	*charcoal stripped*
CT	*computed tomography*
CTLH	*C-terminal to lissencephaly type-1-like homology domain*
$CuSO_4$	*copper sulphate*
Da	*daltons*
dATP	*deoxyadenine triphosphate*
dCTP	*deoxycytosine triphosphate*
DCX	*doublecortin*
ddH_2O	*deionised distilled water*
DDO	*double dropout*
DEPC	*diethyl pyrocarbonate*
DES	*diethylstilbestrol*
dGTP	*deoxyguanine triphosphate*
DHT	5α-*dihydrotestosterone*
DMF	*dimethyl formamide*
DMSO	*dimethyl sulphoxide*
DNA	*deoxyribonucleic acid*
dNTP	*deoxynucleotide triphosphate*
DO	*dropout*
DRE	*digital rectal examination*
ds	*double stranded*
dT	*deoxythymidine*
DTT	*dithiothreitol*
dTTP	*deoxythymidine triphosphate*
Dyrk	*dual-specificity tyrosine phosphorylation-regulated kinases*
ECL	*enhanced chemiluminescence*
EDTA	*ethylenediaminetetra-acetic acid*
EHD	*extended homeodomain*
ER	*endoplasmic reticulum*
EST	*expressed sequence tag*
FBPase	*fructose-1,6-bisphosphatase*

FCS	*foetal calf serum*
FLJ	*full long Japanese*
FMR1	*fragile X mental retardation 1*
FTZ	*Fushi tarazu*
FTZ-1	*Fushi tarazu factor 1*
G	*gauge*
Gal	*galactosidase*
GFP	*green fluorescent protein*
GID	*glucose induced degradation*
GO	*gene ontology*
GR	*glucocorticoid receptor*
Gro	*groucho*
GST	*glutathione S-transferase*
GTF	*general transcription factors*
HA	*haemaglutinin*
HAT	*histone acetyl transferase*
HB	*homeobox*
HCl	*hydrochloric acid*
HD	*homeodomain*
HDAC	*histone deacetylase*
HECT	*homologous to E6-AP carboxyl terminus*
HES	*hairy enhancer of split*
HIPK	*homeodomain interacting protein kinase*
His	*histidine*
HnRNPs	*heterogeneous ribonucleoproteins*
HNRPAB	*heterogenous nuclear ribonucleoprotein A/B*
HP	*hexapeptide*
HPVs	*human papillomaviruses*
HRP	*horseradish peroxidase*
Ig	*immunoglobulin*
IPTG	*isopropyl-β-D-thiogalactoside*
Kb	*kilobase*
KCl	*potassium chloride*

kDa	*kilodaltons*
LB	*Luria Bertani*
LBD	*ligand binding domain*
LD	*long distance*
Leu	*leucine*
LH-RH	*luteinising hormone-releasing hormone*
LiAc	*lithium acetate*
LisH	*lissencephaly type-1-like homology*
LOH	*loss of heterozygosity*
Luc	*luciferase*
MAEA	*macrophage erythroblast attacher*
MCS	*multiple cloning site*
MGC	*mammalian gene collection*
$MgCl_2$	*magnesium chloride*
MGMT	*O^6-methyl-guanine-DNA methyltransferase*
$MgSO_4$	*magnesium sulphate*
MITOP	*mitochondrial proteome database*
MOPS	*3-[N-Morpholino]propanesulphonic acid*
MMLV	*Moloney Murine Leukaemia Virus*
mRNA	*messenger ribonucleic acid*
MS	*mass spectrometry*
N	*amino*
N4BP3	*Nedd4 binding protein 3*
NaCl	*sodium chloride*
NaOH	*sodium hydroxide*
NCBI	*National Center for Biotechnology Information*
NCI	*National Cancer Institute*
N-CoR	*nuclear receptor co-repressor*
NDE1	*nuclear distribution gene E homolog 1*
NDEL1	*NDE1-Like 1*
$(NH_4)_2SO_4$	*ammonium sulphate*
NK2-SD	*NK2-specific domain*
NLS	*nuclear localisation signal*
NOLA2	*nucleolar protein family A member 2*

NP-40	*nonidet P-40*
NPAT	*ataxia-telangiectasia locus protein*
NRB	*nuclear receptor box*
OD	*optical density*
OFD1	*oral-facial-digital syndrome type I*
OPN	*osteopontin*
ORC	*origin recognition complex*
ORF	*open reading frame*
PAFAH1B1	*platelet-activating factor acetylhydrolase, isoform 1b, alpha subunit*
PBS	*phosphate buffered saline*
p.c.	*post coitum*
PCD	*programmed cell death*
PCR	*polymerase chain reaction*
PDEF	*prostate derived Ets factor*
PEG	*polyethylene glycol*
PFK	*phosphofructokinase*
Pfu	*Pyrococcus furiosus*
Phe	*phenylalanine*
PIN	*prostatic intraepithelial neoplasia*
PIPES	piperazine-*N,N'*-bis *(2-ethanesulphonic acid)*
PKC	*protein kinase C*
PLB	*passive lysis buffer*
PMSF	*phenylmethylsulphonylfluoride*
P/S	*penicillin/streptomycin*
PSA	*prostate specific antigen*
QDO	*quadruple dropout*
RANBP9	*RAN-binding protein 9*
RAR	*retinoid acid receptor*
Rb	*retinoblastoma*
RING	*really interesting new gene*
RIPA	*radioimmunoprecipitation assay*
RNA	*ribonucleic acid*
rpm	*revolutions per minute*
rRNA	*ribosomal ribonucleic acid*

RT	*reverse transcription*
RXR	*retinoid X receptor*
S	*sense*
SAAB	*selection and amplification binding assay*
SDS	*sodium dodecyl sulphate*
siRNA	*small interfering RNA*
SMART	*simple modular architecture research tool*
SMGA	*smooth muscle gamma-actin*
SMRT	*silencing mediator of retinoid and thyroid hormone receptor*
SNP	*single nucleotide polymorphism*
SRF	*serum response factor*
ss	*single stranded*
SSC	*sodium chloride, sodium citrate*
TAE	*TRIS, [ethylenedinitrilo] tetra-acetic acid*
Taq	*Thermus aquaticus*
TBL1X	*transducin β-like 1X*
TBS	*TRIS buffered saline*
TBST	*TRIS buffered saline/TWEEN 20*
TCA	*tricarboxylic acid*
TGCTs	*testicular germ cell tumours*
TDO	*triple dropout*
TE	*TRIS/EDTA*
TEMED	*[N, N, N', N'-Tetra-methyl]-ethylenediamine*
TIF	*tagged image format*
TK	*thymidine kinase*
TLE	*transducin-like enhancer of split*
TNM	*tumour, node, metastases classification*
TR	*thyroid receptor*
TRIS	*tris(hydroxymethylaminomethane)*
Triton X-100	*Octylphenoxypolyethoxyethanol*
Trp	*tryptophan*
TRUS	*transrectal ultrasound*
TSG	*tumour suppressor gene*

TSS	*transcription start site*
UAS	*upstream activating sequence*
UTR	*untranslated region*
UV	*ultraviolet*
V	*volts*
WD	*tryptophan-asparagine*
X-Gal	*5-bromo-4 chloro-3-indolyl-β-D-galactopyranoside*
X-α-Gal	*5-bromo-4 chloro-3-indolyl-α-D-galactopyranoside*
Y2H	*yeast two-hybrid*
YPDA	*yeast peptone dextrose agar*