MURDOCH
UNIVERSITY
PERTH, WESTERN AUSTRALIA

INVESTIGATION OF THE MOLECULAR FUNCTION OF THE NUCLEAR ONCOPROTEIN HOX11 IN HUMAN T-CELL LEUKAEMIA

THESIS

PRESENTED FOR THE AWARD OF THE DEGREE

OF DOCTOR OF PHILOSOPHY (Ph.D)

BY

MANSOUR HEIDARI

(B.Sc, M.Sc)

DIVISION OF BIOMEDICAL SCIENCE
MURDOCH UNIVERSITY

2003

DECLARATION

Apart from the assistance stated in the acknowledgments, and where due reference is made in the text, the work presented in this thesis was performed by myself. Segments of the work have been published under the author's name, as shown in the publications and scientific communications.

Mansour Heidari

AKNOWLEDGMENTS

First and foremost, I would like to thank Dr Wayne Greene for supervising my Ph.D studies with insight and enthusiasm, and for his support that made the few difficult times bearable. These investigations would not have been possible without his consistent help, kind encouragement, and interest in the progress of the study. Special thanks are also due to Professor Ursula Kees, Children's Leukaemia and Cancer Research, ICHR for enhancing my academic experience and for efforts in revision, comment, and constructive criticism during my studies. Thanks also to Dr David Berryman, Manager/Research and Development Officer, Western Australian State Agricultural Biotechnology Centre, Murdoch University for guiding me through the use of the laboratory equipment.

I am indebted to several colleagues for their help and advice during my postgraduate work. Firstly, Dr Mohammad Bassami for critical advice provided during the expression and purification of HOX11. Deep appreciation is also expressed to Dr Peter Dallas for providing advice and for his suggestions regarding the chromatin immunoprecipitation technique. I also thank Dr Jacqueline Phillips for providing antibody conjugate and for her critical advice about confocal microscopy. Dr Geoffrey Dwyer was always helpful and provided me with many suggestions on an academic and technical level. A big thank-you also goes to Jette Ford for generously supplying the leukaemic T-cell lines used in this study, Dr John H. Blinco for his help during the immunocytochemical study and Dr John Murphy for his help with the fluorescent microscopy work. I also acknowledge the help and advice on the proteomic work provided by Dr Richard Lipscombe and colleagues of Proteomics International.

On a personal note, I would like to thank my Ph.D student colleagues Kim Rice and Darcelle Dixon for their help and cooperation during the course of this dissertation.

This work would not have been possible without financial assistance. Therefore, I deeply thank the Iranian Ministry of Health and Medical Education for supporting my study by providing a PhD. Scholarship.

I am deeply thankful to my wife for her understanding patience, for her inspiration and constant encouragement. Finally, a deeply heartfelt thank-you to my children, Masoud and Matin, for tolerating me during these studies.

PUBLICATIONS

1. Heidari M., Kim L. Rice, Ursula R. Kees, and Wayne K. Greene. (2002). Expression and purification of the human homeodomain oncoprotein HOX11. Protein Expression & Purification 25; 313-318.

2. Heidari M., J.K. Phillips, U.R. Kees, and W.K. Greene (2003). The Nuclear Oncoprotein HOX11 Associates with Pericentromeric Heterochromatin in Leukaemic T-cells (manuscript in preparation).

3. Heidari M., U.R. Kees and W.K. Greene (2003). Identification of proteins within HOX11-containing nuclear complexes by mass spectrometry (manuscript in preparation).

CONFERENCE ABSTRACTS

1. Heidari M., J.K. Phillips, U.R. Kees and W.K. Greene (2003). The Nuclear Oncoprotein HOX11 Associates with centromeric DNA in Leukaemic T-cells. 3rd Annual Medical Research Symposium, Perth, Western Australia.

2. Heidari M., Kees U.R. and Greene W.K. (2003). The Nuclear Oncoprotein HOX11 Associates with Pericentromeric Heterochromatin in Leukaemic T-cells. 15th Lorne Cancer Conference Lorne, Victoria, Australia.

3. Heidari M., J.K. Phillips, U.R. Kees and W.K. Greene (2003). The Nuclear Oncoprotein HOX11 Associates with Pericentromeric Heterochromatin in Leukaemic T-cells. Keystone Symposium, Chromatin: Organizing the Genome for Patterns of Gene Expression in Health and Disease, Big Sky, Montana, USA.

4. Heidari M., U.R. Kees and W.K. Greene (2001). Identification of direct chromosomal target sequences of HOX11, a nuclear oncoprotein misexpressed in human T-cell leukaemia. The Second Iranian Medical Sciences Postgraduate Students Conference, Teheran, Iran.

ABSTRACT

HOX11, the prototypical member of the HOX11 family (HOX11, HOX11L1 and HOX11L2) was originally discovered as a transcriptional regulator aberrantly expressed in tumours with an immature T-cell phenotype (T-ALL) as a result of specific chromosomal translocations involving T-cell receptor loci. Subsequently, it was revealed that HOX11 is required for normal spleen development since newborn *Hox11-/-* mice exhibit asplenia. In both its normal and abnormal roles, HOX11 has been postulated to function by binding regulatory elements within specific target genes to control gene transcription. However, very few genomic targets of HOX11 have been identified and little is known about its mode of action. In this study, we sought to further understand the role of HOX11 in controlling differentiation and cell growth by 1) determining the identity of genomic sequences that are directly bound by HOX11 and 2) determining the identity of proteins which exist within HOX11-containing nuclear complexes.

To identify direct HOX11 target sequences, a whole genome PCR-based screening method was employed using immobilised recombinant HOX11 that had first been expressed as a biologically active GST fusion protein. Using this approach, restriction enzyme-cleaved human genomic DNA was selected for high-affinity HOX11 binding sites. Unexpectedly, almost all clones isolated contained sequences derived from satellite 2 DNA that, together with related satellite 3 DNA, is found on most chromosomes at transcriptionally inactive pericentromeric heterochromatin. The specific binding of HOX11 to satellite 2 DNA was verified by bandshift assays using both recombinant HOX11 protein and nuclear extract derived from the T-ALL cell line,

ALL-SIL. DNA-protein complexes containing HOX11 were identified by their ablation upon addition of HOX11 antibody.

To confirm that HOX11 associates with pericentromeric heterochromatin *in vivo*, HOX11 was characterised in terms of its nuclear localisation during interphase in unsynchronised leukaemic T-cells (ALL-SIL) harbouring a translocation involving the *HOX11* locus. Using indirect immunofluorescence and confocal microscopy, HOX11 antibody produced a punctate pattern of staining in the nucleus with discrete areas of dense staining superimposed on a diffuse distribution of HOX11 protein. By dual staining, the bright HOX11 foci correlated with centromeres since they overlapped with signals detected by an antibody specific for the centromeric protein CENP-B. Further evidence for a direct interaction of HOX11 with satellite 2 DNA was provided by chromatin immunoprecipitation assay. In the presence of HOX11 antibody, DNA fragments containing satellite 2 sequences were immunoprecipitated from sheared, cross-linked ALL-SIL chromatin but not from chromatin isolated from the HOX11-negative T-cell line PER-117. Finally, using a combination of immunoprecipitation with HOX11 antibody, gel electrophoresis and mass peptide fingerprinting, a set of nuclear proteins were identified as potential HOX11 interactors which are known to either localise to centromeric regions or act as regulators of gene expression. Together, these results implicate HOX11 in a functional interaction with centromeric heterochromatin, which may be a key feature of this oncoprotein in terms of both its T-cell transformation and transcriptional regulatory functions.

LIST OF ABBREVIATIONS

aa	amino acid
Ab	antibody
ALL	acute lymphoblastic leukaemia
AML	acute myeloid leukaemia
APS	ammonium persulphate
B-ALL	B-cell acute lymphoblastic leukaemia
BP	broad pass
bp, kb	(base pair) length of DNA, kilobase pairs
BSA	bovine serum albumin
cDNA	complementary DNA
ChIP	chromatin immunoprecipitation
CLL	chronic lymphocytic leukaemia
CML	chronic myeloid leukaemia
CO_2	carbon dioxide
Da, kDa	dalton, kilodalton
DDW	double deionised H_2O
DMSO	dimethyl sulfoxide
DNase	deoxyribonuclease
dNTP	deoxynucleotide triphosphate
DTT	dithiothreitol
E.coli	*Escherichia coli*
EDTA	ethylene diamine tetra-acetic acid
EMSA	electrophoretic mobility shift assay
FRET	fluorescence resonance energy transfer
gm;mg;µg;ng;pg	gram, milligram, microgram, nanogram, picogram
GST	glutathione *S*-transferase
HCl	hydrochloric acid
HD	homeodomain
hr, min	hour, minute
IF	immunofluorescence
Ig	immunoglobulin
IP	immunoprecipitation
IPTG	isopropyl thio-β-D-galactoside
LB	Luria-Bertani medium
M;mM;µM;nM	molar; millimolar; micromolar; nanomolar
M	marker of DNA
Mr	relative molecular mass
ml;µl	millilitre, microlitre
MALDI-TOF	matrix-assisted laser desorption/ionisation-time of flight
nm	nanometre
NS	non-specific
OD	optical density
PBS	phosphate-buffered saline
PCR	polymerase chain reaction
PMSF	phenymethylsulfonyl fluoride
Poly dI-dC	polydeoxyinosinic-deoxycytidylic acid
rHOX11	recombinant HOX11
RNA	ribonucleic acid
RNase	ribonuclease

rpm	revolutions per minute
RT	room temperature
SDS-PAGE	sodium dodecyl sulphate-polyacrylamide gel electrophoresis
SH	short pass
SN	supernatant
TAE	Tris-acetate-EDTA
T-ALL	T-cell acute lymphoblastic leukaemia
TCR	T-cell receptor
TE	Tris-EDTA
TBS	Tris buffered saline
TEMED	N,N,N',N'-tetra methyl ethylene diamine
Tris	Tris (hydroxymethyl) aminomethane
Tris-HCl	Tris (hydroxymethyl) aminomethane hydrochloride
uCi	microcurie
UV	ultraviolet
v/v	volume per volume
w/w	weight per weight
w/v	weight per volume
WG-PCR	whole genome PCR
X-gal	5-bromo-4-chloro-3-indolyl-β-D-galactoside
g	relative centrifugal field

LIST OF TABLES

TABLE OF CONTENTS

CHAPTER 2: Expression and Purification of HOX11 as GST Fusion Protein

CHAPTER 3: Identification of Genomic DNA Target Sequences of HOX11 by Whole Genome PCR

CHAPTER 4: Confirmation of the Physical Association HOX11 with Pericentromeric Satellite 2 DNA