Brief report

Recurring chromosomal abnormalities in leukemia in *PML-RARA* transgenic mice identify cooperating events and genetic pathways to acute promyelocytic leukemia

Michelle M. Le Beau, Elizabeth M. Davis, Bhumi Patel, Vernon T. Phan, Jastinder Sohal, and Scott C. Kogan

Acute promyelocytic leukemia (APL) is characterized by the *PML-RARA* fusion gene. To identify genetic changes that cooperate with *PML-RARA*, we performed spectral karyotyping analysis of myeloid leukemias from transgenic *PML-RARA* mice and from mice coexpressing *PML-RARA* and *BCL2*, *IL3*, activated *IL3R*, or activated *FLT3*. A cooperating mutation that enhanced survival (*BCL2*) was not sufficient to complete transformation and was associated with multiple numeric abnormalities, whereas cooperating mutations that deregulated growth and enhanced survival were associated with normal karyotypes (*IL3*) or simple karyotypic changes (*IL3R, FLT3*). Recurring abnormalities included trisomy 15 (49%), trisomy 8 (46%), and −X/−Y (54%). The most common secondary abnormality in human APL is +8 or partial trisomy of 8q24, syntenic to mouse 15. These murine leukemias have a defined spectrum of changes that recapitulates, in part, the cytogenetic abnormalities found in human APL. Our results demonstrate that different cooperating events may generate leukemia via different pathways. (Blood. 2003;102:1072-1074)

Introduction

Acute promyelocytic leukemia (APL) is a distinct clinicopathologic entity characterized by the t(15;17)(q22;q11.2-12) and the resultant *PML-RARA* fusion gene.[1,2] Expression of PML-RARA in transgenic mice initiates myeloid leukemias with features of human APL.[3] We have reported a transgenic mouse model for APL, in which the human *MRP8* promoter drives expression of *PML-RARA* in myeloid cells.[4] The long latency before disease onset (median, 8.5 months), together with the incomplete penetrance (64% at 1 year), suggest that leukemogenesis initiated by PML-RARA requires additional genetic changes. This hypothesis is consistent with other transgenic mouse models of acute myeloid leukemia (AML), in which cooperating mutations are required for leukemogenesis by fusion genes (eg, *RUNX1/ETO*, *CBFB/MYH11*, and *MLL-ELL*).[5]

An important aspect of leukemia biology is the elucidation of the spectrum of chromosomal abnormalities and molecular mutations that cooperate in the pathways leading to leukemogenesis.[6] To identify genetic changes that cooperate with PML-RARA in vivo, we performed spectral karyotyping analysis of leukemias arising in *PML-RARA* mice and mice expressing *PML-RARA* plus cooperating mutations. Our results suggest that different cooperating events may generate acute leukemia via different pathways and that the particular combination leads to a specific pattern of additional mutations that complete leukemic transformation.

Study design

Generation of leukemias

Mice (FVB/N background) were bred and maintained at the University of California at San Francisco in accordance with institutional guidelines. *PML-RARA/BCL2* mice were reconstituted with *MRP8–PML-RARA*/MRP8-*BCL2* doubly transgenic bone marrow or with *MRP8–PML-RARA* bone marrow that had been transduced with a retroviral vector expressing *BCL2*.[4,7] *PML-RARA/FLT3*^{W51}, *PML-RARA/IL3*, and *PML-RARA/IL3R* mice were reconstituted with MRP8–*PML-RARA* bone marrow that had been transduced with retroviral vectors expressing an activated version of FMS-related tyrosine kinase 3 (FLT3; murine stem cell virus [MSCV]-FLT3^{W51}-internal ribosomal entry site [IRES]-green fluorescent protein [GFP]),[8,9] interleukin 3 (IL3; pMPZen-IL-3),[10,11] or an activated *IL3R* (βcV449E),[11,12] respectively.

Spectral karyotyping (SKY) analysis

Cytogenetic analysis was performed on fresh or cryopreserved spleen or bone marrow cells obtained at the time of development of leukemia. The initiation of short-term (24-72 hours) cultures, metaphase cell preparation, and spectral karyotyping were performed as described previously.[13] A minimum of 10 metaphase cells were analyzed per leukemia.

Results and discussion

Cytogenetic analysis of murine leukemias initiated by *PML-RARA*

The results of SKY analysis and features of the murine leukemias are given in Table 1. The distribution of chromosome abnormalities

From the Section of Hematology/Oncology, University of Chicago, IL; and Department of Laboratory Medicine, University of California, San Francisco.

Submitted January 17, 2003; accepted March 23, 2003. Prepublished online as *Blood* First Edition Paper, April 10, 2003; DOI 10.1182/blood-2003-01-0155.

Supported by National Institutes of Health (NIH) grants U01 CA84221 (M.M.L., S.C.K.), K08 CA75986 (S.C.K.), and CA 95274 (S.C.K.). S.C.K. is the 32nd Edward Mallinckrodt Junior Scholar and is a recipient of a Burroughs Wellcome Fund Career Award.

Reprints: Michelle M. Le Beau, Section of Hematology/Oncology, University of Chicago, 5841 S Maryland Ave, MC2115, Chicago, IL 60637; e-mail: mlebeau@medicine.bsd.uchicago.edu.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 U.S.C. section 1734.

Table 1. Characteristics of murine leukemias initiated by *PML-RARA* or *PML-RARA* and cooperating mutations

Parameter	Cooperating mutations				
	None	*BCL2*	*FLT3*	*IL3R*	*IL3*
Leukemia, %	64	100	70	100	100
Median latency, d	257	127	105	49	14-21
Disease	AML	AML	AML	AML	AML*
Leukocytosis	No	No	Yes	Yes	Yes
Anemia	Yes	Yes	Yes	Yes	Yes
Thrombocytopenia	Yes	Yes	Yes	Yes	Yes
Disseminated disease	Yes	Yes	Yes	Yes	Yes
Transplantable	Yes	Yes	Yes	Yes	No
ATRA response	Yes	Yes	Yes	Yes	Yes
SKY analysis					
No. cases	11	8	7	9	6
Abnormal (%)	10 (91)	8 (100)	7 (100)	7 (78)	1 (17)†
Karyotype complexity	Intermediate	Complex	Simple	Simple	Normal
Numeric abnormality (%)	10 (91)	8 (100)	7 (100)	7 (78)	0
Structural Abnormality (%)	2 (18)	1 (13)	0	0	1 (17)†
Recurring abnormalities (%)					
+4	1 (9)	7 (88)	0	0	0
+6	1 (9)	4 (50)	0	0	0
+7	1 (9)	7 (88)	0	0	0
+8	5 (45)	5 (63)	2 (29)	7 (78)	0
+10	3 (27)	6 (75)	3 (43)	1 (11)	0
+14	3 (27)	5 (63)	0	0	0
+15	7 (64)	8 (100)	3 (43)	2 (22)	0
+16	4 (36)	3 (38)	0	0	0
−X/−Y	6 (55)	6 (75)	6 (86)	4 (44)	0

ATRA indicates all–*trans*-retinoic acid.

*Diseased animals show pathologic features of AML.

†Robertsonian translocation (centric fusion) noted in 2 cells has no known phenotypic consequences.

in the genetic cohorts is illustrated in Figure 1; the complete karyotypes are provided on the laboratory's website (http://LeBeauLab.uchicago.edu, Table D), and in previous reports.[9,11,13] Each of the additional mutations cooperated with PML-RARA, as evidenced by the increase in leukemia incidence from 64% to between 70% and 100% and the decrease in latency period from 257 days to between 14 and 127 days. The incidence of clonal chromosomal abnormalities was high, ranging from 80% to 100% of cases, in all but the cohort expressing PML-RARA and IL3, in which all cases had a normal karyotype. The most common abnormality was loss of a sex chromosome, an X in females or a Y chromosome in males, noted in 22 (54%) of 41 cases. A gain of chromosome 15 was observed in 20 (49%) cases, and trisomy 8 was observed in 19 (46%) cases.

Of the 11 cases analyzed in *PML-RARA* transgenic mice, clonal abnormalities were identified in 10 (91%). The most common abnormality was a gain of chromosome 15 (7 cases, 64%; Figure 1A). Loss of a sex chromosome was noted in 6 cases (55%), and trisomy 8 was observed in 5 (45%) cases.

BCL2 is expressed in most APLs.[14] In the *PML-RARA/BCL2* cohort, all leukemias had a complex karyotype and were characterized by multiple abnormal clones with multiple numeric abnormalities in each clone (Table 1; Figure 1B). All but 2 cases had at least 45 chromosomes in the abnormal clone(s), compared with 1 of 11 leukemias arising in *PML-RARA* mice and none in the other genetic cohorts. All 8 cases were characterized by trisomy 15 (100%). Of interest is that 6 of 8 cases had the identical pattern of +4, +7, +10, and +15.

The FLT3 receptor tyrosine kinase enhances the proliferation and survival of hematopoietic progenitors in response to the FLT3 ligand. Activating *FLT3* mutations have been identified in approximately 35% of APLs, most of which result from an internal tandem duplication (ITD) of the juxtamembrane domain.[15] All of the

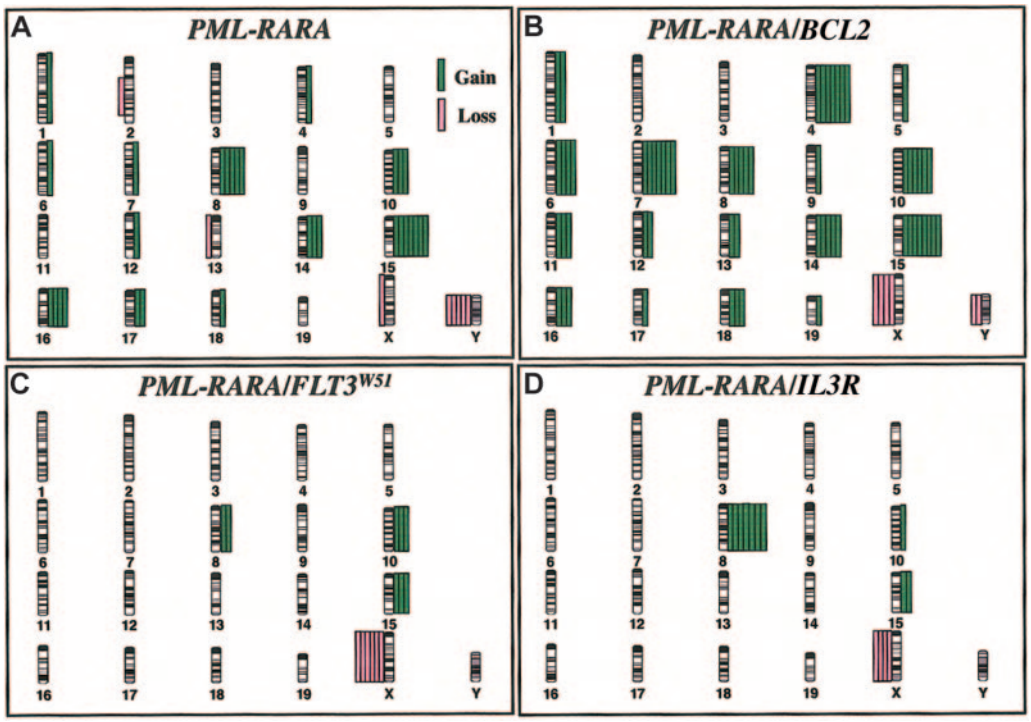

Figure 1. The distribution of chromosomal abnormalities in murine leukemias. The distribution of chromosomal abnormalities in (A) *PML-RARA*, (B) *PML-RARA/BCL2*, (C) *PML-RARA/FLT3^{W51}*, and (D) *PML-RARA/IL3R* (βcV449E) mice reveals a defined spectrum of numeric abnormalities. Chromosome gain is depicted by green bars on the right of each chromosome, and chromosome loss is depicted by pink bars on the left. Each bar represents a single case.

leukemias arising in the *PML-RARA/FLT3*^{W51} mice had abnormal karyotypes; however, the karyotypes were very simple with 6 (86%) of 7 showing loss of an X chromosome, together with either +10 or +15 (each in 3 of 7 cases, 43%; Figure 1C).[9]

Activating mutations of the common β chain of the IL3, IL5, and granulocyte-macrophage colony-stimulating factor (GM-CSF) receptors confer cytokine independence.[10,12] In the *PML-RARA/IL3R* (βcV449E) leukemias, 7 (78%) of 9 cases were abnormal with simple karyotypes. Trisomy 8 (7 [78%] of 9 cases) was most common, followed by loss of an X chromosome (4 [44%] of 9 cases), and trisomy 15 (2 [22%] of 9 cases; Figure 1D).[11]

We have reported that complete trisomy 8 or partial trisomy of bands 8q23-24 (12.4%) are the most common secondary chromosomal abnormalities in human APL.[13] Trisomy 15 is the most frequent abnormality in murine T-cell lymphomas (40%-90%), as well as in B/myeloid leukemias arising in irradiated *Eμ-BCL2* transgenic mice.[16,17] The smallest region gained is bands 15D2-3; this region is syntenic to human 8q23-24 and contains the *Myc* and *Pvt1* genes.[17] At present, the identity of the relevant gene(s) on human 8q is unknown; however, AMLs characterized by trisomy 8 overexpress genes on chromosome 8, suggesting that gene dosage effects mediate leukemogenesis.[18] Perhaps the best candidate gene is *MYC*, which is overexpressed in a number of human tumors.

Genetic pathways to APL

We have demonstrated that the particular combination of cooperating events leads to an obligate pattern of additional mutations that complete transformation. The initiating event, the PML-RARA fusion protein, impairs differentiation; however, leukemogenesis requires additional mutations. The addition of a cooperating mutation that enhances survival, such as *BCL2* expression, is not sufficient to complete transformation and is associated with instability and the acquisition of multiple numeric abnormalities. In contrast, cooperating mutations that deregulate growth, such as activated cytokine signaling pathways (FLT3^{W51}, IL3R, and IL3), are associated with the rapid development of leukemia and simple karyotypic changes or normal karyotypes. There is redundancy in the pathways activated by FLT3 and IL3/IL3R. Both induce *MYC* expression via RAS/MAPK (rat sarcoma viral oncogene/mitogen-activated protein kinase) activation, BCL_{XL} expression via STAT5 (signal transducer and activator of transcription 5) and PI3K/Akt (phosphatidylinositol 3–kinase/v-akt murine thymoma viral oncogene homolog), and BCL2 phosphorylation via ERK1/2 (extracellular signal-related kinase 1/2) or PLCγ (phospholipase C–γ) promoting its potent antiapoptotic function.[19] Moreover, FLT3-ITD mutants induce a transcriptional program that resembles activation of the IL3R, rather than that of wild-type FLT3.[20] Overexpression of *MYC* induces apoptosis, and tumor cell viability is dependent upon cooperating overexpression of *BCL2* family members.[21] We speculate that the increase in MYC levels by activation of the IL3 receptor (IL3R) or FLT3 might decrease selective pressure for gain of a third *MYC* allele via acquisition of an additional chromosome 15. The observation of trisomy 15 in 100% of *PML-RARA/BCL2* leukemias, but in only 43% of *PML-RARA/FLT3*^{W51}, 22% of *PML-RARA/IL3R*, and 0% of *PML-RARA/IL3* mice, is consistent with this hypothesis. Identifying the genes that drive chromosome gains or losses in human and murine myeloid leukemias may offer insights into the pathogenesis or progression of leukemias and may provide new therapeutic targets.

Acknowledgments

We thank Drs Kevin Shannon, James Downing, Nigel Killeen, David Largaespada, Nancy Jenkins, and Neal Copeland for helpful discussions.

References

1. Jaffe ES, Harris NL, Stein H, Vardiman J, eds. Pathology and genetics of tumours of haematopoietic and lymphoid tissues: WHO classification of tumours. Lyon, France: IARC Press; 2001.
2. Melnick A, Licht JD. Deconstructing a disease: RARalpha, its fusion partners, and their roles in the pathogenesis of acute promyelocytic leukemia. Blood. 1999;93:3167-3215.
3. Pollock JL, Westervelt P, Walter MJ, Lane AA, Ley TJ. Mouse models of acute promyelocytic leukemia. Curr Opin Hematol. 200l;8:206-211.
4. Brown D, Kogan S, Lagasse E, et al. A PMLRAR alpha transgene initiates murine acute promyelocytic leukemia. Proc Natl Acad Sci U S A. 1997; 94:2551-2556.
5. Higuchi M, O'Brien D, Kumaravelu P, Lenny N, Yeoh EJ, Downing JR. Expression of a conditional AML1-ETO oncogene bypasses embryonic lethality and establishes a murine model of human t(8;21) acute myeloid leukemia. Cancer Cell. 2002;1:63-74.
6. Kelly L, Clark J, Gilliland DG. Comprehensive genotypic analysis of leukemia: clinical and therapeutic implications. Curr Opin Oncol. 2002;14:10-18.
7. Kogan SC, Brown DE, Schultz DB, et al. BCL-2 cooperates with promyelocytic leukemia retinoic acid receptor α chimeric protein (PMLRARα) to block neutrophilic differentiation and initiate acute leukemia. J Exp Med. 2001;193:531-543.
8. Kelly LM, Liu Q, Kutok JL, Williams IR, Boulton CL, Gilliland DG. FLT3 internal tandem duplication mutations associated with human acute myeloid leukemias induce myeloproliferative disease in a murine bone marrow transplant model. Blood. 2002;99:310-318.
9. Sohal J, Phan VT, Cham PV, et al. A model of human APL with FLT3 mutations is responsive to retinoic acid and a receptor tyrosine kinase inhibitor, SU11657. Blood. 2003;101:3188-3197.
10. Chang JM, Metcalf D, Lang RA, Gonda TJ, Johnson GR. Nonneoplastic hematopoietic myeloproliferative syndrome induced by dysregulated multi-CSF (IL-3) expression. Blood. 1989;73: 1487-1497.
11. Phan VT, Shultz DB, Truong B-TH, et al. Cooperation of cytokine signaling with chimeric transcription factors in leukemogenesis: PML-RARα blocks growth factor mediated differentiation. Mol Cell Biol. In press.
12. Jenkins BJ, D'Andrea R, Gonda TJ. Activating point mutations in the common beta subunit of the human GM-CSF, IL-3, and IL-5 receptors suggest the involvement of beta subunit dimerization and cell type-specific molecules in signaling. EMBO J. 1995;14:4276-4287.
13. Le Beau MM, Bitts S, Davis EM, Kogan SC. Recurring chromosomal abnormalities in leukemia in PML-RARA transgenic mice parallel human acute promyelocytic leukemia. Blood. 2002;99:2985-2991.
14. Wuchter CL, Karawajew L, Ruppert V, et al. Clinical significance of CD95, Bcl-2 and Bax expression and CD95 function in adult de novo acute myeloid leukemia in context of P-glycoprotein function, maturation stage, and cytogenetics. Leukemia. 1999;13:1943-1953.
15. Reilly JT. Class III receptor tyrosine kinases: role in leukaemogenesis. Br. J. Haematol. 2002;116: 744-757.
16. Gibbons DL, MacDonald D, McCarthy KP, et al. An E mu-BCL-2 transgene facilitates leukaemogenesis by ionizing radiation. Oncogene. 1999; 18:3870-3877.
17. Silva S, Babonits M, Wiener F, Klein G. Further studies on chromosome 15 trisomy in murine T-cell lymphomas: mapping of the relevant chromosome segment. Int J Cancer. 1988;41:738-743.
18. Virtaneva K, Wright FA, Tanner SM, et al. Expression profiling reveals fundamental biological differences in acute myeloid leukemia with isolated trisomy 8 and normal cytogenetics. Proc Natl Acad Sci U S A. 2001;98:1124-1129.
19. Hayakawa F, Towatari M, Kiyoi H, et al. Tandem-duplication Flt3 constitutively activates STAT5 and MAP kinase and introduces autonomous cell growth in IL-3 dependent cell lines. Oncogene. 2000;19:624-631.
20. Mizuki M, Schwäble J, Steur C, et al. Suppression of myeloid transcription factors and induction of STAT response genes by AML-specific Flt3 mutations. Blood. 2003;101:3164-3173.
21. Bissonnette RP, Echeverri F, Mahboubi A, Green DR. Apoptotic cell death induced by c-myc is inhibited by bcl-2. Nature. 1992;359:552-554.