Identification of Downstream Target Genes of the T-cell Oncoprotein HOX11 by Global Gene Expression Profiling

Darcelle Natalie Dixon
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Murdoch University
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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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Darcelle Natalie Dixon
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Abstract

HOX11 is a homeodomain transcription factor that has been implicated in leukaemic transformation associated with T-cell acute lymphoblastic leukaemia (T-ALL). Its role in leukaemogenesis remains enigmatic, nevertheless, *in vitro* and *in vivo* studies have provided additional evidence supporting the role of HOX11 as an oncogene. The mechanism by which HOX11 transforms cells is yet to be elucidated, however, HOX11 has been postulated to function by binding regulatory elements within the promoter regions of specific target genes in order to control gene transcription. The identification of transcriptional targets is thus thought to be critical to our understanding of the pathways controlled by this master gene regulator. To date, only three candidate HOX11 target genes have been reported and given that HOX11 overexpression can have a profound impact on cell behaviour, it is likely that many more exist. In this study, we sought to further understand the role of HOX11 in tumorigenesis by: 1) The identification of novel putative HOX11 target genes by profiling gene expression in response to HOX11 in a number of cell lines using a combination of RDA, cDNA microarray and GeneChip approaches and 2) confirming target gene status by assessing whether the proximal promoters of the leading candidates identified are transcriptionally regulated by HOX11.

To identify genes whose expression was altered by HOX11, three techniques were employed, namely representational difference analysis, cDNA microarray and Affymetrix GeneChip array. Because of the relative novelty of these technologies, all three methods were employed in a complementary manner. While representational difference analysis did not require dedicated equipment and enabled the identification of novel genes, the technique was labour-intensive and also exhibited a number of problems including high levels of background. Emphasis was therefore placed on the more systematic microarray approaches that enabled a global investigation of expression patterns and thus the identification of a range of candidate target genes. Initially, this involved cDNA microarray experiments, however, during the course of this work Affymetrix GeneChip technology became available. The latter was identified as the most appropriate technology for the identification of candidate target genes because of its relative ease of use, as well as its employment of multiple independent probe pairs which greatly improved background noise, increased the range and accuracy of detection, minimized the effects of cross hybridization and drastically reduced the rate of false positives and miscalls.

Using these combined approaches, several genes of interest were identified which were differentially regulated in the presence of HOX11 and thus may represent oncogenically or physiologically relevant target genes. These included **OSTEOPONTIN**, **PAG**, **GUANOSINE DIPHOSPHATE DISSOCIATION INHIBITOR 3**, **SUR8**, **GAS3**, **C-KIT**, **VEGFC**, **NOR1** and
SMARCD3. In order to confirm their role as target genes, four candidates (C-KIT, VEGFC, NOR1 and SMARCD3) were characterized 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 C-KIT and VEGFC by HOX11 was observed, which provided further evidence for their status as target genes. This repression was, however, in stark contrast to the transcriptional activation seen when the C-KIT and VEGFC proximal promoters were co-transfected with a HOX11 mutant lacking the third helix of the DNA-binding homeodomain. This unexpected finding suggested that the transcriptional activity of HOX11 is complex and highly context-dependent, and in particular, highlighted the importance of an intact homeodomain for HOX11 function.

C-KIT and VEGFC are both involved in tyrosine kinase signal transduction pathways, as a receptor tyrosine kinase and tyrosine kinase ligand, respectively. C-KIT plays an important role in the survival and self-renewal of haematopoietic cells. It is a previously identified and relatively well characterized oncogene known to be regulated by other transcription factors (SCL/TAL1 and LMO) implicated in the pathogenesis of T-ALL. VEGFC is a member of the vascular endothelial growth factor family that functions in angiogenesis and lymphangiogenesis. A paracrine loop involving VEGFC and its receptor VEGFR-3 has previously been implicated in leukaemic cell survival. While further work is required in order to confirm the status of VEGFC and C-KIT as oncogenically-relevant HOX11 target genes and to characterize their exact mode of regulation, these findings implicate receptor tyrosine kinases in HOX11-mediated tumorigenesis and underscore their potential importance as therapeutic targets in haematological malignancies.
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Abbreviations

°C Degrees Celsius
A Absorbance
aa Amino Acid
Aldh1a1 Aldehyde Dehydrogenase 1a1
ALL Acute Lymphoblastic Leukaemia
AML Acute Myeloid Leukaemia
Amp Ampicillin Resistance Gene
APL Acute Promyelocytic Leukaemia
B-CLL B-cell Chronic Lymphoblastic Leukaemia
bp Base Pairs
BSA Bovine Serum Albumin
CDC25A Cell Division Cycle 25A
cDNA Complementary DNA
CDP CCAAT Displacement Protein
ChIP Chromatin Immunoprecipitation
cm Centimetre
CML Chronic Myeloid Leukaemia
CNS Central Nervous System
COOH Carboxyl terminus
cpm Counts per Minute
CY Cyanine
Da Dalton
DAF Diaminofluorene
DD Dihydriodiol Dehydrogenase
dNTP Dinucleotide Triphosphate
ddH2O Double-deionised Water
DMEM Dulbecco’s Modified Medium
DNA Deoxyribonucleic Acid
DTT Dithiothreitol
E Embryonic Day
EDTA Ethylenediamine Tetra Acetic Acid
EF Elongation Factor
EFS Event Free Survival
EGTA Ethylene Glycol-bis tetra Acetic Acid
En Engrailed
Epo Erythropoietin
ES Embryonic Stem Cell
EST Expressed Sequence Tag
ETF Electron Transfer Flavoprotein
FCS Foetal Calf Serum
FIL FIL Repression Domain
FISH Fluorescence In Situ Hybridization
g Gram
GAPDH Glyceraldehyde 3-Phosphate Dehydrogenase
GAS3 Growth Arrest Specific Factor 3
Gly-Rich Glycine Rich
h Hour
HD Homeodomain
HEPES Hydroxy Ethyl Piperazine Ethane Sulfonic Acid
HRP Horse Radish Peroxidase
IGFBP10 Insulin Like Growth Factor Binding Protein 10
Kan Kanamycin Resistance Gene
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<th>WBC</th>
<th>White Blood Cell</th>
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<td>ZF67</td>
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Thesis Publications


Thesis Presentations

Oral Presentations

“Dissecting a Role for HOX11 in Tumour Development”
Inaugural 2nd Annual Australian Society for Medical Research Symposium, Perth, Western Australia.

2002

“The Role That the Transcription Factor HOX11 Plays in Tumorigenesis”
Postgraduate Seminar Program
Division of Health Sciences, Murdoch University, Perth, Western Australia.

2003

Poster Presentations

“Utilization of GeneChip Technology to Dissect a Role for HOX11 in Tumour Development”
Lorne Cancer Conference, Lorne, Victoria.

2003

“Guanine Nucleotide Dissociation Inhibitor Beta: A Potential HOX11 Target Gene”
Lorne Cancer Conference, Lorne, Victoria.

2002

“Identification of HOX11 Target Genes Using Microarray Technology and Representational Difference Analysis”
Postgraduate Poster Day
Division of Health Sciences, Murdoch University, Perth, Western Australia.

2001
“Searching for Downstream Target Genes of the Oncoprotein HOX11”
Postgraduate Poster Day
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