The Therapeutic Potential of Lentivector-delivered RNAi

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Abstract

Many forms of leukaemia are caused by chromosomal translocations, which result in specific and characteristic genomic sequences. Where these sequences are unique to the leukaemic cells, they represent good candidates for targeting by sequence-specific techniques, such as RNA-Interference (RNAi). RNAi is a mechanism inherent in eukaryotic cells which silences target mRNAs based on homology to a dsRNA template. This template may be introduced artificially by a number of methods, and so this mechanism can be manipulated to regulate the expression of target genes. One of the most efficient methods of introducing RNAi templates is by expression of short-hairpin RNA (shRNA) cassettes from DNA plasmids or vectors.

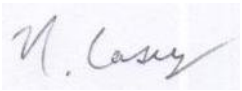
Lentiviral vectors are based on viruses that integrate into the DNA of the host cell, and are a highly efficient class of vectors for transducing and providing stable transgene expression in a range of cell types. They are particularly effective at tansducing haematopoietic cells, which have proven difficult to transduce by other methods. By fine-tuning the methods of vector production and transduction, a range of human leukaemic cells lines were able to be transduced with unprecedented efficiency in the present study. Lentiviral transduction was combined with rapid puromycin selection to generate a pure population of transduced cells with minimal expansion of the cell population.

The strategy of expressing shRNAs from retroviral and lentiviral vectors combined with puromycin selection was used to target three well-characterised fusion genes; Bcr-Abl, PML/RARα and RUNX1/ETO, in three human leukaemic cell lines. In two of these, the shRNA was able to efficiently and effectively down-regulate the target mRNA, and inhibit the proliferation of the transduced leukaemic cells. In the third case, RUNX1/ETO, no effective shRNA design could be identified.

Finally, concerns over the safety of integration targeting by current gene therapy vectors motivated an investigation of the activity of a novel integrase enzyme from the Ty3 retrotransposon found in yeast. In yeast cells, the integration-mediating enzyme of this retrotransposon has very specific targeting characteristcs, which, if retained in human cells, would provide a very safe gene therapy vector. It was found that this enzyme is indeed active in human cells, and therefore has potential in the context of human gene therapy.

Declaration

I certify that this thesis contains no material which has been accepted for a degree or diploma by the University or any other institution, and that to the best of my knowledge and belief, no material previously published or written by another person has been utilised in the preparation of this thesis, except where due acknowledgement is made in the text, nor does this thesis contain any material which infringes copyright.



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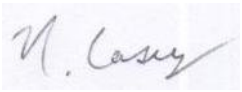

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Publications

Part of the work contained in this thesis has been published or submitted for publication as detailed below:

Brozik, A., N.P. Casey, C. Hegedus, A. Bors, A. Kozma, H. Andrikovics, M. Geiszt, K. Nemet and M. Magocsi (2006). "Reduction of Bcr-Abl function leads to erythroid differentiation of K562 cells via downregulation of ERK." Annals of the New York Academy of Sciences **1090**: 344-54.

Statement of Ethical Conduct

The research associated with this thesis abides by the international and Australian codes on human and animal experimentation, the guidelines of the Australian Government's Office of the Gene Technology Regulator and the rulings of the Safety, Ethics and Institutional Biosafety Committees of the University.

List of Abbreviations

6-FAM	6-CarboxyFluorescein-Aminohexyl Amidite
AAV	Adeno-Associated Virus
Abl	Abelson Leukemia
AE(1/2/3)	AML1-ETO shRNA design (1/2/3)
AIDS	Acquired Immuno Definicency Syndrome
ALL	Acute Lymphoblastic Leukaemia
AML	Acute myeloid Leukaemia
AML1	Acute myeloid Leukaemia-1
AP-1	Activator Protein 1
APL	Acute Promyelocytic Leukemia
ASLV	Avian Sarcoma Leukosis Virus
ASV	Avian Sarcoma Virus
ATCC	American Type Culture Collection
ATO	Arseninc Tetroxide
ATP	Adenosine Triphosphate
ATRA	All Trans-Retinoic Acid
B2M	Beta-2-microglobulin
Bcr	Breakpoint cluster region
BLAST	Basic Local Alignment Search Tool
bp	base pairs
CAG	Cytomegalovirus early enhancer element and chicken beta-actin promoter
CBFA2	Core-Binding Factor, runt domain, Alpha subunit 2
CBP	CREB (cAMP response element-binding)-Binding Protein
CD(nn)	Cluster Differentiation marker number (nn)
CML	Chronic Myelogenous Leukemia
CMV	Cytomegalovirus
CpG	C-phosphate-G
DAI	dsRNA-Activated Inhibitor
DMEM	Dulbecco's Modified Eagle Medium

DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic Acid
ECL	Enhanced Chemiluminescence
EDTA	Ethylenediaminetetraacetic Acid
EF1α	Elongation Factor 1 α
EGFP	Enhanced Green Fluorescent Protein
EGTA	Ethylene Glycol Tetraacetic Acid
EIAV	Equine Infectious Anemia Virus
ERK	Extracellular-signal-Regulated Kinases
ETO	Eight Twenty-One
FAB	French-American-British
FACS	Fluorescence-Activated Cell Sorting
FCS	Foetal Calf Serum
FISH	Fluorescent In Situ Hybridization
FIV	Feline Immunodeficiency Virus
GAPDH	Glyceraldehyde 3-Phosphate Dehydrogenase
G-CSF	Granulocyte Colony-Stimulating Factor
gDNA	Genomic Deoxyribonucleic Acid
GFP	Green Fluorescent Protein
GRB2	Growth Factor Receptor-Bound protein 2
HAT	Histone Acetyl Transferase
HBS	Hepes Buffered Saline
HDAC	Histone Deacetylase
HEK	Human Embryonic Kidney
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HERV	Human Endogenous Retrovirus
HI-FCS	Heat Inactivated Foetal Calf Serum
HIV	Human Immunodeficiency Virus
HSV	Herpes Simplex Virus
IFN	Interferon
IL2	Interleukin-2
IL2RG	Interleukin-2 Receptor subunit gamma

IN	Integrase
IRES	Internal Ribosomal Entry Site
ISG	Interferon-Stimulated Gene
JAK	Janus Kinase
kb	kilo-bases
kDa	kilo-Daltons
K-RAS	Kirsten Rat Sarcoma viral oncogene homolog,
LAM-PCR	Linear Amplification Mediate-Polymerase Chain Reaction
LMO2	LIM domain Only 2
LTR	Long Terminal Repeats
Lys	Lysine
MAPK	MAP (Mitogen-Activated Protein) Kinase
MEK	MAPK/ERK Kinase
MFI	Mean Fluorescence Intensity
miRNA	micro-Ribonucleic Acid
MLV	Murine Leukemia Virus
MoMLV	Moloney Murine Leukemia Virus
mRNA	messenger Ribonucleic Acid
MTG8	Myeloid Translocation Gene 8
N-CoR	Nuclear receptor Co-Repressor 1
nef	negative regulatory factor
NHR	Nuclear Hormone Receptor
NS	Nonsense shRNA design
OAS	Oligoadenylate Synthetase
ORF	Open Reading Frame
PAGE	Poly-Acrylamide Gel Electrophoresis
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
PEBP2αB	Polyomavirus Enhancer Binding Protein 2 alpha B
(p)ERK	(phosphorylated) Extracellular signal-Regulated Kinase
PIWI	P-element Induced Wimpy testis
PKR	Protein Kinase R

PLZF	Promyelocytic Leukemia Zinc Finger
PML	Promyelocytic Leukemia
PNPP	p-Nitrophenyl Phosphate
POD	PML Oncogenic Domain
PolIII	Polymerase class III
PR(1/2)	PML/RARα shRNA design (1/2)
PUROr	Puromycin resistance gene
PVDF	Polyvinylidene Fluoride
qRT-PCR	Quantitative Reverse Transcript Polymerase Chain Reaction
RA	Retinoic Acid
RARα	Retinoic Acid Receptor alpha
RARE	Retinoic Acid Responsive Element
RAS	Rat Sarcoma
RING	Really Interesting New Gene
RISC	RNA-Induced Silencing Complex
RNase	Ribonuclease
RPMI	Roswell Park Memorial Institute
RUNX1	Runt-related transcription factor 1
SDS	Sodium Dodecyl Sulfate
shRNA	short-hairpin Ribonucleic Acid
siRNA	short-interfering Ribonucleic Acid
SMRT	Silencing Mediator for Retinoid and Thyroid-hormone receptors
SNFW	Sterile Nuclease-Free Water
SRC	Sarcoma
SSC	Saline Sodium Citrate
STAT(1/5)	Signal Transducers and Activators of Transcription
SUP2	Suppressor 2
SYBR	Synergy Brands
TBS	Tris Buffered Saline
TFIII	Transcription Factor III
TIF1α	Transcriptional Intermediary Factor 1 alpha
tRNA	transfer RNA

vif	viral infectivity factor
vpr	viral protein R
VSV-G	Vesicular Stomatitis Virus G
WPRE	Woodchuck hepatitis post-transcriptional regulatory element
X-SCID	X-linked Severe Combined Immunodeficiency

Table of Contents

List of Figures