Genetics and Functional Characterization of GATA2, a Novel Cancer Gene in Familial Leukaemia

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ABSTRACT

We first report *GATA2* mutations (heterozygous) in 4 families that are susceptible to MDS/AML (3 large families) and MDS (1 small family). Molecular analysis revealed a germline transmission of a *GATA2* missense mutation (T354M) in MDS/AML families and a *GATA2* deletion mutation (T355del) in MDS family. Neither germline *RUNX1* nor *CEBPA* mutations were found in these families, in 695 non-leukemic ethnically matched controls and 268 sporadic AML samples. The mutations resided within the GATA2 zinc finger 2 domain, a critical region for DNA-binding and protein-protein interactions, but not for nuclear localization. T354M reduced DNA binding ability of GATA2; whereas, T355del bound very little, if any, to the consensus WGATAR DNA motif. T354M and T355del also significantly reduced the transactivation of GATA2 in known GATA2 responsive sequences. Moreover, co-transfection of T354M or T355del with WT reduced WT transactivation ability, suggesting that these mutants act in a dominant negative fashion. Regulatable stable promyelocytic HL-60 cells expressing WT and mutants were generated. Forced expression of WT and T354M inhibited HL-60 cell differentiation when induced with all *trans* retinoic acid. However, when compared to WT, T354M enabled cell proliferation/survival while simultaneously reducing apoptosis. In contrast, T355del was a complete loss-of-function mutant. Microarray studies elucidated that both T354M and T355del significantly decreased the expression of downstream target genes. Together, our data suggest that both T354M and T355del are loss-of-function mutations with some dominant negative attributes.

Recently, we and others have described *GATA2* genetic lesions in other diseases. We further investigated *in vitro* functions of an alllelic series of GATA2 mutants representing the major disease phenotypes: MDS/AML (T354M), MDS (T355del), CML-BC (L359V), Emberger syndrome (R361L and C373R), AML-M5 and biallellic *CEBPA* AML (R362Q), and immunodeficiency syndrome (R398W). We showed that these GATA2 mutants (except L359V) are loss-of-function that reduce DNA binding affinity and transactivation of target genes. Nevertheless, they maintain the ability to bind to known protein binding partners. Intriguingly, T354M and C373R have an enhanced affinity for PU.1, highlighting that these

mutants can influence both DNA-binding and protein-protein interaction. Preliminary transduction of *Gata2* WT or mutant expression constructs into mouse whole bone marrow cells demonstrated that GATA2 mutants did not confer self-renewal capacity, but allowed specific myeloid progenitor differentiation.

We further demonstrated that *Gata2* is expressed in lymphatic endothelial cells and that it can bind to and transactivate a *Prox1* promoter/enhancer element (*PEE*) region. *Prox1* is required for lymphatic development and maintenance, and hence *Gata2* may contribute to lymphoedema throught its action on *Prox1*. Intriguingly, *Gata2* mutants displayed differential binding affinity to two GATA binding sites and reduced transactivation of the *PEE* region. Furthermore, an enhancer region 11.3kb upstream of *Prox1* is activated by GATA2, FOXC2 and SOX18, but repressed by PROX1 itself suggesting that these key lymphatic TFs may co-operate to regulate *Prox1* expression.

In conclusion, I present the experimental work for the landmark discovery of a new MDS/AML predisposition gene. I have also characterized the molecular landscape of *GATA2* mutations where each of the mutations confers specific and major effects on GATA2 function, but where there are also subtle differences between the mutants in the contexts of DNA binding and transactivation.

STATEMENT

I certify that this work 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. In addition, I certify that no part of this work will, in the future, be used in a submission for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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CHAN ENG CHONG

Date: 29 JULY 2013

* THE PUBLISHED ARTICLES

Methods published in the following article were cited in Chapter 3.

Hahn CN, **Chong CE**, Carmichael CL, Wilkins EJ, Brautigan PJ, Li XC, Babic M, Lin M, Carmagnac A, Lee YK, Kok CH, Gagliardi L, Friend KL, Ekert PG, Butcher CM, Brown AL, Lewis ID, To LB, Timms AE, Storek J, Moore S, Altree M, Escher R, Bardy PG, Suthers GK, D'Andrea RJ, Horwitz MS, Scott HS. Heritable GATA2 mutations associated with familial myelodysplastic syndrome and acute myeloid leukemia. *Nat. Genet.* 2011 Sep 4;43(10):1012-7.

Materials, methods and partial results published in the following article were cited in Chapter 4.

Kazenwadel J, Secker GA, Liu YJ, Rosenfeld JA, Wildin RS, Cuellar-Rodriguez J, Hsu AP, Dyack S, Fernandez CV, **Chong CE**, Babic M, Bardy PG, Shimamura A, Zhang MY, Walsh T, Holland SM, Hickstein DD, Horwitz MS, Hahn CN, Scott HS, Harvey NL. *Blood*. 2012 Feb 2;119(5):1283-91.

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LIST OF ABBREVIATIONS

Abbreviations	Description
4HT	4-hydroxytamoxifen
ALL	acute lymphoid leukaemia
ATRA	all *trans* retinoic acid
BFU-E	burst forming unit-erythroid
BM	bone marrow
CFU	colony forming unit
CFU-G	CFU-Granulocyte
CFU-GEMM	CFU-Granulocyte/Erythrocyte/Monocyte/Megakaryocyte
CFU-GM	CFU-Granulocyte/Macrophage
CFU-M	CFU-Macrophage
ChIP	chromatin immunoprecipitation
ChIP-Seq	chromatin immunoprecipitation-sequencing
CLL	chronic lymphoid leukaemia
CLP	common lymphoid progenitor
CML-BC	chronic myeloid leukaemia blast crisis
CMP	common myeloid progenitor
CN-AML	cytogenetically normal AML
Co-IP	co-immunoprecipitation
EMSA	electrophoretic mobility shift assay
ENCODE	ENCyclopedia of DNA Elements
ES cells	Embryonic stem cells
FPD/AML	familial thrombocytopenia with increased risk to develop AML
G-CSF	granulocyte-colony stimulating factor
GFP	green fluorescence protein
GM-CSF	granulocyte-macrophage colony-stimulating factor
GMP	granulocyte-macrophage progenitor

GOF	gain-of-function
HM	haematological malignancies
HPCs	haematopoietic progenitor cells
HSCs	haematopoietic stem cells
KO	knock-out
LEC	lymphatic endothelial cells
LOF	loss-of-function
LSK cells	Lineage negative, SCAL positive, c-KIT positive cells
MDS	myelodysplastic syndrome
MDS/AML	AML with myelodysplasia-related changes
MPN	myeloproliferative neoplasm
OMIM	Online Mendelian Inheritance in Man
PB	peripheral blood
RA	refractory anaemia
REAB-1	refractory anaemia with excess blasts 1
REAB-2	refractory anaemia with excess blasts 2
SEM	standard error mean
SCF	stem cell factor
t-AML	therapy-related AML
TF	transcription factor
TPO	thrombopoietin
TCRD	T cell Receptor Delta
UCSC	University of California, Santa Cruz
WEMSA	Western blotting-electrophoretic mobility shift assay
WT	wild type
ZF1	zinc finger 1
ZF2	zinc finger 2