Identification and Functional Analysis of Gene Expression Changes in Acute Myeloid Leukaemia

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Abbreviations

-7 monosomy 7
-7q deletion of 7q
+8 trisomy 8
a.k.a also known as
AKT protein kinase B
AML acute myeloid leukaemia
AML1 runt-related transcription factor 1
APL acute promyelocytic leukaemia
ATM ataxia telangiectasia mutated
ATRA all-trans retinoic acid
BH Benjamini-Hochberg
BMU bone marrow unit
bp base pairs
C/EBP CCAAT enhancer binding protein
CBF AML core binding factor AML (AML1-ETO and CBFB-MYH11)
CBFB core binding factor beta
CD90 cluster of differentiation 90
ChIP chromatin immunoprecipitation
CHIP microarray chip
CMAP connectivity map
DC dequalinium chloride
DMSO dimethyl sulfoxide
ER endoplasmic reticulum
ERK extracellular signal-regulated kinase
ETO eight twenty one protein
FACS flow cytometry
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>FBS</td>
<td>Fetal Bovine Serum</td>
</tr>
<tr>
<td>FDA</td>
<td>US Food and Drug Administration</td>
</tr>
<tr>
<td>FDR</td>
<td>false discovery rate</td>
</tr>
<tr>
<td>FL</td>
<td>human FLT3 ligand</td>
</tr>
<tr>
<td>FLT3-ITD</td>
<td>FLT3-Internal Tandem Duplication mutation</td>
</tr>
<tr>
<td>FLT3-TKD</td>
<td>FLT3-Tyrosine Kinase Domain mutation</td>
</tr>
<tr>
<td>FLT3-WT</td>
<td>FMS-like Tyrosine Kinase class III receptor</td>
</tr>
<tr>
<td>GEO</td>
<td>gene expression omnibus</td>
</tr>
<tr>
<td>GF</td>
<td>Growth factor</td>
</tr>
<tr>
<td>GM</td>
<td>granulocyte monocyte</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>granulocyte macrophage colony-stimulating factor</td>
</tr>
<tr>
<td>GMR</td>
<td>IL-3/IL-5/GM-CSF hbc receptor</td>
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<tr>
<td>GMR-V449E</td>
<td>FDB1 cells expressing the hβc receptor V449E mutant</td>
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<tr>
<td>GSEA</td>
<td>gene-set enrichment analysis</td>
</tr>
<tr>
<td>h/m</td>
<td>human/mouse</td>
</tr>
<tr>
<td>HDAC</td>
<td>histone deacetylase</td>
</tr>
<tr>
<td>HOX</td>
<td>homeobox gene</td>
</tr>
<tr>
<td>HSC</td>
<td>haemopoietic stem cell</td>
</tr>
<tr>
<td>hβc</td>
<td>human GMR common beta subunit</td>
</tr>
<tr>
<td>IL-3</td>
<td>Interleukin 3</td>
</tr>
<tr>
<td>IMDM</td>
<td>Iscove's modified Dulbecco's medium</td>
</tr>
<tr>
<td>IMDM</td>
<td>Iscove's Modified Dulbecco's Medium</td>
</tr>
<tr>
<td>IPA</td>
<td>Ingenuity Pathway Analysis</td>
</tr>
<tr>
<td>JAK</td>
<td>Janus Kinase</td>
</tr>
<tr>
<td>kDa</td>
<td>kilo dalton</td>
</tr>
<tr>
<td>LIMMA</td>
<td>linear modelling for microarray analysis</td>
</tr>
<tr>
<td>Lod</td>
<td>log of odd ratio score which depicts the differential expression of a gene</td>
</tr>
<tr>
<td>LSC</td>
<td>leukaemic stem cell</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>M</td>
<td>Molar</td>
</tr>
<tr>
<td>M-CSFR</td>
<td>macrophage colony-stimulating factor receptor</td>
</tr>
<tr>
<td>MAPK</td>
<td>Mitogen activating protein Kinase</td>
</tr>
<tr>
<td>miR</td>
<td>micro-RNA</td>
</tr>
<tr>
<td>MLL</td>
<td>mixed-lineage leukaemia</td>
</tr>
<tr>
<td>MNC</td>
<td>mononuclear cells</td>
</tr>
<tr>
<td>MPD</td>
<td>myeloproliferative disorder</td>
</tr>
<tr>
<td>mRNA</td>
<td>messenger RNA</td>
</tr>
<tr>
<td>MTS</td>
<td>(3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium)</td>
</tr>
<tr>
<td>MYH11</td>
<td>myosin, heavy chain 11, smooth muscle</td>
</tr>
<tr>
<td>NBM</td>
<td>normal bone marrow mononuclear cells</td>
</tr>
<tr>
<td>NFkB</td>
<td>nuclear factor of kappa light polypeptide gene enhancer in B-cells</td>
</tr>
<tr>
<td>NK</td>
<td>normal karyotype AML</td>
</tr>
<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
</tr>
<tr>
<td>PI</td>
<td>Propidium Iodide</td>
</tr>
<tr>
<td>PI3K</td>
<td>Phosphatidylinositol3 kinase</td>
</tr>
<tr>
<td>PML</td>
<td>promyelocytic leukaemia</td>
</tr>
<tr>
<td>PSG</td>
<td>Penicillin-Streptomycin-Glutamine</td>
</tr>
<tr>
<td>PTPN11</td>
<td>protein tyrosine phosphatase, non-receptor type 11; a.k.a SHP-2</td>
</tr>
<tr>
<td>Q-PCR</td>
<td>real-time quantitative PCR</td>
</tr>
<tr>
<td>r.p.m</td>
<td>revolutions per minute</td>
</tr>
<tr>
<td>RARA</td>
<td>retinoid acid receptor alpha</td>
</tr>
<tr>
<td>RMA</td>
<td>Robust Multichip Average</td>
</tr>
<tr>
<td>RNA</td>
<td>ribonucleic acid</td>
</tr>
<tr>
<td>ROS</td>
<td>reactive oxygen species</td>
</tr>
<tr>
<td>RTK</td>
<td>receptor tyrosine kinase</td>
</tr>
<tr>
<td>RUNX1</td>
<td>runt-related transcription factor 1</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
</tr>
<tr>
<td>RUNX1T1</td>
<td>runt-related transcription factor 1; translocated to, 1 (cyclin D-related)</td>
</tr>
<tr>
<td>SCF</td>
<td>stem cell factor</td>
</tr>
<tr>
<td>SEM</td>
<td>standard error measurement</td>
</tr>
<tr>
<td>shRNA</td>
<td>short hairpin RNA</td>
</tr>
<tr>
<td>siRNA</td>
<td>small interfering RNA</td>
</tr>
<tr>
<td>SMMHC</td>
<td>a.k.a MYH11</td>
</tr>
<tr>
<td>STAT</td>
<td>Signal Transducer and Activator of Transcription</td>
</tr>
<tr>
<td>TF</td>
<td>Transcription factor</td>
</tr>
<tr>
<td>vs</td>
<td>versus</td>
</tr>
<tr>
<td>Wnt</td>
<td>wingless-type MMTV integration site family</td>
</tr>
<tr>
<td>WT</td>
<td>wild-type</td>
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Abstract

Acute Myeloid Leukaemia (AML) is a malignant blood cancer characterised by uncontrolled growth of leukaemic blasts. This is associated with constitutive activation of key signalling molecules such as AKT, ERK1/2, STAT5 and NFκB and with aberrant transcription factor activity, which in many cases is associated with characteristic chromosomal translocations. Aberrant receptor signaling can constitutively activate the pathways associated with the above signaling molecules. For example, autocrine interleukin-3 (IL-3), and over-expression of IL-3 receptor alpha (IL3RA/CD123) have been found in AML, as has constitutive phosphorylation of the common beta subunit (hβc) for IL-3 and granulocyte-macrophage colony-stimulating factor receptor (GMR). Also mutation of the FMS-like tyrosine kinase 3 (FLT3) receptor is common in AML (~30% of patients) and the resultant aberrant FLT3 signaling contributes to enhanced survival, growth and a block in differentiation.

A focus in this thesis is the identification and dissection of the signaling pathways and downstream genes activated by a leukaemic mutant of GMR (GMR-V449E) and by the FLT3 activated mutants associated with AML. For these studies we make extensive use of the murine bi-potential myeloid cell line model FDB-1 in which these mutants induce factor-independent growth and survival and a block in differentiation. The use of this experimental approach together with bioinformatics has provided leads with regard to the role of the AKT/mTOR and ERK pathways downstream of these receptors, and important for cell proliferation, survival and differentiation. Additionally, we focused on the role of the Growth Arrest and DNA Damage 45a (Gadd45a) gene, repression of which is important for cell survival and the block in differentiation induced by the activated mutants.

A second focus has been extending the bioinformatic approaches to define the gene expression and pathways associated with the abnormal growth characteristics of AML. In
particular, we studied AML cases with numerical chromosomal abnormalities and translocation events. Extensive use is made of the Connectivity Map (CMAP) resource together with publicly available gene expression datasets to define agents with anti-leukaemic potential. We have tested drugs, selected using the inv(16) (CBFβ-MYH11) and MLL AML translocation signatures, for specificity and sensitivity on AML patient samples.
Declaration

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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1. Powell et al, Blood, 2009 (Appendix B)
2. Perugini et al, Leukemia, 2009 (Appendix D)
3. Kok et al, Leukemia, 2010 (Appendix E)

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