REFERENCES

1. Mullighan CG. Molecular genetics of B-precursor acute lymphoblastic leukemia. *J Clin Invest*. 2012;122(10):3407-3415.
2. Wiemels JL, Ford AM, Van Wering ER, Postma A, Greaves M. Protracted and variable latency of acute lymphoblastic leukemia after TEL-AML1 gene fusion in utero. *Blood*. 1999;94(3):1057-1062.
3. Papaemmanuil E, Rapado I, Li Y, et al. RAG-mediated recombination is the predominant driver of oncogenic rearrangement in ETV6-RUNX1 acute lymphoblastic leukemia. *Nat Genet*. 2014;46(2):116-125.
4. Mori H, Colman SM, Xiao Z, et al. Chromosome translocations and covert leukemic clones are generated during normal fetal development. *Proc Natl Acad Sci USA*. 2002;99(12):8242-8247.
5. Eguchi-Ishimae M, Eguchi M, Ishii E, et al. Breakage and fusion of the TEL (ETV6) gene in immature B lymphocytes induced by apoptogenic signals. *Blood*. 2001;97(3):737-743.
6. Olsen M, Madsen HO, Hjalgrim H, Gregers J, Rostgaard K, Schmiegelow K. Preleukemic TEL-AML1-positive clones at cell level of 10(-3) to 10(-4) do not persist into adulthood. *J Pediatr Hematol Oncol*. 2006;28(11):734-740.
7. Lausten-Thomsen U, Hjalgrim H, Marquart H, Lutterodt M, Petersen BL, Schmiegelow K. ETV6-RUNX1 transcript is not frequent in early human haematopoiesis. *Eur J Haematol*. 2008;81(2):161-162.
8. Lausten-Thomsen U, Madsen HO, Vestergaard TR, Hjalgrim H, Lando A, Schmiegelow K. Increased risk of ALL among premature infants is not explained by increased prevalence of pre-leukemic cell clones. *Blood Cells Mol Dis*. 2010;44(3):188-190.
9. Lausten-Thomsen U, Madsen HO, Vestergaard TR, Hjalgrim H, Nersting J, Schmiegelow K. Prevalence of t(12;21)[ETV6-RUNX1]-positive cells in healthy neonates. *Blood*. 2011;117(1):186-189.
10. Zuna J, Madzo J, Krejci O, et al. ETV6/RUNX1 (TEL/AML1) is a frequent prenatal first hit in childhood leukemia. *Blood*. 2011;117(1):368-369; author reply 370-371.
11. Olsen M, Hjalgrim H, Melbye M, Madsen HO, Schmiegelow K. RT-PCR screening for ETV6-RUNX1-positive clones in cord blood from newborns in the Danish National Birth Cohort. *J Pediatr Hematol Oncol*. 2012;34(4):301-303.
12. Škorvaga M, Nikitina E, Kubeš M, et al. Incidence of common preleukemic gene fusions in umbilical cord blood in Slovak population. *PLoS One*. 2014;9(3):e91116.
13. Ornelles DA, Gooding LR, Garnett-Benson C. Neonatal infection with species C adenoviruses confirmed in viable cord blood lymphocytes. *PLoS One*. 2015;10(3):e0119256.
14. Kosik P, Skorvaga M, Durdik M, et al. Low numbers of pre-leukemic fusion genes are frequently present in umbilical cord blood without affecting DNA damage response. *Oncotarget*. 2017;8(22):35824-35834.
15. Fueller E, Schaefer D, Fischer U, et al. Genomic inverse PCR for exploration of ligated breakpoints (GIPFEL), a new method to detect translocations in leukemia. *PLoS One*. 2014;9(8):e104419.
16. Hovorkova L, Zaliova M, Venn NC, et al. Monitoring of childhood ALL using BCR-ABL1 genomic breakpoints identifies a subgroup with CML-like biology. *Blood*. 2017;129(20):2771-2781.
17. Janz S, Potter M, Rabkin CS. Lymphoma- and leukemia-associated chromosomal translocations in healthy individuals. *Genes Chromosomes Cancer*. 2003;36(3):211-223.
18. Greaves MF, Maia AT, Wiemels JL, Ford AM. Leukemia in twins: lessons in natural history. *Blood*. 2003;102(7):2321-2333.
19. Sun C, Chang L, Zhu X. Pathogenesis of ETV6/RUNX1-positive childhood acute lymphoblastic leukemia and mechanisms underlying its relapse. *Oncotarget*. 2017;8(21):35445-35459.
20. Hauer J, Borkhardt A, Sánchez-García I, Cobaleda C. Genetically engineered mouse models of human B-cell precursor leukemias. *Cell Cycle*. 2014;13(18):2836-2846.
21. Wiemels JL, Greaves M. Structure and possible mechanisms of TEL-AML1 gene fusions in childhood acute lymphoblastic leukemia. *Cancer Res*. 1999;59(16):4075-4082.
22. Wiemels JL, Alexander FE, Cazzaniga G, Biondi A, Mayer SP, Greaves M. Microclustering of TEL-AML1 translocation breakpoints in childhood acute lymphoblastic leukemia. *Genes Chromosomes Cancer*. 2000;29(3):219-228.
23. Andersen MT, Nordentoft I, Hjalgrim LL, et al. Characterization of t(12;21) breakpoint junctions in acute lymphoblastic leukemia. *Leukemia*. 2001;15(5):858-859.
24. Rodríguez-Hernández G, Hauer J, Martín-Lorenzo A, et al. Infection exposure promotes ETV6-RUNX1 precursor B-cell leukemia via impaired H3K4 demethylases. *Cancer Res*. 2017;77(16):4365-4377.
25. Jin Y, Wang X, Hu S, Tang J, Li B, Chai Y. Determination of ETV6-RUNX1 genomic breakpoint by next-generation sequencing. *Cancer Med*. 2016;5(2):337-351.

DOI 10.1182/blood-2017-09-808402

TO THE EDITOR:

Loss of RKIP is a frequent event in myeloid sarcoma and promotes leukemic tissue infiltration

Veronica Caraffini,[1] Bianca Perfler,[1] Johannes Lorenz Berg,[1] Barbara Uhl,[1] Silvia Schauer,[2] Karl Kashofer,[2] Nassim Ghaffari-Tabrizi-Wizsy,[3] Herbert Strobl,[3] Albert Wölfler,[1] Gerald Hoefler,[2] Heinz Sill,[1] and Armin Zebisch[1]

[1]Division of Hematology, [2]Institute of Pathology, and [3]Institute of Pathophysiology and Immunology, Medical University of Graz, Graz, Austria

Myeloid sarcoma (MS) is a subtype of acute myeloid leukemia (AML), in which leukemic cells invade extramedullary tissues and form solid tumors.[1-3] MS may manifest as an isolated event or with concomitant involvement of leukemic bone marrow (BM), the latter affecting up to 20% to 30% of all AML cases.[2,4-8] Clinical data about the prognostic relevance of MS are still conflicting, mainly because of variable clearing of extramedullary leukemic blasts by conventional chemotherapy.[2,7,9] Hence, knowledge of the pathogenetic mechanisms, which endow leukemic blasts with an invasive potential and thereby cause the formation of MS, will help to improve therapeutic regimens for MS patients. RAF kinase inhibitor protein (RKIP) is a negative regulator of RAS-MAPK/ERK signaling.[10-12] A somatic loss of RKIP has recently been described as a frequent event in AML. It has been shown to be associated with monocytic AML phenotypes and proven to be of functional relevance for leukemogenesis.[13-15] It is interesting to note that RKIP loss has also been observed in a variety of solid cancers and has been described as a bona fide

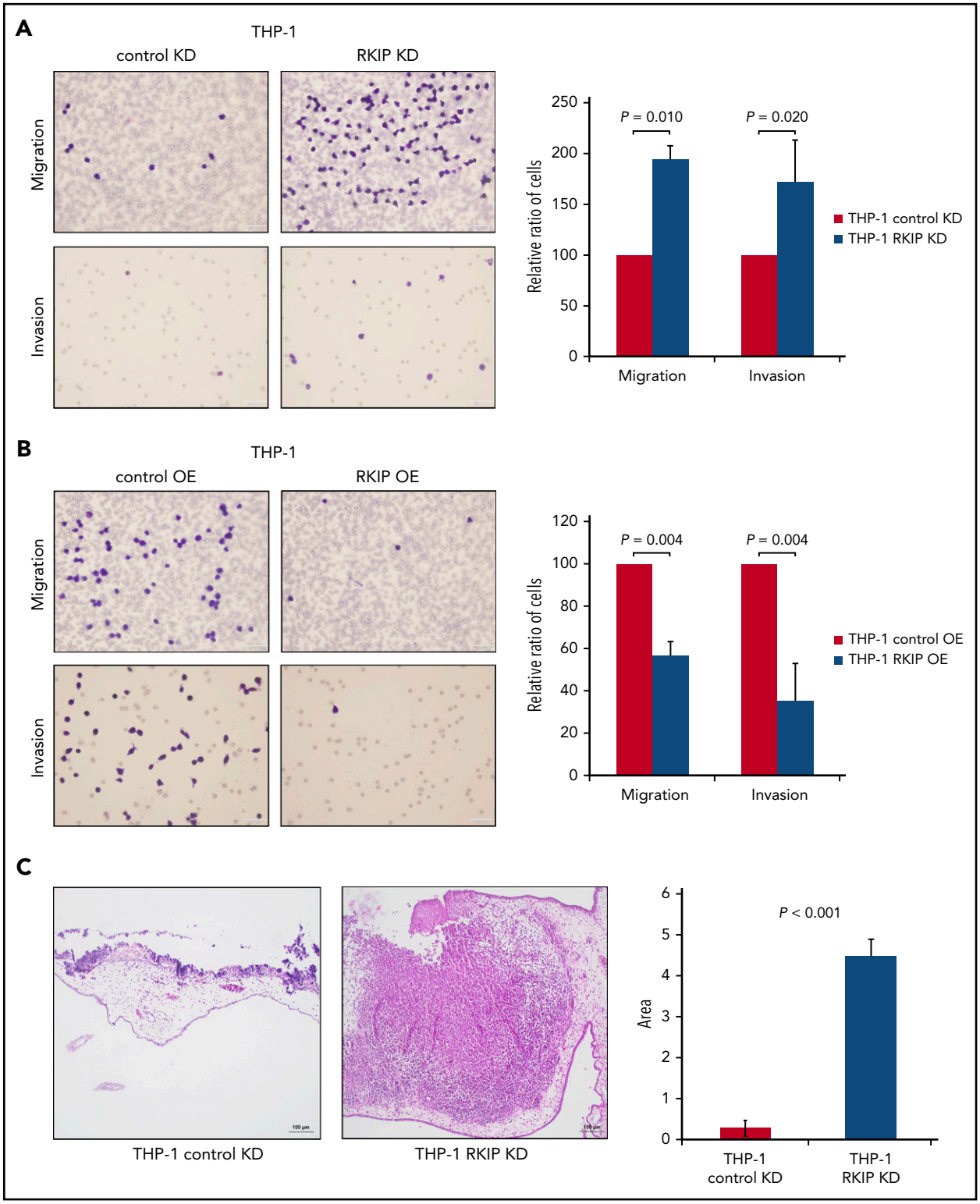

Figure 1. RKIP loss is involved in the development of MS. (A-B) Results of migration and invasion experiments using THP-1 AML cells with RKIP short hairpin RNA KD (A) and FLAG-RKIP OE (B). Representative images (40× magnification) of PET membranes with Giemsa-stained cells are displayed. The number of cells was counted with an inverted microscope. In all cases, cells carrying the empty control vectors (control KD and control OE, respectively) have been arbitrarily set to a value of 100, and the x-fold change in cells transduced with either RKIP short hairpin RNA (THP-1 RKIP KD) or FLAG-RKIP (THP-1 RKIP OE) was calculated using the ratio "number of cells RKIP KD/number of cells control KD" and "number of cells RKIP OE/number of cells control OE," respectively. Graphs summarize the results of at least 3 independent experiments. Data are expressed as mean values ± standard deviation, and *P* values have been calculated using the Student *t* test. (C) Representative hematoxylin and eosin staining of chicken CAMs after seeding of THP-1 AML cells with RKIP KD (THP-1 RKIP KD) and without (THP-1 control KD) showing invasion and tumor formation in the THP-1 RKIP KD condition (10× magnification). The graph displays the area of invading cells/tumors as assessed by ImageJ and summarizes the results of 4 independent experiments. Data are expressed as mean values ± standard deviation, and *P* values have been calculated using the Student *t* test.

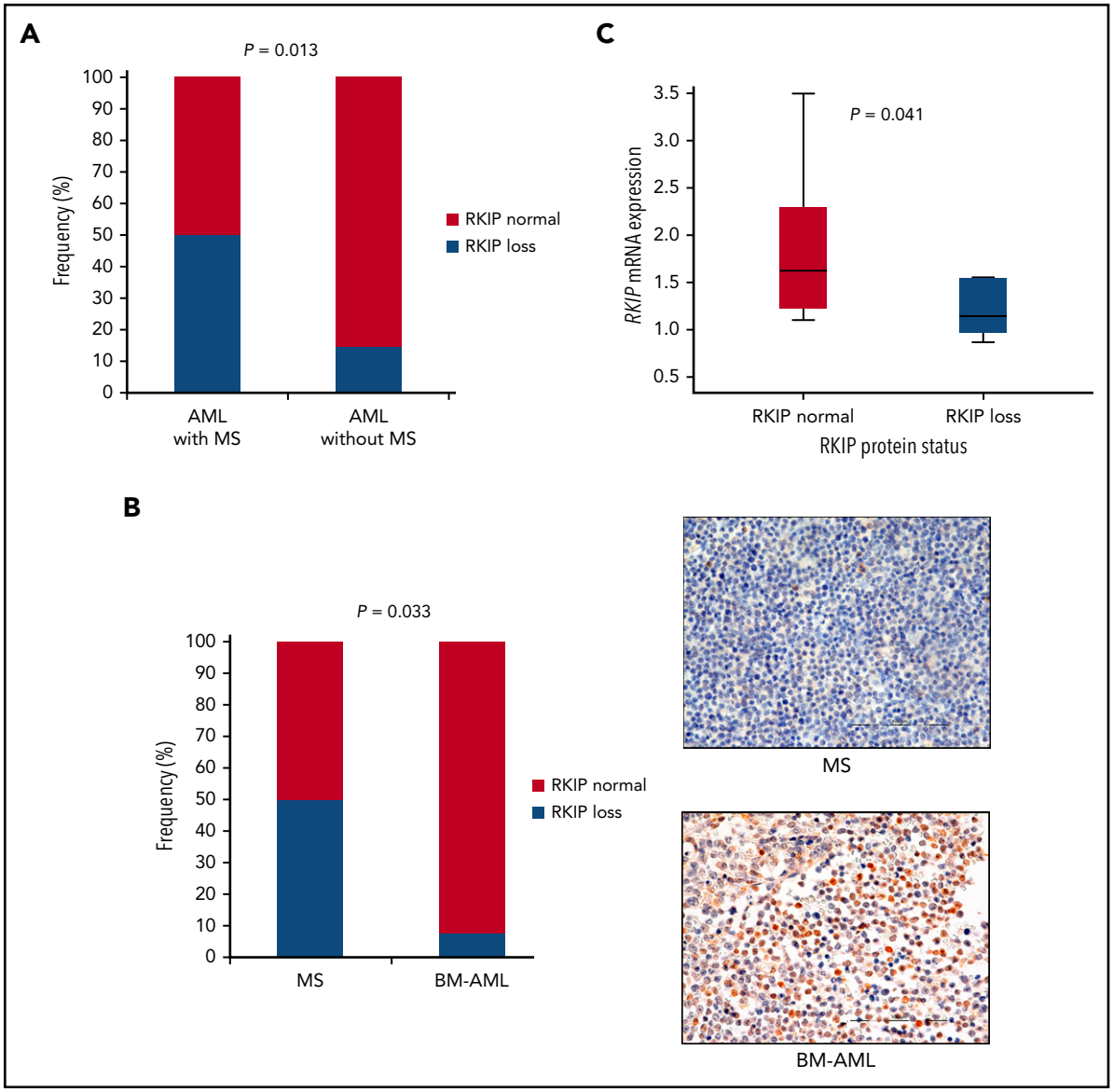

Figure 2. Loss of RKIP is a frequent event in MS, both at the protein and mRNA levels. (A) Clinical evaluation of extramedullary involvement in 58 AML patients, who have been analyzed in respect to RKIP protein expression in the leukemic BM previously (cohort I).[15] The frequency of specimens defined as RKIP loss was significantly increased in AML patients with MS compared with those without, as assessed by Fisher's exact test. (B) RKIP loss was also associated with MS in an independent cohort, where MS was confirmed by biopsy (cohort II). The frequency of RKIP losses was significantly increased in 14 samples of MS compared with 14 specimens of BM from AML patients without any evidence of MS (BM-AML), as calculated by Fisher's exact test. A representative immunohistochemical analysis showing loss of RKIP expression in a specimen of MS and normal RKIP expression in BM-AML is also shown (40× magnification). (C) *RKIP* mRNA expression analysis by quantitative polymerase chain reaction demonstrating that samples with RKIP loss at the protein level also exhibit decreased levels of *RKIP* mRNA. NB4 AML cells served as a calibrator, and statistical significance was calculated using the Mann-Whitney-Wilcoxon test.

metastasis suppressor in these entities.[10,16,17] Because of the similarities between metastasis formation and tissue infiltration of AML cells in the development of MS, we now aimed to identify the role of RKIP in the pathogenesis of this AML subtype.

Initially, we studied the functional role of RKIP in leukemic tissue infiltration in vitro by assessing the migration and invasion potential of THP-1 AML cells with stable RKIP knockdown (THP-1 RKIP KD) and overexpression (THP-1 RKIP OE), respectively (supplemental Methods; supplemental Figure 1A-B, available on the *Blood* Web site).[15,18] In these experiments, RKIP KD significantly increased both migration and invasion of THP-1 cells ($P = .010$ and $P = .020$, respectively; Figure 1A), whereas RKIP OE caused the opposite effects ($P = .004$ and $P = .004$, respectively; Figure 1B). The same phenomenon was observed in U937, an additional AML cell line (supplemental Methods; supplemental Figure 2 and 3). To further corroborate these findings in vivo, we performed a chorioallantoic membrane (CAM) assay (supplemental Methods). In this assay, we evaluated the potential of THP-1 cells with and without RKIP KD to invade the CAM of chicken embryos and, consequently, to form solid tumor masses. In agreement with our in vitro data, this was the case in all RKIP KD experiments, whereas only occasional and sparse infiltrations of single THP-1 cells were observed in control transfected cells ($P < .001$; Figure 1C). Taken together, these data demonstrate that RKIP is indeed involved in the tissue infiltration of leukemic blasts and, consequently, in the formation of MS.

Next, we aimed to shed more light on the mechanisms by which RKIP loss promotes MS development. Unexpectedly, although RKIP is an inhibitor of RAS-MAPK/ERK signaling, inhibition of MEK with U0126 failed to rescue the effects of RKIP KD ($P = .889$; supplemental Methods; supplemental Figure 4), indicating that MS formation in AML with RKIP loss is mediated via other RAS-MAPK/ERK–independent effectors. To identify potential candidate genes, we performed messenger RNA (mRNA) microarrays (supplemental Methods) in THP-1 cells with and without RKIP KD. It is interesting to note that KD of RKIP thereby induced a distinct gene expression profile, which included the prominent deregulation of networks involved in degradation of connective tissues and migration as well as in the interaction, binding, and engulfment of hematopoietic cells (supplemental Figure 5; supplemental Table 1).

Having proven that decreased expression of RKIP indeed plays a role in tissue infiltration of leukemic blasts, we then aimed to clarify the clinical relevance of this finding (supplemental Methods; supplemental Figure 6). Therefore, we initially screened the medical records of 103 patients with AML, whose BM specimens have previously been characterized in respect to RKIP expression,[15] for clinical evidence of MS as previously described[7,19] ("cohort I"). This retrospective clinical evaluation was possible in 58 patients, with MS being present in 16 (28%). In agreement with our functional data, specimens with RKIP loss were significantly enriched in AML cases with concomitant occurrence of MS (8/16 [50%] vs 6/42 [14%]; $P = .013$; Figure 2A; supplemental Table 2). We then tried to additionally corroborate our findings in an independent cohort, where the presence of MS was confirmed by biopsy ("cohort II"; supplemental Table 3 and 4). Therefore, we studied RKIP protein expression by immunohistochemistry in a series of formalin-fixed, paraffin-embedded patient specimens of MS (n = 14) and corresponding leukemic BM samples (n = 6). In brief, we classified RKIP expression as either "RKIP normal" or "RKIP loss" by implementing a previously described scoring system that incorporates both the amount and intensity of positively stained cells.[20] Loss of RKIP was observed in 7 of 14 cases (50%) with no differences in RKIP expression between MS and corresponding leukemic BM specimens, as well as between AML/MS diagnostic and relapse material (supplemental Figure 7). It is important to realize that RKIP loss was detected in only 1 of 14 BM specimens from AML patients without any evidence of MS (7%; $P = .033$; Figure 2B; supplemental Figure 8), which further supports the association between RKIP loss and MS. These data also suggest that RKIP expression could serve as a potential AML biomarker, which can easily be detected in leukemic BM and which aids in identifying cases with additional extramedullary manifestations. As a limitation of our study, it has to be noted that the small sample size of the cohort analyzed did not enable us to test whether MS, and particularly its RKIP expression status, might be of prognostic relevance for AML. Therefore, analysis of larger, preferably prospective clinical cohorts in future studies will be necessary.

To gain more insight into the molecular landscape of MS patients with RKIP loss, we performed next-generation sequencing of 39 leukemia-associated genes in all formalin-fixed, paraffin-embedded MS specimens with sufficient DNA quality for this analysis (n = 11; supplemental Figure 9) as previously described.[21] Mutations in *RAS* signaling were enriched in samples with RKIP loss, with 5 of 6 cases (83%) carrying 1 or more of these substitutions, whereas only 2 of 5 cases (40%) with normal RKIP expression were affected. Unfortunately, statistical analysis was precluded by the small sample size. However, because a functional synergism in leukemogenesis between mutant *RAS* and RKIP loss has been shown previously,[15] these data might suggest a potential relevance of this interaction for MS formation as well. It is interesting to note that in accordance with this hypothesis, mutations in *NPM1* and *DNMT3A* were almost exclusively detected in cases with normal RKIP expression. Both aberrations are frequently detected in MS and have been shown to promote *RAS*-independent MS formation in vitro and in vivo.[2,21-25] Finally, when examining the mechanisms that cause RKIP loss in MS, we observed that quantitative polymerase chain reaction analyses delineated that samples harboring RKIP loss at the protein level also showed decreased levels of *RKIP* mRNA ($P = .041$; Figure 2C). This finding is in agreement with previous data of our own group, where we did show that RKIP loss in myeloid malignancies is caused by miR-23a–induced downregulation of its mRNA.[13,15,18]

In conclusion, we show that loss of the metastasis-suppressor RKIP is a frequent event in MS and that decreased expression of RKIP in leukemic BM of AML patients might serve as a biomarker for the occurrence of additional extramedullary manifestations. Finally, we demonstrate that RKIP is functionally involved in tissue infiltration of leukemic cells and in the development of MS, which makes it an attractive target for future MS-directed therapies.

Acknowledgments

The authors would like to thank Karin Wagner for excellent technical support at microarray experiments. The authors also thank SFL Technologies (Stallhofen, Austria) for providing Olympus microscopes used in this study.

This study was supported by research funding from the Austrian Science Fund (grant P26619-B19) (A.Z.). Work in the laboratories of A.Z., A.W., and H. Sill is further supported by Leukämiehilfe Steiermark. PhD candidate V.C. received funding from the Austrian Science Fund (grant P26619-B19 [A.Z.]) and was trained within the frame of the PhD Program Molecular Medicine of the Medical University of Graz. PhD candidate J.L.B. received funding from the Medical University of Graz within the PhD Program Molecular Medicine.

Authorship

Contribution: A.Z. designed and supervised the study; V.C., B.P., J.L.B., S.S., G.H., H. Strobl, K.K., N.G.-T.-W., H. Sill, and A.Z. acquired data; B.U., G.H., H. Sill, A.W., and A.Z. provided patient samples and clinical data; V.C., J.L.B., K.K., G.H., H. Strobl, A.W., H. Sill, and A.Z. analyzed and interpreted the data; V.C. and A.Z. wrote the manuscript; and all authors reviewed and approved the manuscript.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

ORCID profiles: A.W., 0000-0002-3112-9857; G.H., 0000-0002-9056-3063; H. Sill, 0000-0003-0993-4371.

Correspondence: Armin Zebisch, Division of Hematology, Medical University of Graz, Auenbruggerplatz 38, 8036 Graz, Austria; e-mail: armin.zebisch@medunigraz.at.

Footnote

The online version of this article contains a data supplement.

REFERENCES

1. Vardiman JW, Thiele J, Arber DA, et al. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. *Blood*. 2009;114(5):937-951.
2. Ohanian M, Faderl S, Ravandi F, et al. Is acute myeloid leukemia a liquid tumor? *Int J Cancer*. 2013;133(3):534-543.
3. Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood*. 2016;127(20):2391-2405.
4. Chang H, Brandwein J, Yi QL, Chun K, Patterson B, Brien B. Extramedullary infiltrates of AML are associated with CD56 expression, 11q23 abnormalities and inferior clinical outcome. *Leuk Res*. 2004;28(10):1007-1011.
5. Klco JM, Welch JS, Nguyen TT, et al. State of the art in myeloid sarcoma. *Int J Lab Hematol*. 2011;33(6):555-565.
6. Zebisch A, Cerroni L, Beham-Schmid C, Sill H. Therapy-related leukemia cutis: case study of an aggressive disorder. *Ann Hematol*. 2003;82(11):705-707.
7. Ganzel C, Manola J, Douer D, et al. Extramedullary disease in adult acute myeloid leukemia is common but lacks independent significance: analysis of patients in ECOG-ACRIN cancer research group trials, 1980-2008. *J Clin Oncol*. 2016;34(29):3544-3553.
8. Gornicec M, Wölfler A, Stanzel S, Sill H, Zebisch A. Evidence for a role of decitabine in the treatment of myeloid sarcoma. *Ann Hematol*. 2017;96(3):505-506.
9. Bakst RL, Tallman MS, Douer D, Yahalom J. How I treat extramedullary acute myeloid leukemia. *Blood*. 2011;118(14):3785-3793.
10. Al-Mulla F, Bitar MS, Taqi Z, Yeung KC. RKIP: much more than Raf kinase inhibitory protein. *J Cell Physiol*. 2013;228(8):1688-1702.
11. Zebisch A, Troppmair J. Back to the roots: the remarkable RAF oncogene story. *Cell Mol Life Sci*. 2006;63(11):1314-1330.
12. Yeung K, Seitz T, Li S, et al. Suppression of Raf-1 kinase activity and MAP kinase signalling by RKIP. *Nature*. 1999;401(6749):173-177.
13. Zebisch A, Haller M, Hiden K, et al. Loss of RAF kinase inhibitor protein is a somatic event in the pathogenesis of therapy-related acute myeloid leukemias with C-RAF germline mutations. *Leukemia*. 2009;23(6):1049-1053.
14. Zebisch A, Staber PB, Delavar A, et al. Two transforming C-RAF germ-line mutations identified in patients with therapy-related acute myeloid leukemia. *Cancer Res*. 2006;66(7):3401-3408.
15. Zebisch A, Wölfler A, Fried I, et al. Frequent loss of RAF kinase inhibitor protein expression in acute myeloid leukemia. *Leukemia*. 2012;26(8):1842-1849.
16. Escara-Wilke J, Keller JM, Ignatoski KM, et al. Raf kinase inhibitor protein (RKIP) deficiency decreases latency of tumorigenesis and increases metastasis in a murine genetic model of prostate cancer. *Prostate*. 2015;75(3):292-302.
17. Lamiman K, Keller JM, Mizokami A, Zhang J, Keller ET. Survey of Raf kinase inhibitor protein (RKIP) in multiple cancer types. *Crit Rev Oncog*. 2014;19(6):455-468.
18. Hatzl S, Geiger O, Kuepper MK, et al. Increased expression of miR-23a mediates a loss of expression in the RAF kinase inhibitor protein RKIP. *Cancer Res*. 2016;76(12):3644-3654.
19. Langer C, Marcucci G, Holland KB, et al. Prognostic importance of MN1 transcript levels, and biologic insights from MN1-associated gene and microRNA expression signatures in cytogenetically normal acute myeloid leukemia: a cancer and leukemia group B study. *J Clin Oncol*. 2009;27(19):3198-3204.
20. Chatterjee D, Sabo E, Tavares R, Resnick MB. Inverse association between Raf Kinase Inhibitory Protein and signal transducers and activators of transcription 3 expression in gastric adenocarcinoma patients: implications for clinical outcome. *Clin Cancer Res*. 2008;14(10):2994-3001.
21. Kashofer K, Gornicec M, Lind K, et al. Detection of prognostically relevant mutations and translocations in myeloid sarcoma by next generation sequencing. *Leuk Lymphoma*. 2018;59(2):501-504.
22. Li Z, Stölzel F, Onel K, et al. Next-generation sequencing reveals clinically actionable molecular markers in myeloid sarcoma. *Leukemia*. 2015;29(10):2113-2116.
23. Xian J, Shao H, Chen X, et al. Nucleophosmin mutants promote adhesion, migration and invasion of human leukemia THP-1 cells through MMPs up-regulation via Ras/ERK MAPK signaling. *Int J Biol Sci*. 2016;12(2):144-155.
24. Xu J, Zhang W, Yan XJ, et al. DNMT3A mutation leads to leukemic extramedullary infiltration mediated by TWIST1. *J Hematol Oncol*. 2016;9(1):106.
25. Pastoret C, Houot R, Llamas-Gutierrez F, et al. Detection of clonal heterogeneity and targetable mutations in myeloid sarcoma by high-throughput sequencing. *Leuk Lymphoma*. 2017;58(4):1008-1012.

DOI 10.1182/blood-2017-09-804906

TO THE EDITOR:

ABO zygosity, but not secretor or Fc receptor status, is a significant risk factor for IVIG-associated hemolysis

Donald R. Branch,[1-3] Åsa Hellberg,[4,5] Christine W. Bruggeman,[6] Jill R. Storry,[4,5] Darinka Sakac,[3] Megan Blacquiere,[3] Tik Nga Tong,[2,3] Emeralda Burke-Murphy,[3] Beth Binnington,[3] Nagina Parmar,[7] Lorna Sampson Riden,[7] Kezia Willie,[7] Chantal Armali,[8] Jiwajee Aziz,[9] Lani Lieberman,[2,7] Vincent Laroche,[10] Jeannie Callum,[2,8] Yulia Lin,[2,8] Nadine Shehata,[2,11,12] Katerina Pavenski,[2,9] Wendy Lau,[2,11,13] Barbara Hannach,[12] Taco W. Kuijpers,[6,14] Martin L. Olsson,[4,5,*] Christine Cserti-Gazdewich,[1,2,7,12,*] and Jacob Pendergrast[1,2,7,*]

[1]Department of Medicine and [2]Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, ON, Canada; [3]Centre for Innovation, Canadian Blood Services, Toronto, ON, Canada; [4]Division of Hematology and Transfusion Medicine, Department of Laboratory Medicine, Lund University, Lund, Sweden; [5]Department of Clinical Immunology and Transfusion Medicine, Laboratory Medicine, Office of Medical Services, Region Skåne, Lund, Sweden; [6]Department of Blood Cell Research, Sanquin Research and Landsteiner Laboratory, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands; [7]Laboratory Medicine Program, University Health Network, Toronto, ON, Canada; [8]Department of Clinical Pathology, Sunnybrook Health Sciences Centre, Toronto, ON, Canada; [9]Department of Laboratory Medicine, St. Michael's Hospital, Toronto, ON, Canada; [10]Institut Universitaire de Cardiologie et Pneumologie de Québec and CHU de Québec and Hôpitaux Enfant-Jésus et Saint-Sacrement, Québec City, QC, Canada; [11]Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, Toronto, ON, Canada; [12]Canadian Blood Services, Toronto, ON, Canada; [13]Department of Transfusion Medicine, Pediatric Laboratory Medicine, Hospital for Sick Children, Toronto, ON, Canada; [14]Pediatric Hematology, Immunology, Rheumatology, and Infectious Diseases, Emma Children's Hospital, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

Although frequently effective[1,2] and usually benign, high-dose (2 g/kg) intravenous immunoglobulin (IVIG) therapy can result in marked red blood cell (RBC) hemolysis, which in some cases is life threatening in severity.[3-5] The mechanism by which this hemolysis occurs is not completely understood but appears to involve the binding of isohemagglutinins within the product (anti-A,