



MURDOCH UNIVERSITY

**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*

***Kim L. Rice***

## 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 HOX11 $\Delta$ H3 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 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
<b>A</b>	Absorbance
<b>aa</b>	Amino Acid
<b>Abd</b>	Abdominal
<b>ADH</b>	Alcohol Dehydrogenase
<b>AF-2</b>	Activation Function Domain-2
<b>ALDH</b>	Aldehyde Dehydrogenase
<b>Aldh1a1</b>	Aldehyde Dehydrogenase 1a1
<b>ALL</b>	Acute Lymphoblastic Leukaemia
<b>AML</b>	Acute Myeloid Leukaemia
<b>Amp</b>	Ampicillin Resistance Gene
<b>Antp</b>	Antennapedia
<b>AP</b>	Anterior-Posterior
<b>APC</b>	Allophycocyanin
<b>APL</b>	Acute Promyelocytic Leukaemia
<b>APS</b>	Ammonium Persulfate
<b>ARC</b>	Animal Resource Centre
<b>AS</b>	Antisense
<b>ATRA</b>	All-trans Retinoic Acid
<b>B-ALL</b>	B-cell Acute Lymphoblastic Leukaemia
<b>bap</b>	Bagpipe
<b>BD</b>	Becton Dickinson
<b>bHLH</b>	Basic Helix-Loop-Helix
<b>Bio</b>	Biotin
<b>bp</b>	Base Pairs
<b>BSA</b>	Bovine Serum Albumin
<b>Bx</b>	Bithorax
<b>CAT</b>	Chloramphenicol Acetyl Transferase
<b>CD</b>	Cluster of Differentiation
<b>cDNA</b>	Complementary DNA
<b>CDP</b>	CCAAT-Displacement Protein
<b>CDK</b>	Cyclin Dependent Kinase
<b>C/EBP<math>\beta</math></b>	CCAAT/Enhancer-Binding Protein $\beta$
<b>CFU-GM</b>	Colony Forming Unit-Granulocyte Macrophage
<b>cGy</b>	Centigrays
<b>chIP</b>	Chromatin Immunoprecipitation
<b>CIP</b>	Calf Intestinal Phosphatase
<b>CKI</b>	Cyclin Kinase Inhibitor
<b>CLCR</b>	Children's Leukaemia and Cancer Research
<b>CLP</b>	Common Lymphoid Progenitor
<b>cm</b>	Centimetre
<b>CMP</b>	Common Myeloid Progenitor
<b>CNS</b>	Central Nervous System
<b>Co-IP</b>	Co-Immunoprecipitation
<b>COOH</b>	Carboxyl Terminus

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

<b>HOM-C</b>	Homeotic Complex
<b>HOX</b>	Homeobox
<b>HRP</b>	Horseradish Peroxidase
<b>HSC</b>	Haematopoietic Stem Cell
<b>IFN</b>	Interferon
<b>IL</b>	Interleukin
<b>Inr</b>	Initiator
<b>IPTG</b>	Isopropyl $\beta$ -d-Thiogalactopyranoside
<b>IRES</b>	Internal Ribosome Entry Site
<b>kb</b>	Kilobase
<b>kDa</b>	Kilodalton
<b>L</b>	Litre
<b>lab</b>	Labial
<b>LB</b>	Luria-Bertani
<b>LBD</b>	Ligand Binding Domain
<b>lbe</b>	Ladybird early
<b>lbl</b>	Ladybird late
<b>LIM</b>	Lin-11, Isl-1 and Mec-3
<b>LMO</b>	LIM-Only
<b>LT-HSC</b>	Long-Term Haematopoietic Stem Cells
<b>LTR</b>	Long Terminal Repeat
<b>lym</b>	Lymphoid Progenitor
<b>M</b>	Molar
<b>M/E</b>	Myeloerythroid Progenitor
<b>MED-1</b>	Multiple Start Site Element Downstream-1 Element
<b>MEIS</b>	Myeloid Ectopic Integration Site
<b>mg</b>	Milligram
<b>min</b>	Minute
<b>MIS</b>	Mullerian Inhibiting Substance
<b>mL</b>	millilitre
<b>MLL</b>	Mixed Lineage Leukaemia
<b>MLP</b>	Multilineage Progenitor
<b>mM</b>	Millimolar
<b>MOPS</b>	3-[N-morpholino] Propanesulfonic Acid
<b>MPD</b>	Myeloproliferative Disease
<b>mRNA</b>	Messenger RNA
<b>MSCV</b>	Murine Stem Cell Virus
<b>MW</b>	Molecular Weight
<b>MWM</b>	Molecular Weight Marker
<b>MZF-1</b>	Myeloid Zinc Finger-1
<b>NcoR</b>	Nuclear receptor CoRepressor
<b>NES</b>	Nuclear Export Sequence
<b>ng</b>	Nanogram
<b>NGFR</b>	Nerve Growth Factor Receptor
<b>NH<sub>2</sub></b>	Amino Terminus
<b>NK</b>	Natural Killer Cell
<b>NKL</b>	NK-Like
<b>NLS</b>	Nuclear Localisation Signal
<b>nm</b>	Nanometre
<b>nmol</b>	Nanmoles

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

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

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