Blood First Edition Paper, prepublished online April 8, 2013; DOI 10.1182/blood-2012-12-471300

Tribbles in Acute Leukaemia

Kai Ling Liang[2], Loveena Rishi[1] and Karen Keeshan[1]

[1] Paul O'Gorman Leukaemia Research Centre, Institute of Cancer Sciences, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, G12 0YN, UK
[2] University College Cork, Cork, Ireland.

Corresponding author: Karen Keeshan. Tel: 0141 301 7895. karen.keeshan@glasgow.ac.uk

Abstract

There is growing research interest in the mammalian Trib family of serine/threonine pseudokinases and their oncogenic association with acute leukaemias. This review is to understand the role of *Trib* genes in haematopoietic malignancies and their potential as targets for novel therapeutic strategies in acute myeloid leukaemia (AML) and acute lymphoblastic leukaemia (ALL). We discuss the role of Tribs as central signalling mediators in different subtypes of acute leukaemia and propose that inhibition of dysregulated Trib signalling may be therapeutically beneficial.

Introduction

The mammalian Trib family, *Trib1*, *Trib2* and *Trib3*, are classified as pseudokinases and have important roles in many cellular processes.[1] Trib1-3 are postulated to act as adaptor molecules to regulate and integrate a wide range of signalling pathways. However, the functional interpretation of the pseudokinase classification remains unclear. Here, we review the Trib family members in acute leukaemias and discuss Tribs as potential therapeutic targets in acute leukaemias.

Trib1-3 in human haematopoiesis

We examined *Trib1-3* expression in human haematopoiesis[2] using the Leukaemia Gene Atlas (LGA) platform[3] (Figure 1A). Statistical analyses showed that *Trib1* expression increased significantly in Granulocyte/Monocyte (GRAN/MONO) and B cell lineages compared to other lineages and indeed Trib1 knockout mice have a defect in macrophage and eosinophil differentiation.[4] However, *Trib2* and *Trib3* expressions were found to increase significantly in T cell and Erythrocyte (ERY) lineages respectively and interestingly FOG and GATA2 both bind the *Trib2* promoter in preMegEs to drive Megakaryocytic/Erythroid lineages.[5] *Trib1* expression increased in MONO sub-lineage whereas *Trib2* and *Trib3* expressions increased in GRAN sub-lineage (Figure 1B). Only *Trib2* was differentially expressed in $CD4^{+ve}$ T cells (Figure 1C). We observed similarities in human haematopoiesis of Trib1-3 expression, as described above e.g. T cells, monocytes, and dissimilarities e.g. HSC population, using the HemaExplorer platform[6] (Supplemental Figure 1). As opposed to LGA platform analysis (using one study), HemaExplorer combined data sets of human haematopoiesis originated from several studies.

Role of Tribbles in AML

Trib1

Previous studies have identified that only *Trib1* expression increases in AML due to amplification of chromosome 8q24 region as double minutes (dmin) despite other genes, including *MYC*, present in 8q24-dmin, and the implications of this are yet to be determined.[7,8] Using the LGA, we analyzed gene expression data from five different studies[9-13] where samples with known AML karyotypes and/or FAB subtypes were available. *Trib1* expression was increased significantly in AML with inv(16) or t(16;16) and of FAB M4 and M5 compared to other karyotypes and FAB subtypes (Table 1), and healthy controls (Table 2).

Trib1 was first discovered as a cooperating gene in a murine model of *HOXA9/MEIS1* myeloid leukemogenesis.[14] *Trib1* overexpression alone is able to induce AML in mice by promoting degradation of C/EBPα.[15] Interaction between Trib1 and MEK1 leads to ERK phosphorylation and degradation of C/EBPα.[16] A gain of function mutation, R107L in Trib1 was identified in human acute megakaryocytic leukaemia[17] and its overexpression accelerates the onset of murine AML.

Trib2

Trib2 was the first family member to be identified as an oncogene, where it induced potent AML in mice through inactivation of C/EBPα.[18] Trib2 induces proteasomal-dependent degradation of C/EBPα via the E3 ligase COP1[18,19], changing the C/EBPα isoform ratio in favour of the truncated oncogenic form. We did not observe elevated expression of *Trib2* in any AML

karyotypes and FAB subtypes (Table 1). *Trib2* expression is generally low[20], but upregulated specifically in a biologically and epigenetically distinct subset of immature AML[21] with silenced *CEBPA* and a mixed myeloid/T-lymphoid phenotype[18,22]. *Trib1* expression is lower in this cluster[20] which could potentially be attributed to the different roles of *Trib1* and *Trib2* in myeloid and lymphoid haematopoiesis.

All-trans retinoic acid is a hugely successful differentiation therapy for acute promyelocytic leukaemia APL (M3) with t(15;17) translocation (*PML-RARα*) and *Trib2* expression levels albeit overall low, are at higher levels in the M0 and M3 subtypes, and in *PML-RARα* positive leukaemias (cluster 12) compared to *PML-RARα* negative leukaemias (Figure 2A-B). Interestingly, *Trib1* levels are lower in *PML-RARα* positive leukaemias compared to *Trib2* (Figure 2A). Approximately 37% of *PML-RARα* APL patients have mutations that constitutively activate *FLT3*.[23] Cluster 12 identified originally by Valk et al[9] can be divided into two subgroups that correspond to the *FLT3-ITD* mutation status. Increased *Trib2* expression in this cluster was associated with *FLT3-TKD* but not *FLT3-ITD* mutations (Figure 2C). *FLT3-ITD* has been shown to induce a myeloproliferative disease and to cooperate with *PML-RARα* to induce an APL-like disease.[24] However, *FLT3-TKD* was shown to induce a murine lymphoid disease[25] and the cooperative relationship between *FLT3-TKD* and *PML-RARα* has not been examined. In contrast to *FLT3-ITD*, *FLT3-TKD* could not induce aberrant activation of STAT5, and repression of C/EBPα and Pu.1.[26] Thus Trib1 and 2, known to inactivate C/EBPα, may be important in *PML-RARα* positive leukaemias that harbour *FLT3-TKD* mutations.

Both *Trib1*[14] and *Trib2*[27] are target genes of *HOX*-mediated leukaemogenesis, but they are activated in different context of murine AML. *Trib1* is activated in *HOXA9/MEIS1*-AML[14] whereas *Trib2* is activated in *NUP98-HOXD13/MEIS1*-AML[27]. Differential activation of *Trib1* and *Trib2* might underlie the dissimilarities identified previously in *HOXA9*- and *NUP98-HOXD13*-mediated leukaemogenesis.[28,29] *Trib2* was shown to cooperate also with HOXA9 and accelerate the onset of murine AML[30], indicating that both elevated Trib1 and Trib2 are cooperative events in HOXA9 positive AMLs.

Trib3

Our analysis showed that *Trib3* expression was increased significantly in AML with t(8;21) and t(15;17), and of FAB M2 and M3 (Table 1, 2). While overexpression of Trib3 was unable to drive AML in the murine model[15], future study should determine if *Trib3* is a cooperating leukaemogen with *AML1-ETO* and *PML-RARα* as it may contribute to leukaemogenesis via a different mechanism.

Role of Tribbles in ALL

No evidence is currently available to implicate involvement of *Trib1* and *Trib3* in ALL, whereas there is growing evidence for a role of *Trib2* in ALL (Supplemental Figure 2).

Trib2

Trib2 was differentially expressed in $CD4^{+ve}$ T cells (Figure 1C), and is highest in T-ALL with normal karyotype and lowest in ALL with t(12;21).[31] *Trib2* was first identified in a screen for downstream effectors of NOTCH1 signalling in T-ALL[18] and is associated with activating

NOTCH1 mutations.[31] Subsequent study showed that NOTCH1 binds to the *Trib2* promoter and upregulates its expression.[22] We identified putative NOTCH1 binding sites in the promoter of *Trib1*, but not *Trib3* (data not shown), which is interesting as Trib3 cannot drive murine AML in the bone marrow transplant model.[15] Malignant thymocytes in NOTCH1-associated T-ALL are arrested at the double positive ($CD4^{+ve}CD8^{+ve}$ /DP) stage of development. In normal T cell development, expansion and differentiation of double negative (DN) to DP thymocytes requires pre-TCR signalling. High *Trib2* expression in T-ALL subset was shown to enrich for gene sets that define TCR signalling.[31] We found gradual increase of *Trib2* expression from DN to DP thymocytes using the ImmGen Data Browsers[32] (Supplemental Figure 3). Hence, it is likely that *Trib2* is a T-cell-specific cooperative signal required by aberrant NOTCH1 signalling to drive transformation, proliferation and survival of malignant thymocytes.

In T-ALL, *Trib2* appears to be a downstream target of multiple oncogenic transcription factors. As well as NOTCH1[22,31], PITX1[33] and TAL1[34] were also found to upregulate *Trib2*. *PITX1* is recurrently activated in T-ALL to deregulate genes involved in T-cell development, including *Trib2*.[33] *TAL1* is aberrantly activated in 50-60% of human T-ALL patients[35] and 40% of these patients also develop activating mutations in *NOTCH1*.[36] *Trib2* was identified in a knockdown screen in T-ALL as one of the critical targets of the core transcriptional regulatory circuit controlled by the TAL1 complex and importantly Trib2 was shown to be essential for the growth and survival of human T-ALL cell lines.[34] A *TAL1/LMO2* mouse model of T-ALL showed that 75% of the T-ALL mice develop spontaneous activating mutations in *NOTCH1*.[37] Thus, further studies are warranted to examine the role of *Trib2* and its potential cooperation in $TAL1^{+ve}$ and *NOTCH1* mutant T-ALL pathogenesis.

Trib2 is also potentially involved in ALL with t(1;19) as the expression level of *Trib2* in this subset of ALL was higher than that in T-ALL.[31] t(1;19, E2A-PBX1) is present in about 6% of all B-ALLs, 25% of paediatric pre-B-ALL, and in rare cases of myeloid and T-cell leukaemias.[38] E2A-PBX1 was shown to cooperate with NOTCH1 and HOXA9, which have established relationships with Trib2, to induce T-Cell Lymphoma/Leukaemia[39] and AML[40] in murine models. Given the strong link between NOTCH1 and Trib2 in T-ALL the development of Trib inhibitors may prove to be potentially therapeutic.

Targeting Trib in AML and ALL therapy

Structurally, Trib family members have three clearly distinguishable regions, a C-terminal region that contains a MEK1 and COP1 E3 ligase-binding sites, a central serine/threonine kinase like domain with an ATP binding motif, and a N terminal region not required for oncogenicity.[19] The binding of COP1, and another E3 ligase TRIM21[41], to Trib is essential for Trib-induced proteasomal degradation of C/EBPα and AML suggesting that potential inhibitors which may act by inhibiting the Trib-E3 ligase (COP1/TRIM21) relationship using proteasome inhibitors or molecules that interfere with the binding of the E3 ligase to the Trib C-terminal region would be effective therapeutics for Trib-induced AML. Current clinical trials using bortezomib (proteasome inhibitor) with other standard chemotherapeutics are underway in myelodysplastic syndrome and AML.

In comparison to conventional kinases, Tribs are considered pseudokinases as they contain an N-terminal lobe in the central kinase region that contains a lysine residue critical for ATP

binding and contains other atypical kinase motifs.[1] Although Tribs lack demonstrable serine/threonine kinase activity to date, the intact kinase domain is required for leukemogenesis.[19] Thus, small molecule inhibitors which target the Trib kinase domain ATP binding pocket specifically for example may be good therapeutics for AML and ALL involving upregulated Tribs. All three Tribs contain a conserved motif which is required for the MEK1 binding in the C-terminus of the kinase domain and results in enhanced ERK phosphorylation required for the degradation of C/EBPα in AML.[15,16] Thus MEK1 inhibitors could be considered as potential chemotherapeutic agents targeting Trib function in AML and ALL. As it appears that Tribs may be central mediators of signalling pathways, they may be good therapeutic downstream targets in T-ALL and AML driven by other oncogenes (e.g. NOTCH1, FLT3, HOX).

Perspectives

The specific subtypes of acute leukaemia that each *Trib* associates with have differentiation arrest at different stages of hematopoiesis. Hence, understanding the regulation of *Trib1-3* lineage specific expression and their roles in differentiation is important. This will pave the way to understanding the molecular aberrations and cooperative signalling pathways that occur during leukaemic transformation.

It is important to note the differences seen in the human and murine leukaemias. Our analyses of human AML consists solely of mRNA expression levels, whereas in murine overexpression models elevated protein expression would not be subject to transcriptional regulation. Indeed the myeloid versus lymphoid differences seen in the murine versus human disease may be due to the origin of cells that overexpress *Trib2*. These discrepancies may be resolved through the use of conditional knock-in Trib models, or murine transplantations using lineage specific donor cells. A more relevant Trib-induced leukaemia murine model is necessary to gain functionally and clinically relevant data.

Though studies so far suggest Trib proteins act as adaptor molecules or decoy kinases in signalling pathways and the proteasome degradation pathway, it remains possible that Trib proteins are atypical kinases that can directly impact substrate protein activity. To date, direct substrate phosphorylation via Trib proteins has not been described, and indeed screens for novel Trib substrates have not been documented.

This review highlights the contribution of Trib proteins in AML and ALL signalling pathways and presents them as potential targeted therapies. Inhibitors that target activated oncogenes with essential functions in normal cells are likely to have narrow therapeutic windows and serious side effects. Available knockout mice for Trib2 and Trib3 have not shown significant phenotypic manifestations[42] however a critical role for Trib1 in macrophage differentiation leading to adipose tissue maintenance and suppression of metabolic disorders has been demonstrated in Trib1 knockout mice.[4] Future work in knockout mice are required to clarify the physiological and/or redundant roles for each Trib family member in normal and malignant haematopoiesis. Nevertheless, this indicates that selective targeting of Trib family members might be the favourable approach.

Author Contributions

K.L.L analysed data, wrote the paper and made the figures. L.R wrote the paper. K.K designed the study and wrote the paper. K.L.L is supported by Health Research Board Ireland. L.R and K.K are supported by the Howat Foundation and Children with Cancer, UK.

Conflict of interest

The authors report no potential conflict of interest.

References

1. Hegedus Z, Czibula A, Kiss-Toth E. Tribbles: a family of kinase-like proteins with potent signalling regulatory function. *Cell Signal*. 2007;19(2):238-250.
2. Novershtern N, Subramanian A, Lawton LN, et al. Densely interconnected transcriptional circuits control cell states in human hematopoiesis. *Cell*. 2011;144(2):296-309.
3. Hebestreit K, Grottrup S, Emden D, et al. Leukemia gene atlas--a public platform for integrative exploration of genome-wide molecular data. *PLoS One*. 2012;7(6):e39148.
4. Satoh T, Kidoya H, Naito H, et al. Critical role of Trib1 in differentiation of tissue-resident M2-like macrophages. *Nature*. 2013.
5. Mancini E, Sanjuan-Pla A, Luciani L, et al. FOG-1 and GATA-1 act sequentially to specify definitive megakaryocytic and erythroid progenitors. *EMBO J*. 2012;31(2):351-365.
6. Bagger FO, Rapin N, Theilgaard-Mönch K, et al. HemaExplorer: a Web server for easy and fast visualization of gene expression in normal and malignant hematopoiesis. *Blood*. 2012;119(26):6394-6395.
7. Rothlisberger B, Heizmann M, Bargetzi MJ, Huber AR. TRIB1 overexpression in acute myeloid leukemia. *Cancer Genet Cytogenet*. 2007;176(1):58-60.
8. Storlazzi CT, Fioretos T, Paulsson K, et al. Identification of a commonly amplified 4.3 Mb region with overexpression of C8FW, but not MYC in MYC-containing double minutes in myeloid malignancies. *Hum Mol Genet*. 2004;13(14):1479-1485.
9. Valk PJ, Verhaak RG, Beijen MA, et al. Prognostically useful gene-expression profiles in acute myeloid leukemia. *N Engl J Med*. 2004;350(16):1617-1628.
10. Gutierrez NC, Lopez-Perez R, Hernandez JM, et al. Gene expression profile reveals deregulation of genes with relevant functions in the different subclasses of acute myeloid leukemia. *Leukemia*. 2005;19(3):402-409.
11. Verhaak RG, Wouters BJ, Erpelinck CA, et al. Prediction of molecular subtypes in acute myeloid leukemia based on gene expression profiling. *Haematologica*. 2009;94(1):131-134.
12. Haferlach T, Kohlmann A, Wieczorek L, et al. Clinical utility of microarray-based gene expression profiling in the diagnosis and subclassification of leukemia: report from the International Microarray Innovations in Leukemia Study Group. *J Clin Oncol*. 2010;28(15):2529-2537.
13. Eppert K, Takenaka K, Lechman ER, et al. Stem cell gene expression programs influence clinical outcome in human leukemia. *Nat Med*. 2011;17(9):1086-1093.
14. Jin G, Yamazaki Y, Takuwa M, et al. Trib1 and Evi1 cooperate with Hoxa and Meis1 in myeloid leukemogenesis. *Blood*. 2007;109(9):3998-4005.
15. Dedhia PH, Keeshan K, Uljon S, et al. Differential ability of Tribbles family members to promote degradation of C/EBPalpha and induce acute myelogenous leukemia. *Blood*. 2010;116(8):1321-1328.
16. Yokoyama T, Kanno Y, Yamazaki Y, Takahara T, Miyata S, Nakamura T. Trib1 links the MEK1/ERK pathway in myeloid leukemogenesis. *Blood*. 2010;116(15):2768-2775.
17. Yokoyama T, Toki T, Aoki Y, et al. Identification of TRIB1 R107L gain-of-function mutation in human acute megakaryocytic leukemia. *Blood*. 2012;119(11):2608-2611.
18. Keeshan K, He Y, Wouters BJ, et al. Tribbles homolog 2 inactivates C/EBPalpha and causes acute myelogenous leukemia. *Cancer Cell*. 2006;10(5):401-411.
19. Keeshan K, Bailis W, Dedhia PH, et al. Transformation by Tribbles homolog 2 (Trib2) requires both the Trib2 kinase domain and COP1 binding. *Blood*. 2010;116(23):4948-4957.

20. Gilby DC, Sung HY, Winship PR, Goodeve AC, Reilly JT, Kiss-Toth E. Tribbles-1 and -2 are tumour suppressors, down-regulated in human acute myeloid leukaemia. *Immunol Lett*. 2010;130(1-2):115-124.
21. Figueroa ME, Wouters BJ, Skrabanek L, et al. Genome-wide epigenetic analysis delineates a biologically distinct immature acute leukemia with myeloid/T-lymphoid features. *Blood*. 2009;113(12):2795-2804.
22. Wouters BJ, Jorda MA, Keeshan K, et al. Distinct gene expression profiles of acute myeloid/T-lymphoid leukemia with silenced CEBPA and mutations in NOTCH1. *Blood*. 2007;110(10):3706-3714.
23. Kottaridis PD, Gale RE, Frew ME, et al. The presence of a FLT3 internal tandem duplication in patients with acute myeloid leukemia (AML) adds important prognostic information to cytogenetic risk group and response to the first cycle of chemotherapy: analysis of 854 patients from the United Kingdom Medical Research Council AML 10 and 12 trials. *Blood*. 2001;98(6):1752-1759.
24. Kelly LM, Kutok JL, Williams IR, et al. PML/RARalpha and FLT3-ITD induce an APL-like disease in a mouse model. *Proc Natl Acad Sci U S A*. 2002;99(12):8283-8288.
25. Grundler R, Miething C, Thiede C, Peschel C, Duyster J. FLT3-ITD and tyrosine kinase domain mutants induce 2 distinct phenotypes in a murine bone marrow transplantation model. *Blood*. 2005;105(12):4792-4799.
26. Choudhary C, Schwable J, Brandts C, et al. AML-associated Flt3 kinase domain mutations show signal transduction differences compared with Flt3 ITD mutations. *Blood*. 2005;106(1):265-273.
27. Argiropoulos B, Palmqvist L, Yung E, et al. Linkage of Meis1 leukemogenic activity to multiple downstream effectors including Trib2 and Ccl3. *Exp Hematol*. 2008;36(7):845-859.
28. Pineault N, Abramovich C, Humphries RK. Transplantable cell lines generated with NUP98-Hox fusion genes undergo leukemic progression by Meis1 independent of its binding to DNA. *Leukemia*. 2005;19(4):636-643.
29. Pineault N, Buske C, Feuring-Buske M, et al. Induction of acute myeloid leukemia in mice by the human leukemia-specific fusion gene NUP98-HOXD13 in concert with Meis1. *Blood*. 2003;101(11):4529-4538.
30. Keeshan K, Shestova O, Ussin L, Pear WS. Tribbles homolog 2 (Trib2) and HoxA9 cooperate to accelerate acute myelogenous leukemia. *Blood Cells Mol Dis*. 2008;40(1):119-121.
31. Hannon MM, Lohan F, Erbilgin Y, et al. Elevated TRIB2 with NOTCH1 activation in paediatric/adult T-ALL. *Br J Haematol*. 2012;158(5):626-634.
32. Heng TS, Painter MW, Immunological Genome Project C. The Immunological Genome Project: networks of gene expression in immune cells. *Nat Immunol*. 2008;9(10):1091-1094.
33. Nagel S, Venturini L, Przybylski GK, et al. Activation of Paired-homeobox gene PITX1 by del(5)(q31) in T-cell acute lymphoblastic leukemia. *Leuk Lymphoma*. 2011;52(7):1348-1359.
34. Sanda T, Lawton LN, Barrasa MI, et al. Core transcriptional regulatory circuit controlled by the TAL1 complex in human T cell acute lymphoblastic leukemia. *Cancer Cell*. 2012;22(2):209-221.
35. Ferrando AA, Neuberg DS, Staunton J, et al. Gene expression signatures define novel oncogenic pathways in T cell acute lymphoblastic leukemia. *Cancer Cell*. 2002;1(1):75-87.
36. Weng AP, Ferrando AA, Lee W, et al. Activating mutations of NOTCH1 in human T cell acute lymphoblastic leukemia. *Science*. 2004;306(5694):269-271.

37. Tatarek J, Cullion K, Ashworth T, Gerstein R, Aster JC, Kelliher MA. Notch1 inhibition targets the leukemia-initiating cells in a Tal1/Lmo2 mouse model of T-ALL. *Blood*. 2011;118(6):1579-1590.
38. Bijl J, Krosl J, Lebert-Ghali CE, Vacher J, Mayotte N, Sauvageau G. Evidence for Hox and E2A-PBX1 collaboration in mouse T-cell leukemia. *Oncogene*. 2008;27(49):6356-6364.
39. Feldman BJ, Hampton T, Cleary ML. A carboxy-terminal deletion mutant of Notch1 accelerates lymphoid oncogenesis in E2A-PBX1 transgenic mice. *Blood*. 2000;96(5):1906-1913.
40. Thorsteinsdottir U, Krosl J, Kroon E, Haman A, Hoang T, Sauvageau G. The oncoprotein E2A-Pbx1a collaborates with Hoxa9 to acutely transform primary bone marrow cells. *Mol Cell Biol*. 1999;19(9):6355-6366.
41. Grandinetti KB, Stevens TA, Ha S, et al. Overexpression of TRIB2 in human lung cancers contributes to tumorigenesis through downregulation of C/EBPalpha. *Oncogene*. 2011;30(30):3328-3335.
42. Yokoyama T, Nakamura T. Tribbles in disease: Signaling pathways important for cellular function and neoplastic transformation. *Cancer Sci*. 2011;102(6):1115-1122.

Tables and Table Footnotes

Table 1. Expression of Tribbles increased in specific AML karyotypes and FAB subtypes

Studies	*Trib1* (202241_at)		*Trib2* (202478_at)		*Trib3* (218145_at)	
	Karyotype	FAB	Karyotype	FAB	Karyotype	FAB
Valk et al[9]*	inv(16) or t(16;16)†	M4† M5‡	NS	NS	t(8;21)† t(15;17)†	M2† M3‡
Gutierrez et al[10]	inv(16)(p12;q13)†	M4Eo†	NS	NS	t(15;17)(q12;q21)†	M3†
Verhaak et al[11]	idt(16)† inv(16)‡ t(6;9)†	M4‡ M5‡	NS	RAEB† RAEB-t†	t(6;9)† t(8;21)‡ t(15;17)‡	M2‡ M3‡
Haferlach et al[12]*	complex† t(8;21)†	—	complex† inv(16) or t(16;16)†	—	complex‡ t(15;17)†	—
Eppert et al[13]	+13† inv(16)(p13;q22)†	NS	inv(16)(p13;q22)†	M4Eo†	NS	NS

AML samples with unknown karyotype or FAB subtype were excluded from statistical analysis provided by the LGA.
NS indicates no statistically significant increased expression found.
— indicates unavailable data for statistical analysis.
*AML samples with normal karyotype were not able to be included in the statistical analysis.
†Statistically significant increased expression with adjusted $P<0.05$ in Welch's t-Test.
‡Statistically highly significant increased expression with adjusted $P<0.001$ in Welch's t-Test.

Table 2. Expression of Tribbles increased in subsets of AML compared to healthy samples

Studies	*Trib1* (202241_at)		*Trib2* (202478_at)		*Trib3* (218145_at)	
	Karyotype	FAB	Karyotype	FAB	Karyotype	FAB
Valk et al[9]	inv(16) or t(16;16)*	M4* M5*	NS	NS	t(8;21)* t(15;17)†	M2* M3†
Haferlach et al[12]	NS	—	NS	—	complex† t(15;17)*	—

Elevated expression of Tribbles in karyotypes and FAB subytypes identified in Table 1 were compared to that of healthy samples. Healthy samples were not available in Gutierrez et al[10], Verhaak et al[11] and Eppert et al[13].
Abbreviations are explained in Table 1.
*Statistically significant increased expression with adjusted $P<0.05$ in Welch's t-Test.
†Statistically highly significant increased expression with adjusted $P<0.001$ in Welch's t-Test.

Figure Legends

Figure 1. Distributions of *Trib1-3* expression in human haematopoietic system. Expressions of *Trib1-3* in (A) different haematopoietic cell lineages, and among the different cell populations of (B) GRAN/MONO and (C) T cell lineages were examined by using the Leukaemia Gene Atlas (LGA) based on the gene expression data set from Novershtern et al[2]. Statistically significant increase of *Trib1-3* expressions, marked by *adjusted $P<0.05$ and **adjusted $P<0.001$, were determined by Welch's t-Test.

Figure 2. Elevated expression of *Trib2* in *PML-RARα* positive leukaemias is associated with *FLT3-TKD* but not *FLT3-ITD* mutations. Expressions of *Trib1* and *Trib2* in (A) different FAB subtypes, and (B) between *PML-RARα* positive and negative leukaemias were examined by using the LGA based on the gene expression data set from Valk et al[9]. (C) *Trib2* expression in *PML-RARα* positive leukaemias was further stratified based on *FLT3-ITD* and -*TKD* mutation status, and compared to that of *PML-RARα* negative leukaemias.

Figure 1

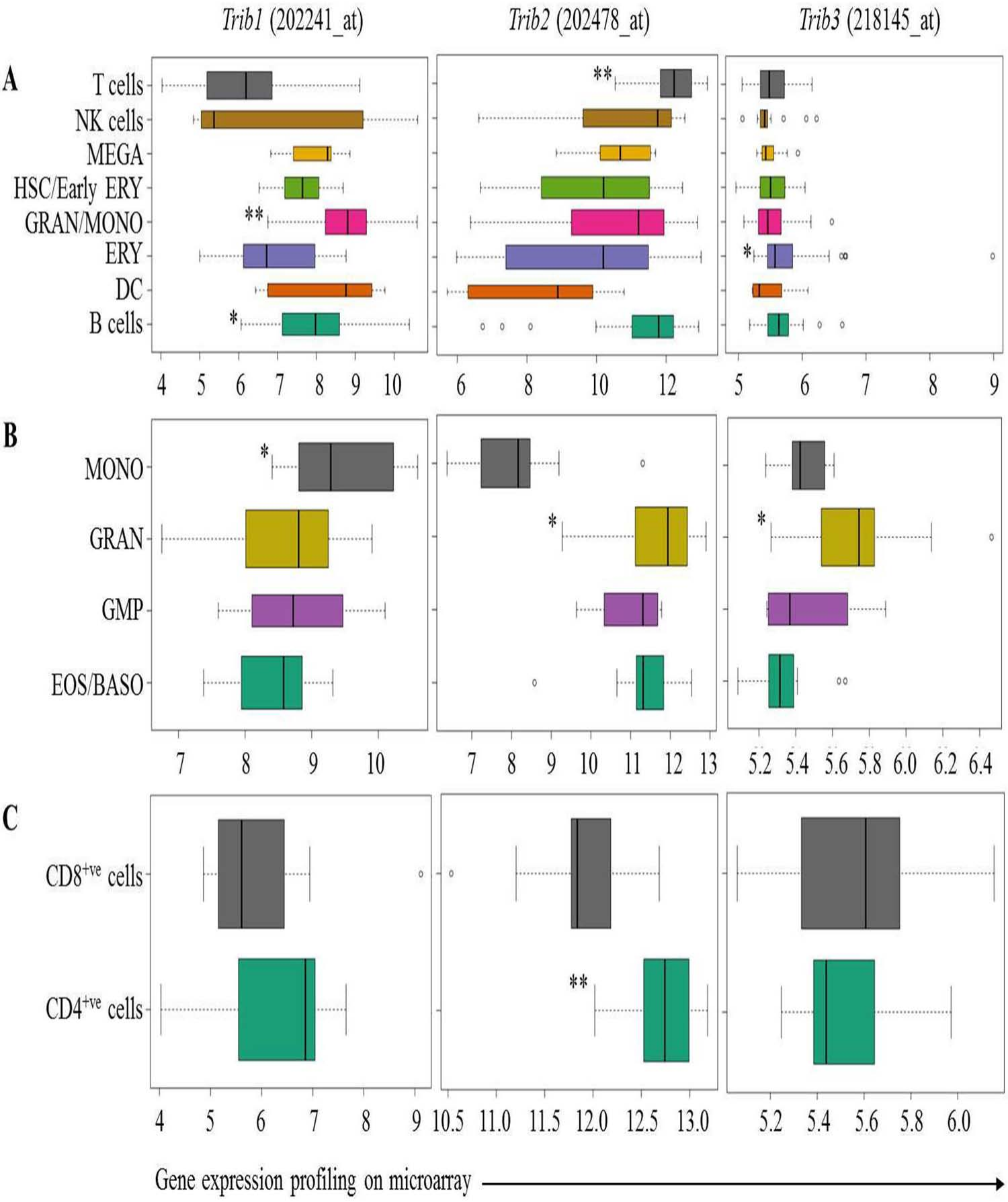
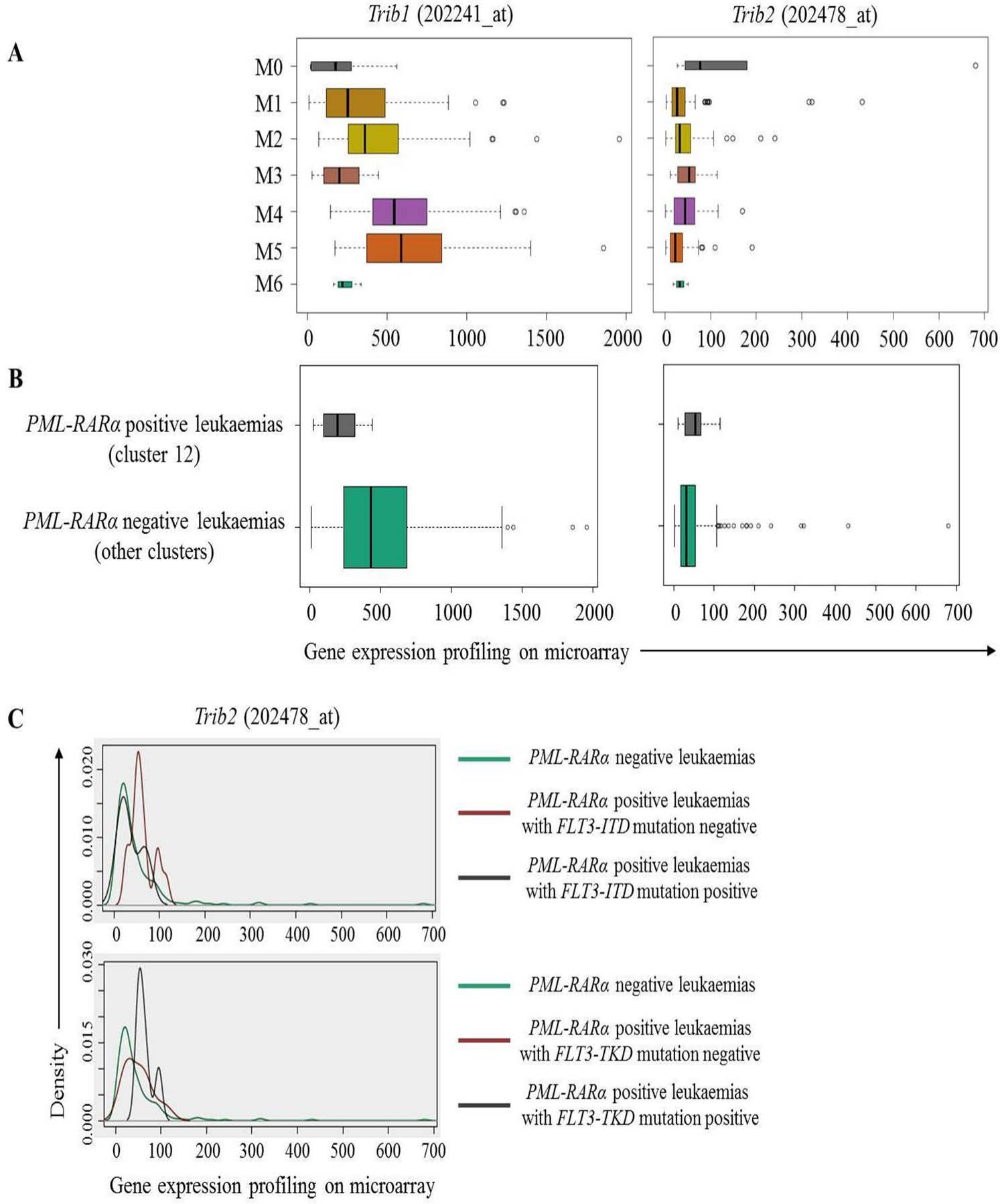

Trib1 (202241_at)
Trib2 (202478_at)
Trib3 (218145_at)
A
T cells
NK cells
MEGA
HSC/Early ERY
GRAN/MONO
ERY
DC
B cells
B
MONO
GRAN
GMP
EOS/BASO
C
CD8+ve cells
CD4+ve cells
Gene expression profiling on microarray

Figure 2


A
Trib1 (202241_at)
Trib2 (202478_at)
M0
M1
M2
M3
M4
M5
M6
0
500
1000
1500
2000
0
100
200
300
400
500
600
700
B
PML-RARα positive leukaemias
(cluster 12)
PML-RARα negative leukaemias
(other clusters)
Gene expression profiling on microarray
C
Trib2 (202478_at)
Density
0.000
0.010
0.020
0.030
0.015
PML-RARα negative leukaemias
PML-RARα positive leukaemias
with FLT3-ITD mutation negative
PML-RARα positive leukaemias
with FLT3-ITD mutation positive
PML-RARα negative leukaemias
PML-RARα positive leukaemias
with FLT3-TKD mutation negative
PML-RARα positive leukaemias
with FLT3-TKD mutation positive
Gene expression profiling on microarray

Prepublished online April 2, 2013;
doi:10.1182/blood-2012-12-471300 originally published online
April 2, 2013

Tribbles in acute leukemia

Kai Ling Liang, Loveena Rishi and Karen Keeshan

Blood (print ISSN 0006-4971, online ISSN 1528-0020), is published weekly by the American Society of Hematology, 2021 L St, NW, Suite 900, Washington DC 20036.