Regulation of the Tumour Suppressor PP2A by Oncogenic Tyrosine Kinases

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STATEMENT OF ORIGINALITY

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ABSTRACT

Reversible protein phosphorylation plays a central role in the regulation of intracellular signalling, and is controlled by the opposing activities of protein kinases and phosphatases. Deregulation of these mechanisms can result in increased proliferation and enhanced survival, which is a hallmark feature of malignant transformation. For example, over 90% of chronic myeloid leukaemia (CML) patients express the BCR/ABL oncoprotein, which exhibits unrestrained tyrosine kinase activity. In addition, activating mutations within the receptor tyrosine kinase, c-KIT, contribute to the pathogenesis of gastrointestinal stromal tumours (GIST), systemic mastocytosis, acute myeloid leukaemia (AML), testicular seminoma and melanoma. The advent of small molecule tyrosine kinase inhibitors, such as imatinib, has revolutionised the treatment of malignancies driven by these oncogenic kinases. However, a proportion of patients are either unresponsive or develop resistance, and as such, relapse and disease progression is a major clinical problem. In order to improve the treatment outcome for these patients, a greater understanding of the signalling pathways regulated downstream of BCR/ABL and c-KIT is required.

The data presented in this thesis indicates that oncogenic BCR/ABL and mutant c-KIT both require inhibition of the tumour suppressor, protein phosphatase 2A (PP2A), to induce tumourigenesis. PP2A is a large family of serine/threonine phosphatases that provide the fine control on signalling pathways by governing the rate and duration of phosphorylation. The heterotrimeric PP2A enzyme is comprised of a structural subunit (PP2A Aα and Aβ), a catalytic subunit (PP2Ac_α and c_β) and a regulatory subunit, which consists of three unrelated families: B55 (α, β, γ, δ), B56 (α, β, γ, δ, ε) and B" (PR72/130 / PR70/48). Binding of the regulatory subunit to the core PP2A AC dimer directs both the substrate specificity and cellular localisation of the enzyme. The combinatorial assembly of these individual components permits the formation of distinct complexes which have been implicated in numerous cellular functions such as proliferation, survival and mitosis. In particular, important roles for PP2A in various aspects of malignant transformation are beginning to emerge.

Recent work demonstrates that PP2A is functionally inactivated by BCR/ABL in myeloid progenitor cells. Using the mouse myeloid progenitor cell line, FDC-P1, these

observations were confirmed in the current study. Detailed investigation into the underlying mechanisms have demonstrated for the first time that active BCR/ABL increases the expression of the PP2A structural and certain regulatory subunits. This alters the PP2A holoenzyme composition and results in the abundance of complexes containing B55α and B56α. Consequently, B56γ, a known tumour suppressive subunit, appears to be simultaneously displaced. To investigate which subunits are functionally important for BCR/ABL-mediated leukaemogenesis, individual PP2A subunits were targeted with shRNA sequences in WT BCR/ABL FDC-P1 cells. Subsequent evaluation identified B56α as a key player which facilitates the leukaemic phenotype. In accordance with an increase in PP2A activity, knockdown of B56α significantly inhibited the cellular growth and reduced the clonogenic potential of BCR/ABL$^+$ myeloid progenitors. Furthermore, suppression of the B56δ subunit in WT BCR/ABL FDC-P1 cells appears to delay progression through the cell cycle. Together, these findings provide new insights into the biology of PP2A and begin to define the precise mechanisms by which BCR/ABL induces leukaemogenesis via PP2A in CML.

Investigation of the regulation of PP2A was also extended to the oncogenic tyrosine kinase, c-KIT. Using FDC-P1 cells expressing imatinib-sensitive (V560G) or –resistant (D816V) mutant c-KIT, this work demonstrates for the first time that constitutive activation of c-KIT impairs the activity of PP2A, and this is essential for tumourigenesis. Pharmacological reactivation of PP2A with FTY720 significantly reduced the proliferation, impaired the clonogenic potential and induced apoptosis of oncogenic c-KIT cells, whilst having no effect on empty vector controls or WT c-KIT cells stimulated with stem cell factor (SCF). These cytotoxic effects of FTY720 are mediated, in part, by the rapid dephosphorylation, and hence inactivation, of oncogenic c-KIT receptors. These promising *in vitro* findings were translated into an *in vivo* model, where the daily administration of FTY720 significantly delayed the growth of mutant c-KIT$^+$ tumours. Furthermore, FTY720 markedly prevented the infiltration of D816V c-KIT tumour cells into secondary lymphoid organs, such as the spleen and bone marrow. As a result, the survival of FTY720-treated mice was significantly prolonged compared to saline-treated controls.

Overall, this body of work greatly enhances our understanding of PP2A function and identifies the complex mechanisms of PP2A regulation by the oncogenic tyrosine kinases, BCR/ABL and c-KIT. Taken together, the data suggests that inhibition of PP2A may represent a general mechanism employed by constitutively active kinases to facilitate tumour growth. As such, this work supports the future application of PP2A-activating agents in a broad range of human malignancies.

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day, even if we ran into each other in the corridor, he was more than happy to stop and give advice, which is something I really appreciate.

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PUBLICATIONS

Roberts KG, Smith AM, McDougall F, Carpenter H, Neviani P, Perrotti D, Sim ATR, Ashman LK, Verrills NM (2009) Essential requirement for PP2A inhibition by c-KIT: FTY720-mediated reactivation of PP2A as a treatment strategy for c-KIT^{+} cancers. Submitted to *Cancer Research* #CAN-09-2544

Patents

International PCT Patent: Inhibition of c-KIT Cancers *Filed March 2009*

Oral Conference Presentations

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Roberts KG, Smith AM, Carpenter, H, Santhanam R, Ashman LK, Sim ATR, Perrotti D, Verrills NM (2009) Reactivation of PP2A as a treatment strategy for c-KIT^{+} cancers. *Australian Society for Medical Research Annual Meeting.* Sydney, Australia.
Awarded winner for best student poster.

Roberts KG, Smith AM, Carpenter, H, Santhanam R, Ashman LK, Sim ATR, Perrotti D, Verrills NM (2008) Inhibition of the tumour suppressor PP2A by c-KIT in acute myeloid leukaemia. *HMRI Conference on Translational Cancer Research*, Newcastle, Australia. **Awarded winner for best student poster.**

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Roberts KG, Smith AM, Carpenter, H, Santhanam R, Ashman LK, Sim ATR, Perrotti D, Verrills NM (2008) c-Kit functionally inactivates the tumour suppressor PP2A in AML. *New Directions for Leukaemia Research*, Sunshine Coast, Australia.
Awarded a travel scholarship for best student abstract submission.

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Roberts KG, Ashman LK, Sim ATR, Verrills NM (2006) Investigating the role of protein phosphatase 2A in chronic myeloid leukaemia, *HMRI Conference on Translational Cancer Research*, Newcastle, Australia.
Awarded runner-up for best student poster.

Awards

Best Poster Presentation – *Australia Society for Medical Research Annual Meeting, Sydney, June 2009.*

GlaxoSmithKline Best Student Poster Presentation – *Hunter Medical Research Institute Conference, Newcastle, September 2008*

Leukaemia Foundation Student Travel Scholarship – *New Directions for Leukaemia Research, Sunshine Coast, April 2008.*

GlaxoSmithKline Runner-up Best Student Poster Presentation – *Hunter Medical Research Institute Conference, Newcastle, September 2008*

Hunter Medical Research Institute Travel Grant – *November 2007*

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ABBREVIATIONS

µg	microgram
µl	microlitre
µM	micromolar
ALL	acute lymphoblastic leukaemia
AML	acute myeloid leukaemia
B-CLL	B-cell chronic lymphocytic leukaemia
bp	base pair
BSA	bovine serum albumin
CBF-AML	core-binding factor AML
CML	chronic myeloid leukaemia
cDNA	complementary DNA
CML-BC	blast crisis CML
CML-CP	chronic phase CML
Ct	cycle threshold
DAB	diaminobenzidine
DMEM	Dulbecco's modified Eagle's medium
DMSO	dimethyl sulphoxide
DNA	deoxyribose nucleic acid
ECL	enhanced chemiluminescence
ERK	extracellular signal-regulated kinase
EtOH	ethanol
FACS	fluorescence-activated cell sorter
FCS	fetal calf serum
FITC	fluorescein isothiocyanate
FLT3	fms-like tyrosine kinase 3
g	gram
g	gravity
GFP	green fluorescent protein
GIST	gastrointestinal stromal tumour
GM-CSF	granulocyte-macrophage colony-stimulating factor
GSK3β	glycogen synthase kinase 3β
HRP	horseradish peroxidise

HSC	haematopoietic stem cell
ID_{50}	concentration of drug that inhibits cells by 50%
IFN	interferon
IL	interleukin
IMDM	Iscove's modified Dulbecco's medium
i.p.	intraperitoneal
JAK	Janus kinases
JMD	juxtamembrane domain
kDa	kilodalton
M	molar
mAb	monoclonal antibody
MAPK	mitogen-activated protein kinase
mg	milligram
ml	millilitre
mM	millimolar
mRNA	messenger RNA
nt	nucleotide
nm	nanometre
pAb	polyclonal antibody
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate buffered saline
PBA	PBS / 0.1%BSA / 0.1% sodium azide
PCR	polymerase chain reaction
Ph^1	Philadelphia chromosome
pH	potential of hydrogen
PI	propidium iodide
PI3K	phosphatidylinositol 3-kinase
p.o.	oral gavage
PP2A	protein phosphatase 2A
pSR	pSUPER.retro.neo+GFP
qRT-PCR	quantitative real time PCR
RIPA	radio-immunoprecipitation assay
RNA	ribonucleic acid
RT-PCR	reverse-transcriptase PCR

RTK	receptor tyrosine kinase
RTV	relative tumour volume
S1PR	sphingosine-1-phosphate receptor
s.c.	subcutaneous
SCF	stem cell factor
SDS	sodium dodecyl sulphate
SEM	standard error of the mean
SFK	Src family kinases
SMP	skim milk powder
SphK	sphingosine kinase
ST	small T antigen
STAT	signal transducer and activator of transcription
SV40	simian virus 40
TBST	tris buffered saline-Tween 0.1%
TUNEL	terminal deoxynucleotidyltransferase-mediated dUTP nick end labelling
U	units
Wnt	wingless/Int
WT	wild-type