Role of matriptase (ST14) in Chronic Myeloid Leukaemia

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Thesis Declaration

I declare that this thesis is the result of my own work carried out in the Department of Haematology, Centre for Cancer Biology, SA Pathology, between 2008 and 2013.

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Ka-chun Cheung and , to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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Abbreviations

Abl	Abelson proto-oncogene
ALL	acute lymphoblastic leukaemia
Allo-SCT	allogeneic haematopoietic stem cell transplantation
AML	acute myeloid leukaemia
Ab	antibody
AP	accelerated phase
APOD	apoliprotein D
ATO	arsenic trioxide
ATP	adenosine triphosphate
BC	blast crisis
BCL2	B-cell CLL/lymphoma 2
BCR	breakpoint cluster region
BM	bone marrow
BMI1	BMI1 polycomb ring finger oncogene
bp	base pair
BSA	bovine serum albumin
BUS	busulfan
cDNA	copy DNA
cGMP	cyclic guanosine monophosphate
CCyR	complete cytogenetic response (0% Ph chromosome)
CHR	complete haematological response
CLP	common lymphoid progenitor
CML	chronic myeloid leukaemia
CMP	common myeloid progenitor
CMR	complete molecular response
CP	chronic phase
Ct	cycle threshold value
CUB	complement protein CIr/CIs, urchin embryonic growth factor and bone morphologenic protein 1 domain
DAB	3,3'-Diaminobenzidine

DAS	dasatinib
DLI	donor lymphocyte infusion
ds	double stranded
DUB1	deubiquitinating enzyme 1
DUB2	deubiquitinating enzyme 2
ECL	enhanced chemiluminescence
ECM	extracellular matrix
EGF	epithelial growth factor
EGR1	early growth response 1
ELA	elastase
ERK	extracellular signal-regulated kinase
FACS	flow cytometry and fluorescence activated cell sorting
FSC	forward scatter
FTI	farnesyl transferase inhibitors
GADD45	growth arrest and DNA-damage-inducible
G-CSF	granulocyte-colony stimulating factor
GFP	green fluorescent protein
GLI	GLI family zinc finger
GM-CSF	granulocyte-macrophage colony stimulating factor
GMP	granulocyte-macrophage progenitor
GUSB	glucuronidase, beta
GVHD	graft versus host disease
HAI-1	hepatocyte growth factor activator inhibitor-1
HDI	histone deacetylase inhibitors
HGF/SF	hepatocyte growth factor/scattering factor
HHT	homoharringtonine
HLA	human leukocyte antigen
HMOX1	heme oxygenase (decycling) 1
HRP	horseradish peroxidise
HSC	haemopoetic stem cells
HSP70	heat shock protein 70 (HSPA1B)
HSP90	heat shock protein 90
HU	hydroxyurea/hydroxycarbamide
IFN	interferon

IgG	immunoglobulin type G
IL-3	interleukin-3
IL-5	interleukin-5
IM	imatinib
IRES	internal ribosome entry site
IVT	in vitro transcription
JAK2	Janus kinase 2
Kb	kilo base
KDa	kilo Dalton
LPS	lipo-polysaccharides
LRIG1	leucine-rich repeats and immunoglobulin-like domains 1
LTR	long terminal repeat
LYN	v-yes-1 Yamaguchi sarcoma viral related oncogene homolog
MACS	magnetic antibody coated bead separation
MAPK	mitogen activated protein kinase
MATK	megakaryocyte associated tyrosine kinase
mCyR	minimal cytogenetic response (35%-95% Ph chromosome)
MEP	megakaryocyte-erythroid progenitor
Met	proto-oncogene (hepatocyte growth factor receptor)
MFI	mean fluorescence intensity
MGG	May-Grunwald-Giemsa stain
miR	micro-RNA
MMP-2	matrix metalloproteinase-2
MMP-9	matrix metalloproteinase-9
MMR	major molecular response
MNC	mononuclear cells
MPP	multipuripotent progenitor
mRNA	messenger RNA
mTOR	mammalian target of rapamycin
MT-SP1	membrane type serine protease 1
MYD116	myeloid differentiation primary response gene 116
NANOS1	nanos homolog 1 (Drosophilia)
NDR1	N-myc downstream regulated gene 1 (NDRG1)
NF-κB	nuclear factor kappa B

NIL	nilotinib
NPM	nucleophosmin
Oligos	oligonucleotides
OS	overall survival
PAR2	protease-activated receptor 2
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PCyR	partial cytogenetic response (1%-35% Ph chromosome)
PDGF	platelet-derived growth factor
PFS	progression free survival
Ph	Philadelphia chromosome
Ph-pos	Philadelphia positive
Ph-neg	Philadelphia negative
PI	propidium iodide
PI-3K	phosphatidylinositol-3 kinase
PMSF	phenylmethylsulfonyl fluoride
POLK	polymerase (DNA directed) kappa
PPV	positive predictive value
PR3	protease 3
PTCH1	patched 1 (Drosophilia)
RHOJ	Ras homolog gene family, membrane J (ARHJ)
RISC	RNA-induced silencing complex
RNAi	RNA interference
ROS	reactive oxygen species
rpm	rotations per minute
RQ-PCR	quantitative real time reverse transcriptase polymerase chain reaction
RT	room temperature
SCT	stem cell transplantation
sc-uPA	single chain urokinase-type plasminogen activator
SDS-PAGE	sodium dodecyl sulphate-polyacrylamide gel electrophoresis
SEA	sea urchin sperm protein, enterokinase and agrin domain
shRNA	short hairpin RNA
siRNA	short interfering RNA

SLC2A1	solute family carrier 2 (facilitated glucose transporter), member 1 (GLUT1)
Smo	smoothened, frizzled family receptor
SOCS2	suppressor of cytokine signalling 2
ss	single stranded
ST14	suppression of tumorigenicity 14 (colon carcinoma)
TAF1	TAF1 RNA polymerase II, TATA box binding protein –associated factor
TBP	TATA box binding protein
TE	Tris-EDTA buffer
TGF	transforming growth factor
TKTL1	transketolase-like 1
TLDA	Taqman Low Density Array
TLE6	transducin-like enhancer of split 6 homolog (Drosophilia)
TNF	tumour necrosis factor
TNFRSF6	tumour necrosis factor superfamily member 6 (FAS)
WBC	white blood cells count
UV	ultraviolet

Abstract

Chronic myeloid leukaemia (CML) is characterised by a reciprocal chromosomal translocation that gives rise to a 22q-, or Philadelphia (Ph) chromosome and a derivative 9+. The translocation results in a chimaeric *BCR-ABL* gene which is expressed as a 210 kDa protein. The tyrosine kinase inhibitor imatinib mesylate (IM) specifically blocks the enzymatic activity of the BCR-ABL fusion protein and has been successfully employed for the treatment of CML. However, some patients develop resistance to IM and progress to blastic crisis (BC) which is usually fatal. Previous microarray screening of murine cell clones ectopically expressing graded amounts of BCR-ABL showed that ST14 mRNA levels correlated with those of BCR-ABL. *ST14*, encoding matriptase, acts as a tumour suppressor gene in solid tumours, but has also been linked to metastasis and invasion. The aim of my project is to understand the function of ST14 in CML. Taqman real time quantitative PCR showed that there was downregulation of *ST14* expression in CD34 cells from CML chronic phase (CP) as compared to those from healthy individuals. These findings were further confirmed by experiments demonstrating upregulation of *ST14* expression in Ph-pos cell lines upon IM treatment, a phenomenon not observed in Ph-neg lines. In order to study the effect of ST14 overexpression in CML cell lines, a retroviral vector encoding *ST14* was used to infect Ph-pos cell lines (i.e. K562, KCL22 and LAMA84). Overexpression of ST14 in CML cell lines and primary cells significantly enhanced their migratory and invasion capacities. Intriguingly, it restored IM sensitivity in the KCL22 cell line which is intrinsically resistant to IM. The rescue of IM sensitivity in KCL22 overexpressing ST14 was independent of the level of *BCR-ABL* expression and unrelated to intracellular IM uptake, *LYN* and *MET* expression. The increased sensitivity to IM of KCL22 overexpressing ST14 was found to be associated with downregulation of BCL2 expression, a pro-survival protein. Furthermore, the analysis of *ST14* expression in a group of IM treated CML CP patients from a clinical trial showed that a lower *ST14* expression was associated with poorer molecular responses. The ST14 percentage at baseline was found to be a better predictor of achieving complete cytogenetic response (CCyR) at 12 months than the Sokal score. Overall, our findings were consistent with a

tumour suppressor role for *ST14*. Future studies should focus on delineating the role of ST14 as a prognostic indicator in CML patients and its role in the mechanism of disease progression as well as IM resistance.