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 CBFβ-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	extracelullar signal-regulated kinase
ETO	eight twenty one protein
FACS	flow cytometry

FBS	Fetal Bovine Serum
FDA	US Food and Drug Administration
FDR	false discovery rate
FL	human FLT3 ligand
FLT3-ITD	FLT3-Internal Tandem Duplication mutation
FLT3-TKD	FLT3-Tyrosine Kinase Domain mutation
FLT3-WT	FMS-like Tyrosine Kinase class III receptor
GEO	gene expression omnibus
GF	Growth factor
GM	granulocyte monocyte
GM-CSF	granulocyte macrophage colony-stimulating factor
GMR	IL-3/IL-5/GM-CSF hbc receptor
GMR-V449E	FDB1 cells expressing the hβc receptor V449E mutant
GSEA	gene-set enrichment analysis
h/m	human/mouse
HDAC	histone deacetylase
HOX	homeobox gene
HSC	haemopoietic stem cell
hβc	human GMR common beta subunit
IL-3	Interleukin 3
IMDM	Iscove's modified Dulbecco's medium
IMDM	Iscove's Modified Dulbecco's Medium
IPA	Ingenuity Pathway Analysis
JAK	Janus Kinase
kDa	kilo dalton
LIMMA	linear modelling for microarray analysis
Lod	log of odd ratio score which depicts the differential expression of a gene
LSC	leukaemic stem cell

M	Molar
M-CSFR	macrophage colony-stimulating factor receptor
MAPK	Mitogen activating protein Kinase
miR	micro-RNA
MLL	mixed-lineage leukaemia
MNC	mononuclear cells
MPD	myeloproliferative disorder
mRNA	messenger RNA
MTS	(3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium)
MYH11	myosin, heavy chain 11, smooth muscle
NBM	normal bone marrow mononuclear cells
NFkB	nuclear factor of kappa light polypeptide gene enhancer in B-cells
NK	normal karyotype AML
PCR	polymerase chain reaction
PI	Propidium Iodide
PI3K	Phosphatidylinositol3 kinase
PML	promyelocytic leukaemia
PSG	Penicillin-Streptomycin-Glutamine
PTPN11	protein tyrosine phosphatase, non-receptor type 11; a.k.a SHP-2
Q-PCR	real-time quantitative PCR
r.p.m	revolutions per minute
RARA	retinoid acid receptor alpha
RMA	Robust Multichip Average
RNA	ribonucleic acid
ROS	reactive oxygen species
RTK	receptor tyrosine kinase
RUNX1	runt-related transcription factor 1

RUNX1T1	runt-related transcription factor 1; translocated to, 1 (cyclin D-related)
SCF	stem cell factor
SEM	standard error measurement
shRNA	short hairpin RNA
siRNA	small interfering RNA
SMMHC	a.k.a MYH11
STAT	Signal Transducer and Activator of Transcription
TF	Transcription factor
vs	versus
Wnt	wingless-type MMTV integration site family
WT	wild-type

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

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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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