Regulation of BCR-ABL expression via its 3'untranslated region

Bradley Chereda

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School of Medicine
Faculty of Health Sciences
University of Adelaide

Leukaemia Biology Group
Centre for Cancer Biology
IMVS, SA Pathology

Declaration

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Abstract

In patients with chronic myeloid leukaemia (CML), a translocation between chromosomes 9 and 22 fuses the *BCR* gene with the *ABL1* gene, and gives rise to the *BCR-ABL* gene. Expression of the BCR-ABL protein initiates and drives CML. The level of BCR-ABL expression is associated with disease progression and response to therapy, yet control of BCR-ABL expression is poorly understood. This study has added to this limited knowledge-base by investigating the role that the *BCR-ABL* 3'untranslated region (3'UTR) plays in controlling BCR-ABL expression. Due to the nature of the translocation, the *BCR-ABL* 3'UTR is contributed by the *ABL1* gene. We found that *ABL1* and *BCR-ABL* have similar, but shorter half-lives than *BCR*. This suggests that the *ABL1* moiety influences the stability of the *BCR-ABL* transcript. Addition of the *ABL1* 3'UTR to a *Renilla* reporter gene strongly repressed reporter expression. Furthermore, insertion of a premature poly-adenylation site in the 3'UTR resulted in a rescue of reporter expression, demonstrating that the 3'UTR is required in the transcript for full activity, and thus is indicative of post-transcriptional control. Generation of *ABL1* reporters containing various regions of the 3'UTR revealed that discrete regions of the 3'UTR could strongly influence gene expression. Following these results, we attempted to identify factors involved in the regulation of BCR-ABL expression. We focused on microRNAs-29, 30, 125, 141, 196 and 203, predicted by bioinformatics to interact with the 3'UTR. Although some of these microRNAs interacted with *ABL1* reporters, they did not modulate endogenous BCR-ABL expression. In parallel, we developed an assay that was aimed at identifying RNA-binding proteins that bind to the *ABL1* 3'UTR. Finally, using publically-available datasets, we found data suggesting that RNA-binding proteins; TTP, hnRNP-C, ELAVL-1, TIA-1 and TIAL-1 interact with functional regulatory regions of the *ABL1* 3'UTR.

Taken together, we have shown that the *BCR-ABL* 3'UTR sequence is repressive, and contains discrete regions that can influence gene expression. The 3'UTR is located within a region of the *BCR-ABL* transcript that is responsible for controlling *BCR-ABL* mRNA stability. Although many attempts were made to discover functional *ABL1* 3'UTR binding factors, their identities remain unknown. Further research is required to identify the binding factors involved. We envisage that a comprehensive understanding of how BCR-ABL is controlled will contribute to a better understanding of the biology of CML, and pave the way for innovative forms of targeted therapy.

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Abbreviations

2D	2-dimensional
7mG	7'-methyl guanine cap
AANAT	Aralkylamine N-acetyltransferase
ABL1	Abelson gene
AGO	Argonaute
ALL	Acute lymphoblastic leukaemia
AML	Acute myeloid leukaemia
ANKFN1	Ankyrin-repeat and fibronectin type III domain containing 1
AP	Alkaline phosphatase
ASF	see SFRS1
ATP	Adenosine tri-phosphate
AU-RE	AU-rich element
Aza	5-Azacytidine
BC	Blast crisis
BCL2	B-cell lymphoma 2
BCR	Breakpoint cluster region
bone fide	*in good faith, genuine*
BSA	Bovine serum albumin
C/EBPα	CCAAT/enhancer-binding protein alpha
CAGE	Capped analysis of gene expression
CDC42	Cell division control protein 42 homolog
CDCA	Chenodeoxycholic acid
CDK4	Cyclin-dependent kinase 4
cDNA	complementary DNA
CHOP	CCAAT/enhancer-binding protein homologous protein
CID	Collision-induced dissociation
CIP	Alkaline phosphatase
cis	on the same side
CLL	Chronic lymphocytic leukaemia
CML	Chronic myeloid leukaemia
CMP	Common myeloid progenitor
CNL	Chronic neutrophilic leukaemia
COLIa1	Collagen type I alpha1
CP	Chronic phase
CRB3	Crumbs homolog 3
DEPC	Diethylpyrocarbonate
DGCR8	DiGeorge syndrome critical region gene 8
DNA	Deoxy-ribose nucleic acid
DNA-PKcs	DNA-dependent protein kinase, catalytic subunit

DNMT3A	DNA (cytosine-5-)-methyltransferase 3 alpha
DSB	Double-stranded breaks
ELAVL	(Embryonic lethal, abnormal vision, Drosophila)-like
EST	Expressed sequence tag
et al	*and others*
etc	ecetera
ex vivo	*out of the living*
FACS	Fluorescence-activated cell sorting, Flow cytometry
FOXO3	Forkhead box O3
FUS	Fused in sarcoma
GFP	Green fluourescent protein
GMP	Granulocyte-macrophase progenitor
GRP58	see PDIA3
GUS-B	Glucuronidase beta
HNF4α	Hepatocyte nuclear factor 4 alpha
hnRNP	heterogeneous nuclear ribonucleoprotein
HOX-B7	Homeobox B7
HPLC	High-performance liquid chromatography
HSC	Haemopoietic stem cell
HuR	ELAVL-1
IFNγ	Interferon gamma
IGF2BP	Insulin-like growth factor 2 binding protein
IL	Interleukin
IMP	see IGF2BP
IMPDH2	Inosine-5'-monophosphate dehydrogenase 2
in silico	*performed on computer*
in vitro	*in glass*
in vivo	*within the living*
JAK	Janus kinase
kb	kilobases
kDa	kilo dalton
KH	K-homology
KRAS	Kirsten rat sarcoma viral oncogene homolog
LB	Luria broth
LC-eSI-IT	Liquid chromatography-electrospray ionisation ion-trap mass spectrometry
LDLR	Low density lipoprotein receptor
LT-HSC	Long-term HSC
LTR	Long terminal repeat
m/z	mass/charge
max	maximum
MCL	Mantle-cell lymphoma
Me	Methylation

Meg^K	Megakaryocyte
MEP	Megakaryocyte-erythroid progenitor
MMR	Major molecular response
modus operandi	*mode of operation*
MR	Molecular response
mRNA	messenger RNA
MS-MS	Tandem mass spectrometry
next-gen	next-generation
NPM1	Nucleophosmin
nt	nucleotide
NUMB	Numb homolog
Oct4	octamer-binding transcription factor 4
PACT	protein activator of PKR
PAS	Poly adenylation signal
PBA	4-Phenylbutyric acid
PBMNC	Perhiperal blood mononuclear cell
PCR	Polymerase chain reaction
PDIA3	Protein disulphide-isomerase A3
Ph	Philadelphia
PhD	Doctor of philosophy
PI3K	Phosphoinositide 3-kinase
PKCβII	Protein kinase C βII
PMSF	Phenylmethylsulfonyl fluoride
poly-A	poly-adenosine
PP2A	Protein phosphatase 2A
Pre-miRNA	Precursor-microRNA
Pri-miRNA	Primary-microRNA
PTEN	Phosphatase and tensin homolog
PTENP1	PTEN pseudogene 1
qRT-PCR	Quantitative real-time PCR
RACE	Rapid amplification of cDNA ends
RB	Retinoblastoma (gene)
RBC	Red blood cell
RBD	RNA-binding domain
RIP-SEQ	Immunoprecipitation of proteins coupled with next-gen sequencing
RISC	RNA-induced silencing complex
RNA	Ribonucleic acid
ROS	Reactive oxygen species
RRM	RNA-recognition motif
SD	Standard deviation
SEM	Standard error of the mean
SFRS1	Splicing factor, arginine/serine-rich 1

SH1	Src homology domain 1
siRNA	silencing RNA
SILAC	Stable isotope labelling with amino acids in cell culture
SNP	Single nucleotide polymorphisms
ssRNA	single-stranded RNA
STAT5	Signal transducer and activator of transcription 5
ST-HSC	Short-term HSC
TIA-1	T-cell-restricted intracellular antigen-1
TIAL-1	TIA-like-1
TKI	Tyrosine kinase inhibitor
TLS	Translocated in liposarcoma
TNF	Tumour necrosis factor
trans	opposite side
TRBP	HIV transactivating response RNA binding protein
TTP	Tristertaprolin
UTR	Untranslated region
UV	Ultraviolet
vice versa	*the other way around*
vs.	*versus*
ZFP36	see TTP