

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



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Table of Contents

List of Figures	i
List of Tables	v
List of Appendix	vii
Abbreviations	viii
Abstract	xii
Declaration	xiv
Acknowledgement	xv
Chapter 1: Introduction	1
1.1 Acute Myeloid Leukaemia	1
1.1.1 The classification and the prognostic outcome of AML	1
1.1.2 Targeted therapies on AML	7
1.2 Haemopoiesis: interplay between growth factor signalling and lineage-specific transcription factors	12
1.2.1 The importance of growth factors in haemopoiesis	12
1.2.2 Transcription factors that determine haemopoietic cell fates	16
1.3 AML biology and pathogenesis	18
1.3.1 Genetic alteration of transcription factors in AML	21
1.4 Leukaemic stem cell	22
1.5 Receptor signalling in haemopoiesis	24
1.5.1 IL-3/IL-5/GM-CSF receptor.....	24
1.5.2 FMS-like tyrosine kinase 3 receptor (FLT3).....	25
1.5.3 c-Kit receptor.....	27
1.6 Activated receptor mutants in AML	28
1.6.1 Constitutive activation of GMR induces AML	28
1.6.1.1 GMR-V449E mutation in common beta chain	28
1.6.1.2 A critical motif in hβc regulates proliferation and survival.....	30
1.6.2 FLT3.....	31
1.6.2.1 FLT3-ITD mutation.....	32
1.6.2.2 FLT3-TKD mutations.....	34
1.6.3 The c-Kit-TKD mutation.....	35
1.7 Downstream signal transducers	36
1.7.1 The PI3K/AKT/mTOR pathway	36
1.7.2 RAS/MAPK/ERK1/2 signalling.....	37

1.7.3	JAK/STAT signalling.....	39
1.8	Application of gene expression profiling technology to AML	40
1.8.1	Gene expression profiling for diagnostic and prognostic predictions	41
1.8.2	Gene expression profiling in target-based drug discovery	43
1.9	Cell line models to study AML	44
1.9.1	A cell line model of granulocyte-monocyte growth and differentiation, FDB1.....	45
1.10	Aims of the project.....	46
1.10.1	Overall Aims:.....	46
1.10.1.1	Specific aims:	46
Chapter 2: Regulation of myeloid proliferation, differentiation and survival signals		
by the human GM-CSF/IL-3/IL-5 common beta chain		
47		
2.1	Introduction	47
2.2	Materials and methods	49
2.2.1	Reagents	49
2.2.2	Antibodies	49
2.2.3	Cell lines and culture conditions	50
2.2.4	Flow cytometry	50
2.2.5	Differentiation, cell viability, apoptosis and proliferation assays	51
2.2.6	Cell cycle analysis.....	51
2.2.7	Western blotting	52
2.2.8	Bioinformatics analysis	52
2.2.8.1	Gene-set enrichment analysis using the Wilcoxon rank sum test.....	52
2.2.8.2	Microarray dataset re-analysis.....	53
2.2.8.3	Connectivity map, pathway and gene ontology analysis.....	53
2.2.8.4	Transcription factor prediction using microarray significant gene-set.....	54
2.2.9	Statistical analysis	54
2.3	Results.....	54
2.3.1	V449E Y577F cells fail to proliferate but maintain viability.....	54
2.3.2	The V449E Y577F signature: A proliferation-associated signature	59
2.3.3	The Connectivity Map (CMAP) as a tool to explore the nature of the V449E proliferation signature	68
2.3.4	Experimental validation of CMAP results	72
2.3.5	Treatment of GMR-V449E cells with compounds identified from the CMAP analysis	74
2.3.5.1	Treatment with the PI3K-AKT-mTOR pathway inhibitors.....	74
2.3.5.2	Effects of the pathway inhibitors on survival of V449E FDB1	75
2.3.5.3	Effect of pathway inhibitors on cell cycle status of V449E FDB1 cells.....	76
2.3.5.4	Effects of pathway inhibitors on myeloid differentiation of V449E FDB1 cells.....	77

2.3.6	Gene-set enrichment analysis (GSEA) of the V449E proliferation signature in AML..	80
2.3.7	Analysis of the hβc Ser ⁵⁸⁵ Signature – a signature associated with survival-only signalling	83
2.3.8	Relevance of the survival-only signature to AML	87
2.4	Discussion	87
Chapter 3: Mechanisms associated with FLT3 mutations in AML.....		98
3.1	Introduction	98
3.2	Materials and Methods.....	100
3.2.1	Reagents	100
3.2.2	Cell lines.....	100
3.2.3	Cell viability.....	101
3.2.4	Primers	101
3.2.5	Immunophenotyping by flow cytometry	101
3.2.6	Bioinformatics analysis	102
3.2.6.1	Pathway analysis	103
3.2.7	Statistical analysis	103
3.3	Results.....	103
3.3.1	Association of FLT3 mutations with inv(16) AML: a combined analysis of 734 reported cases of core-binding factor AML.....	103
3.3.2	Gene expression and prognostic analysis of FLT3 mutations in normal karyotype AML blasts	108
3.3.2.1	Experimental design and patient selection	108
3.3.2.2	Prognosis of FLT3 mutations in <i>NPM1</i> ⁺ NK AML.....	112
3.3.2.3	Gene expression profiling of FLT3 mutation in NK AML.....	112
3.3.3	Gene expression profiling of FLT3-ITD in NK acute myeloid leukaemia stem cell (LSC)	121
3.3.3.1	Focus of <i>HOX</i> genes that associated with FLT3-ITD in NK LSC	125
3.3.3.2	Genes selectively up-regulated in HSC and FLT3-ITD NK LSC	127
3.3.3.3	<i>HOX</i> expression pattern in FLT3-ITD resembles normal HSC.....	129
3.3.3.4	Signalling pathways associated with NK LSC	129
3.3.4	Role of FLT3 mutants in the differentiation block in AML.....	135
3.3.4.1	FDB1 cells expressing FLT3 mutants or GMR-V449E are blocked in differentiation	135
3.3.4.2	The ERK1/2 pathway contributes to survival and blocks differentiation of FLT3 mutants in FDB1 cells	137
3.3.5	The role of <i>Gadd45a</i> downstream of FLT3 activated mutants	141
3.3.5.1	Role of <i>Gadd45a</i> in the differentiation block.....	142
3.3.5.2	Regulation of <i>Gadd45a</i> expression level in haemopoiesis	145

3.3.6	The role of <i>GADD45A</i> as a tumour suppressor in AML	148
3.3.6.1	Repression of <i>GADD45A</i> expression is associated with MLL translocations in AML	148
3.3.7	Discussion	150
3.3.7.1	FLT3-ITD and FLT3-TKD in CBF AML	150
3.3.7.2	FLT3-ITD and FLT3-TKD mediated gene expression in NK AML	151
3.3.7.3	Gene expression in NK LSC	152
3.3.7.4	FLT3 mediated <i>HOX</i> gene expression in NK LSC.....	153
3.3.7.5	The association of ATM signalling pathway with FLT3-ITD LSC.....	154
3.3.7.6	<i>Gadd45a</i> and FLT3-ITD signalling.....	155
3.3.7.7	Mechanism of <i>Gadd45a</i> repression or silencing in AML	156
Chapter 4: Use of bioinformatic approaches to determine key pathways and specific therapeutic approaches in AML subgroups		158
4.1	Introduction	158
4.2	Materials and Methods.....	159
4.2.1	Reagents	159
4.2.2	AML patient samples thawing and culturing	160
4.2.3	Apoptosis, cell counts and differentiation	160
4.2.4	Microarray re-analysis.....	161
4.2.5	Connectivity Map analysis (CMAP)	162
4.2.6	Statistical analysis	162
4.3	Results.....	162
4.3.1	Rationale: Comparing AML gene expression to normal bone marrow mononuclear cells (NBM)	162
4.3.2	Identification of 4 specific AML translocation gene lists	163
4.3.3	Identification of gene expression changes associated with multiple translocations....	167
4.3.3.1	Genes common to all 4 translocations	167
4.3.3.2	Gene expression changes common to Core Binding Factor (CBF) AML	168
4.3.4	Identification of specific compounds and drugs using translocation signatures	173
4.3.5	Experimental validation of CMAP results	177
4.3.5.1	The effects of pentoxifyverine on AML patient blasts	177
4.3.5.2	The effects of dequalinium chloride on MLL AML patient blasts.....	185
4.4	<i>HOXA9</i> is over-expressed in Trisomy 8 AML	187
4.4.1	Results presented as manuscript format	187
4.4.1.1	Identification of compounds and drugs using a trisomy 8 signature	188
4.5	Discussion	188
Chapter 5: Final Discussion.....		196
5.1	Receptor signalling in AML.....	196

5.1.1	Signaling from the GMR.....	196
5.1.2	Constitutive activation of FLT3 receptor and the role of <i>Gadd45a</i>	197
5.2	Transcription factors in AML	201
5.2.1	<i>CEBPA</i> mutation	201
5.2.2	Over-expression of <i>HOX</i> genes.....	201
5.3	CMAP	202
Chapter 6:	References.....	204
Chapter 7:	Appendix.....	256
7.1	Appendix A. Reagent Recipes.....	256
7.2	Appendix B. Powell et al, 2009	257
7.3	Appendix C. Effect of FLT3 mutations in CBF leukaemia on overall survival and event-free survival	270
7.4	Appendix D. Perugini et al, 2009.....	272
7.5	Appendix E. Kok et al, 2010	283

List of Figures

Chapter 1:

Figure 1.1. Relative frequencies of the recurrent cytogenetic abnormalities in AML

Figure 1.2. The prognosis of AML is strongly related with the cytogenetic findings

Figure 1.3. Alternative models of haemopoiesis

Figure 1.4. The effects of growth factors in determining cell lineage specification during haemopoiesis

Figure 1.5. The role of transcription factors involved in determining cell fate during haemopoiesis

Figure 1.6. The effects and the frequency of two “hit” hypothesis for AML progression and development

Figure 1.7. The important stem cell properties and low transcription factor activity in leukaemic stem cell

Figure 1.8. Receptor signalling in haemopoiesis and AML

Figure 1.9. The summary of PI3K/AKT/mTOR and MAPK pathways that are activated by RTK

Figure 1.10. Application of gene expression profiling for diagnosis and drug discovery

Chapter 2:

Figure 2.1. Effect of the GMR-V449E Y577F mutation on factor independent proliferation and viability in FDB1 cells

Figure 2.2. Cell cycle analysis of FDB1 cells expressing V449E or V449E Y577F

Figure 2.3. Expression of cell surface myeloid differentiation markers and morphology on FDB1 V449E and V449E Y577F cells

Figure 2.4. Differentially expressed GMR-V449E Y577F genes

Figure 2.5. Significant AML signalling pathways associated with VY577F mutant cells

Figure 2.6. Proliferation signature network

Figure 2.7. Differential activation of the PI3K pathway by V449E and VY577F

Figure 2.8. Inhibition of cell proliferation and survival in FDB1 cells expressing GMR-V449E related to PI3K-AKT-mTOR network

Figure 2.9. Effect of LY294002 on the cell cycle status of FDB1 GMR-V449E cells

Figure 2.10. Expression of Gr-1 and c-FMS on FDB1 cells expressing GMR-V449E after treatment with PI3K and mTOR inhibitors

Figure 2.11. Identification of a PI3K-AKT network by CMAP analysis for the Ser⁵⁸⁵ survival-only signature

Figure 2.12. The mechanism of Wnt/ β -catenin pathway

Figure 2.13. Summary of pathways regulated by GMR common beta chain residue Tyr⁵⁷⁷

Figure 2.14. Summary of pathways regulated by GMR common beta chain residue Ser⁵⁸⁵

Chapter 3:

Figure 3.1. Effect of FLT3 mutations in NPM⁺ NK AML on overall survival and event-free survival.

Figure 3.2. The gene expression differences of FLT3-ITD and FLT3-TKD compared to FLT3-WT in normal karyotype AML.

Figure 3.3. Both FLT3-ITD and FLT3-TKD repress *GATA1* target genes.

Figure 3.4. The network of the commonly up- and down-regulated genes by both FLT3-ITD and FLT3-TKD compared to FLT3-WT in NK AML

Figure 3.5. Differential gene expression of FLT3-ITD linked to a *MYC* network.

Figure 3.6. Differential gene expression of FLT3-TKD linked to a NFκB and *CEBPA* network

Figure 3.7. The microarray strategy to identify genes regulated by FLT3-ITD and/or FLT3-WT LSC in normal karyotype AML

Figure 3.8. Commonly dysregulated genes in NK LSC AML

Figure 3.9. Differential expression of multiple HOX genes in FLT3-mutant LSC

Figure 3.10. Hierarchical clustering of *HOX* gene family expression in LSC and HSC.

Figure 3.11. The gene expression pattern of the *HOX* gene family in mouse haemopoietic cell lineages

Figure 3.12. The network pathways derived from genes differentially expressed in NK LSC

Figure 3.13. The network pathways derived from genes differentially expressed in FLT3-ITD NK LSC

Figure 3.14. Assessment of myeloid differentiation in FDB1 cells expressing activated growth factor receptor mutants

Figure 3.15. Effect of the MEK inhibitor, U0126, on cell viability

Figure 3.16. Treatment with U0126 induced Gr-1 myeloid differentiation on FDB1 cells expressing FLT3 mutants and GMR-V449E

Figure 3.17. The effect of *Gadd45a* over-expression on the block in myeloid differentiation in GMR-V449E and FLT3-ITD FDB1 cells

Figure 3.18. The *Gadd45a* expression pattern at various stages of haemopoiesis

Figure 3.19. Expression of *GADD45A* in AML subtypes defined by karyotype

Figure 3.20. The proposed mechanism and expression pattern of *Gadd45a* in AML and normal haemopoiesis

Chapter 4:

Figure 4.1. Genes that are specific to each of the 4 AML translocation events

Figure 4.2. Identification of genes selectively regulated either in AML with PML-RAR α , MLL or common to all four translocations groups

Figure 4.3. Identification of genes that are selectively regulated in CBF AML

Figure 4.4. The expression of *Caprin2* in several AML microarray dataset

Figure 4.5. The expression pattern of *CAPRIN2* in human and mouse myeloid differentiation

Figure 4.6. Validation of CMAP analysis by identification of ATRA in PML-RAR α

Figure 4.7. CMAP selectively identifies specific compounds for each AML translocation group

Figure 4.8. The effects of inv(16) patient MNC cells treated with pentoxifyverine in the absence of growth factor cocktail

Figure 4.9. The effects of inv(16) patient MNC cells treated with pentoxifyverine in the presence of growth factor cocktails

Figure 4.10. The effects of MLL patient MNC cells treated with pentoxifyverine in the absence of growth factor cocktails

Figure 4.11. The effects of MLL patient MNC cells treated with pentoxifyverine in the presence of growth factor cocktails

Figure 4.12. The effects of MLL patient MNC cells treated with dequalinium chloride in the absence or presence of growth factor cocktails

Figure 4.13. The proposed mechanisms of action of dequalinium chloride

List of Tables

Chapter 1:

Table 1.1. The use of the FAB category based on morphology and cytogenetics to classify AML (adapted from Bennett *et al*, 1976)

Table 1.2. The use of WHO category to classify AML (adapted from Gulley *et al*, 2010)

Table 1.3. The cytogenetic and mutation prognostic risk factors assignments of AML (adapted from Gulley *et al*, 2010)

Table 1.4. The frequency of the AML subgroups and the mutation of the transcription factors in AML (adapted from Rosenbauer and Tenen, 2007)

Table 1.5. The examples of the compound used and the mechanism of the current targeted therapies in AML (adapted from Haferlach, 2008)

Table 1.6. Summary of the transcription factors which determine specific cell lineage and their knockout effect in haemopoiesis (adapted from from Rosenbauer and Tenen, 2007)

Chapter 2:

Table 2.1. Enrichment of gene ontology and canonical pathways associated with the GMR-V449E proliferation signature

Table 2.2. Prediction of transcription factor regulation associated with the GMR-V449E proliferation signature

Table 2.3. Identification of TCF7L2 and CTNNB1 target genes in VY577F gene list

Table 2.4. Top 10 Connectivity Map compounds identified using V449E proliferation signature

Table 2.5. Gene-set enrichment analysis (GSEA) of top 50 V449E proliferation signature genes with indicated Valk *et al* (Valk *et al*, 2004) AML subtypes compared to NBM

Table 2.6. Top 10 compounds predicted from CMAP analysis for Ser⁵⁸⁵ signatures

Table 2.7. Gene-set enrichment analysis (GSEA) of Ser⁵⁸⁵ survival-only signature genes with indicated AML subtypes defined by Valk *et al* (Valk *et al*, 2004) compared to NBM

Chapter 3:

Table 3.1. The summary frequency of FLT3 mutations in CBF leukaemia in the 19 combined studies

Table 3.2. Direct target genes of TCF4/ β -catenin in CBF leukaemia

Table 3.3. Patient clinical characteristic in CBF leukaemia

Table 3.4. The patient clinical characteristic of normal karyotype AML based on FLT3 mutations category

Table 3.5. Top significant canonical pathways regulated by LSC compared to HSC

Chapter 4:

Table 4.1. Significant connectivity scores for the AML translocation gene signatures

Table 4.2. Drugs and/or small molecules that are negatively associated with the trisomy 8 AML signature

List of Appendix

Appendix A. Reagent recipes

Appendix B. Powell *et al*, 2009

Appendix C. Effect of FLT3 mutations in CBF leukaemia on overall survival and event-free survival

Appendix D. Perugini *et al*, 2009

Appendix E. Kok *et al*, 2010

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- <i>trans</i> 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 CBF β -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

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 NF κ B 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 ($\text{h}\beta\text{c}$) 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)* (CBF β -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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