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Functional Analysis of the HOX11 Target Genes *ALDH1A1* and *FHL1*

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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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ABSTRACT

HOX11 is a developmental regulator that plays a crucial role in the normal development of the spleen and is also aberrantly activated by the t(10;14)(q24;q11) and variant t(7;10)(q35;q24) translocations in a subset of T-cell acute lymphoblastic leukaemias (T-ALLs). The recent finding that HOX11 is deregulated in up to 40% of childhood T-ALLs when abnormalities not detected by cytogenetics are included, suggests that the over-expression of HOX11 and subsequent deregulation of downstream target genes are critical events in the progression of this tumour type. To date, three candidate HOX11 target genes have been reported, two of which are *Aldehyde Dehydrogenase 1a1* (*ALDH1A1*) and *Four and a Half LIM domain Protein 1* (*FHL1*). This investigation focused on two aspects of HOX11 function, namely its roles as a transcriptional regulator and as a nuclear oncoprotein capable of inducing neoplastic transformation. More specifically, we sought to further understand the role of HOX11 in tumorigenesis by 1) Confirming target gene status of *ALDH1A1* and *FHL1* by assessing whether their proximal promoter regions are transcriptionally regulated by HOX11, 2) Investigating the regulatory elements/transcriptional complexes involved in the response of *ALDH1A1* to HOX11 in both a T-cell and an erythroid cell line in order to gain an insight into the mechanism(s) responsible for mediating a HOX11 activity and 3) Assessing the ability of *ALDH1A1* and *FHL1* to perturb normal patterns of haematopoiesis, on the basis that the transforming capabilities of HOX11 are thought to derive from its ability to affect haematopoietic cell differentiation.

To confirm *ALDH1A1* and *FHL1* as target genes, they were both characterised in terms of the ability of their proximal promoters to be transcriptionally regulated by HOX11 using luciferase reporter assays. Significant repression of the proximal promoters of *ALDH1A1* and *FHL1* by HOX11 was observed in PER-117 T-cells which provided further evidence for their status as target genes. In the case of *ALDH1A1*, a CCAAT box (-74/-70bp) was identified as the primary *cis*-regulatory element involved in *ALDH1A1* transcription and repression by HOX11 appeared to occur, either directly or indirectly, via interactions at the CCAAT box. Electromobility shift assays (EMSAs) revealed the disruption of a specific complex at this site by HOX11, which also altered the formation of complexes at a non-canonical TATA box (a GATA box at -34/-29bp). Significantly,

HOX11 was shown to have the potential to interact with TFIIB, a member of the basal transcriptional complex. This, together with the presence of a TFIIB responsive element immediately 5' of the GATA box, suggested that HOX11 may repress transcription by interfering with members of a preinitiation complex on the *ALDH1A1* promoter. The transcriptional repression by HOX11 demonstrated in T-cells was dependent on DNA binding helix 3 of the homeodomain, suggesting that repression may require DNA binding. Alternatively, this region may be required for stable protein-protein interactions. In support of this, the *in vitro* association of HOX11 with TFIIB was disrupted upon deletion of helix 3, and the HOX11ΔH3 mutant switched from a transcriptional repressor to a potent activator of transcription. Together, this data supports a model whereby HOX11 represses transcription by interfering with activation complexes at the CCAAT box and at the GATA box possibly via protein-protein interactions involving the homeodomain helix 3, whereas deletion of the region disables repressor-specific interactions, resulting in potent activation by HOX11.

Luciferase reporter gene assays investigating the response of nested deletions of the *ALDH1A1* promoter to HOX11 in the HEL900 erythroleukaemic cell line, also identified the CCAAT box (-74/-70bp) as the primary *cis*-regulatory element involved in *ALDH1A1* transcription. However, in stark contrast to the its effect in T-cells, HOX11 was shown to activate transcription in the HEL cell line, both from the empty pGL3Basic luciferase reporter vector and from the *ALDH1A1* promoter, in a manner independent of the homeodomain DNA binding helix 3. HOX11 thus appears to be a dichotomous regulator, capable of both transcriptional activation and repression depending on the circumstances. The mechanisms underlying these two functions are also appear to be distinct, with repression but not activation requiring the presence of homeodomain helix 3.

ALDH1A1 encodes an enzyme involved in the irreversible conversion of retinaldehyde to the biologically active metabolite, retinoic acid (RA) and appears to be physiologically regulated by Hox11 in the developing spleen. Since RA is a potent modulator of cellular differentiation, proliferation and apoptosis, the dysregulation of RA synthesis is likely to have severe consequences for the cell and may constitute a mechanism whereby overexpression of HOX11 predisposes T-cells to malignant

transformation. *FHL1* also appears to have potential relevance to tumorigenesis, given that it encodes protein isoforms with suspected roles in transcriptional regulation. As a starting point to investigate a possible link between these HOX11 target genes and leukaemogenesis, the effect of overexpressing *ALDH1A1* and *FHL1* on murine haematopoiesis was assessed following reconstitution of lethally irradiated mice with retrovirally-transduced primary murine bone marrow cells. The enforced expression of *ALDH1A1* in bone marrow was associated with a marked increase in myelopoiesis and a decrease in B and T-lymphopoiesis. By contrast, overexpression of *FHL1* was not associated with perturbations in myelopoiesis or lymphopoiesis, although a slight increase in erythropoiesis was observed in the bone marrow. While further work is required to clarify the possible oncogenic roles of both of these HOX11 target genes, these findings have served to identify *ALDH1A1* in particular, as a gene which could potentially be involved in HOX11-mediated tumorigenesis.

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PUBLICATIONS

The following paper has been published in international peer-reviewed journal during the course of this PhD candidature:

Heidari, M., Rice, K.L., Kees, U.R. and Greene, W.K. (2002), Expression and Purification of the Human Homeodomain Oncoprotein HOX11. *Protein Expression and Purification,* (**25**): 313-318.

ABSTRACTS

The following abstract has been presented at a conference proceeding during the course of this PhD candidature:

Heidari, M., Rice, K.L., Kees, U.R., Greene, W.K. (2003) The Nuclear Oncoprotein HOX11 Associates with Pericentromeric Heterochromatin in Leukemic T-cells. *Keystone Symposia - Chromatin: Organising the Genome for Patterns of Gene Expression in Health and Disease*, Big Sky, Montana, USA.

Abbreviations

Abbreviation	Expansion
A	Absorbance
aa	Amino Acid
Abd	Abdominal
ADH	Alcohol Dehydrogenase
AF-2	Activation Function Domain-2
ALDH	Aldehyde Dehydrogenase
Aldh1a1	Aldehyde Dehydrogenase 1a1
ALL	Acute Lymphoblastic Leukaemia
AML	Acute Myeloid Leukaemia
Amp	Ampicillin Resistance Gene
Antp	Antennapedia
AP	Anterior-Posterior
APC	Allophycocyanin
APL	Acute Promyelocytic Leukaemia
APS	Ammonium Persulfate
ARC	Animal Resource Centre
AS	Antisense
ATRA	All-trans Retinoic Acid
B-ALL	B-cell Acute Lymphoblastic Leukaemia
bap	Bagpipe
BD	Becton Dickinson
bHLH	Basic Helix-Loop-Helix
Bio	Biotin
bp	Base Pairs
BSA	Bovine Serum Albumin
Bx	Bithorax
CAT	Chloramphenicol Acetyl Transferase
CD	Cluster of Differentiation
cDNA	Complementary DNA
CDP	CCAAT-Displacement Protein
CDK	Cyclin Dependent Kinase
C/EBPβ	CCAAT/Enhancer-Binding Protein β
CFU-GM	Colony Forming Unit-Granulocyte Macrophage
cGy	Centigrays
chIP	Chromatin Immunoprecipitation
CIP	Calf Intestinal Phosphatase
CKI	Cyclin Kinase Inhibitor
CLCR	Children's Leukaemia and Cancer Research
CLP	Common Lymphoid Progenitor
cm	Centimetre
CMP	Common Myeloid Progenitor
CNS	Central Nervous System
Co-IP	Co-Immunoprecipitation
COOH	Carboxyl Terminus

cpm	Counts Per Minute
cps	Counts Per Second
CRBP	Cellular Retinol Binding Protein
CRAB	Cellular Retinoic Acid Binding Protein
CTF1	CCAAT-box-binding Transcription Factor 1
CYP450	Cytochrome P450
Da	Dalton
DBD	DNA Binding Domain
DC	Dendritic Cell
dE	Embryonic Day
Dfd	Deformed
dH_2O	Deionised Water
DMEM	Dulbecco's Modified Medium
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic Acid
dNTP	Deoxynucleoside Triphosphate
ddNTP	Dideoxynucleotide Triphosphate
dpc	Days Postcoitum
DPE	Downstream Promoter Element
DTT	Dithiothreitol
EB	Embryoid Bodies
EDTA	Ethylenediamine Tetra Acetic Acid
EGTA	Ethylene Glycol-bis Tetra Acetic Acid
EMSA	Electrophoretic Mobility Shift Assay
en	Engrailed
EPCD	Epicardially Derived Cell
ERα	Estrogen Receptor α
EST	Expressed Sequence Tag
eve	Even-Skipped
Exd	Extradenticle
FACS	Fluorescence Activated Cell Sorting
FCS	Foetal Calf Serum
Fhl1	Four and a Half LIM Domain Protein 1
FRET	Fluorescence Resonance Energy Transfer
FSC	Forward Scatter
FTOC	Foetal Thymic Organ Culture
g	Gram
gag	Group Antigen
GFP	Green Fluorescence Protein
GM-CSF	Granulocyte-Macrophage Colony Stimulating Factor
GST	Glutathione S-transferase
GTF	General Transcription Factor
h	Hour
HAT	Histone Acetyltransferase
HBS	Hepes Buffered Saline
HCA	Human Cardiac Alpha Gene
HDAC	Histone Deacetylase
Hdg-1	HOX11 Dependent Gene-1
HEPES	N-2-Hydroxyethylpiperazine-N'-2-Ethanesulfonic Acid
HOM	Homeotic

HOM-C	Homeotic Complex
HOX	Homeobox
HRP	Horseradish Peroxidase
HSC	Haematopoietic Stem Cell
IFN	Interferon
IL	Interleukin
Inr	Initiator
IPTG	Isopropyl β-d-Thiogalactopyranoside
IRES	Internal Ribosome Entry Site
kb	Kilobase
kDa	Kilodalton
L	Litre
lab	Labial
LB	Luria-Bertani
LBD	Ligand Binding Domain
lbe	Ladybird early
lbl	Ladybird late
LIM	Lin-11, Isl-1 and Mec-3
LMO	LIM-Only
LT-HSC	Long-Term Haematopoietic Stem Cells
LTR	Long Terminal Repeat
lym	Lymphoid Progenitor
M	Molar
M/E	Myeloerythroid Progenitor
MED-1	Multiple Start Site Element Downstream-1 Element
MEIS	Myeloid Ectopic Integration Site
mg	Milligram
min	Minute
MIS	Mullerian Inhibiting Substance
mL	millilitre
MLL	Mixed Lineage Leukaemia
MLP	Multilineage Progenitor
mM	Millimolar
MOPS	3-[N-morpholino] Propanesulfonic Acid
MPD	Myeloproliferative Disease
mRNA	Messenger RNA
MSCV	Murine Stem Cell Virus
MW	Molecular Weight
MWM	Molecular Weight Marker
MZF-1	Myeloid Zinc Finger-1
NcoR	Nuclear receptor CoRepressor
NES	Nuclear Export Sequence
ng	Nanogram
NGFR	Nerve Growth Factor Receptor
NH_2	Amino Terminus
NK	Natural Killer Cell
NKL	NK-Like
NLS	Nuclear Localisation Signal
nm	Nanometre
nmol	Nanmoles

nM	Nanomolar
NMR	Nuclear Magnetic Resonance
NP-40	Nonident-40
NS1	Non-Specific Competitor 1
NS2	Non-Specific Competitor 2
NTP	Nucleoside Triphosphate
OD	Optical Density
ONPG	O-N-Galactopyranoside
pb	Proboscipedia
PBS	Phosphate Buffered Saline
Pbx1	Pre-B-cell Leukemia Transcription Factor 1
PCR	Polymerase Chain Reaction
PE	Phycoerythrin
PI	Propidium Iodide
PIM	PBX-Interaction Motif
PLZF	Promyelocytic Leukaemia Zinc Finger
PML	Promyelocytic Leukemia
PMSF	Phenylmethylsulfonyl Fluoride
PMT	Photomultiplier Tube
PNK	Polynucleotide Kinase
PolydIdC	Polydeoxycytidylic Acid
PP1C	Protein Phosphatase 1Catalytic Subunit
PP2AC	Protein Phosphatase 2A Catalytic Subunit
PTC-100	Programmable Thermal Controller-100
PTP	Protein Tyrosine Phosphatase
RA	Retinoic Acid
RAR	Retinoic Acid Receptor
RARE	Retinoic Acid Response Element
RACE	Rapid Amplification of cDNA Ends
RALDH2	Retinaldehyde Dehydrogenase 2
RARE	Retinoic Acid Response Element
RBC	Red Blood Cell
RBP	Retinol Binding Proteins
RDA	Representational Difference Analysis
RLM-RACE	RNA Ligase-Mediated Rapid Amplification of cDNA Ends
RLU	Relative Light Unit
RNA	Ribonucleic Acid
RPH	Royal Perth Hospital
RT	Reverse Transcription
RT-PCR	Reverse Transcriptase-Polymerase Chain Reaction
RXR	Retinoid X Receptor
S	Sense
Sal	Spalt
SCF	Stem Cell Factor
SCL	Stem Cell Leukaemia
SCID	Severe Combined Immunodeficiency
SDH	Short-chain Dehydrogenase Reductase
SDS	Sodium Dodecyl Sulfate
SDS-PAGE	SDS-Polyacrylamide Gel Electrophoresis
sec	Second

SIL	SCL Interupting Locus
SLIM1	Striated Muscle LIM protein 1
SLIMMER	SLIM1 with Extra Regions
slou	Slouch
SMRT	Silencing Mediator of Retinoid and Thyroid Hormone Receptor
SRF	Serum Response Factor
SRY	Sex-Determining Region Y Gene
SSS	Single Start Site
SSC	Side Scatter
TAE	Tris Acetate Ethylenediamine Tetra Acetic Acid
TALE	Three-Amino-Acid Loop Extension
T-ALL	T-Cell Acute Lymphoblastic Leukaemia
TAP	Tobacco Acid Pyrophosphatase
TBE	Tris Borate Ethylenediamine Tetra Acetic Acid
TCR	T-Cell Receptor
TdT	Terminal Deoxynucleotidyl Transferase
TGIF	5'-TG-3' Interacting Factor
tin	Tinman
TLE	Transducin-like Enhancer of Split
TSS	Transcriptional Start Site
U	Units
Ubx	Ultrabithorax
µg	Microgram
µl	Microlitre
UV	Ultraviolet
V	Volts
v/v	Volume/Volume
WT1	Wilm's Tumor Gene
w/v	Weight/Volume

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