Overexpression of *RUNX1* short isoform has an important role in the development of myelodysplastic/myeloproliferative neoplasms

Hiroko Sakurai,¹ Yuka Harada,^{1,2} Yosuke Ogata,² Yuki Kagiya,³ Naoki Shingai,¹ Noriko Doki,⁴ Kazuteru Ohashi,⁴ Toshio Kitamura,⁵ Norio Komatsu,¹ and Hironori Harada^{1,3}

¹Department of Hematology, Juntendo University School of Medicine, Tokyo, Japan; ²Department of Clinical Laboratory Medicine, Bunkyo Gakuin University, Tokyo, Japan;

³Laboratory of Oncology, School of Life Sciences, Tokyo University of Pharmacy and Life Sciences, Hachioji, Japan; ⁴Tokyo Metropolitan Cancer and Infectious Disease Center Komagome Hospital, Tokyo, Japan; and ⁵Division of Cellular Therapy, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan

Key Points

- *RUNX1a*, but not *RUNX1b*, is overexpressed in CD34⁺ cells from patients with myelodysplastic/myeloproliferative neoplasms.
- *SRSF2*P95H mutation induces *RUNX1a* overexpression and a monocytic phenotype in TF-1 cells.

Introduction

RUNX1 mutations have been shown to contribute to the development of myeloid neoplasms and are frequently identified in patients with myelodysplastic syndromes (MDS),¹ myelodysplastic/myeloproliferative neoplasms (MDS/MPN), including chronic monocytic leukemia (CMML),² and acute myeloid leukemia (AML).³ The biological function of these mutations has been elucidated in a mouse model⁴ and in human CD34⁺ cells.⁵ However, the relevance of the level of *RUNX1* expression for the pathogenesis of hematological malignancies has not been explored.

The *RUNX1* gene has several isoforms.^{6,7} Of 3 major isoforms, we focused on both functional full-length isoforms *RUNX1b/c* (lacking exon 7a and including exon 7b and 8), and the short isoform, *RUNX1a* (including exon 7a, which has a stop codon), which is found only in primates.⁸ *RUNX1b* and *RUNX1c* are functionally identical and *RUNX1b* is broadly expressed,⁹ whereas *RUNX1a* has a dominant-negative effect on *RUNX1b*.¹⁰ *RUNX1a* overexpression has been reported in AML.^{10,11} Enforced expression in murine hematopoietic cells enhances engraftment after transplantation.¹² Consistent with this, *RUNX1a* expands hematopoietic stem cells,^{13,14} and dominant inhibition by *RUNX1a* results in monopoiesis.¹⁵ Therefore, expression levels of each *RUNX1* isoform are likely to be important for normal and malignant hematopoiesis.

Accordingly, in the present study, we quantified expression levels of *RUNX1* isoforms in CD34⁺ cells from patients with myelodysplastic disease. We found that *RUNX1a* was overexpressed in some MDS and MDS/MPN patients and that these levels of expression increased as the disease progressed. The aberrant splicing resulting in *RUNX1a* is believed to be caused by splicing factor mutations that are frequently detected in MDS and MDS/MPN.¹⁶ Therefore, we enforced the expression of the *SRSF2* mutant in an MDS-derived cell line, and we show that this results in high expression of *RUNX1a*. Our results indicate that *RUNX1a* may have an important role in the progression of MDS and MDS/MPN and that splicing factor mutations may be one of the mechanisms contributing to *RUNX1a* overexpression.

Methods

Patients

Patients with hematological malignancies were examined as approved by the institutional review board at Juntendo University and Bunkyo Gakuin University. Patients gave written informed consent, in accordance with the Declaration of Helsinki. Gene mutation analysis was performed by Sanger sequencing as described previously⁵ using primer pairs listed in supplemental Table 1.

Quantitative reverse-transcription polymerase chain reaction

CD34⁺ cells were purified and total RNA was harvested as described previously.⁵ Gene expression levels were quantified with SYBR Select Master Mix and the following forward/reverse primer pairs: *RUNX1a* (5'-GAACCACTCCACTGCCTTAAC-3'/5'-ATTCTGAGGGCTGTCATCTTC-3'), *RUNX1b*

Figure 1.

(5'-GAACCACTCCACTGCCTTAAC-3'/5'-GAAAGTTCTGCAGAGAGGGTTG-3'), and *GAPDH* (5'-GGAAGGTGAAGGTCGAGTC-3'/5'-GGAAGATGGTGGTGGGATTC-3').

Retrovirus transduction

Purified CD34⁺ cells were infected with retroviral *RUNX1a* vector or empty (control) vector. After GFP-based sorting, the cells were cultured on MS5 stromal cells as described previously.⁵ The MDS-derived cell line TF-1 was infected with retroviral *SRSF2*P95H vector, and transduced single cells were isolated using FACS Aria. Growing clones were then analyzed separately. Flow cytometry and immunoblot analysis were performed as reported previously.⁵ Details are provided in supplemental Methods.

Statistical analysis

For multiple pairwise comparisons, data were analyzed by 1-way analysis of variance followed by Dunnett's multiple comparison test, or differences between individual groups were estimated using the Steel-Dwass test. $P < .05$ was considered statistically significant.

Results and discussion

Levels of expression of *RUNX1a* and *RUNX1b* in CD34⁺ cells from patients with hematological malignancies, including 10 lymphoma patients with normal bone marrow (controls), were analyzed. The average relative *RUNX1a* expression level in MDS patients ($n = 55$) and MDS/MPN patients ($n = 27$) was 9.6-fold and 11.1-fold that of controls, respectively, whereas *RUNX1b* expression was only approximately twofold that of the controls. In both disease groups, patients with excess blasts displayed a significantly higher level of *RUNX1a* expression (Figure 1A-B). During disease progression, the expression of *RUNX1a* increased, while it decreased during azacitidine treatment (shown for a single patient in Figure 1C-D). Interestingly, some patients had high expression of *RUNX1a* and repression of *RUNX1b*, even though both share a common promoter. RNA processing may explain why the expression levels of these isoforms do not change in parallel.

To evaluate the effect of *RUNX1a* overexpression on long-term cell expansion, we transduced *RUNX1a* into CD34⁺ cells from MDS patients with low-level expression of *RUNX1a*. The *RUNX1a*-transduced cells proliferated more than the control cells (Figure 1E). Therefore, overexpression of *RUNX1a* may provide a growth advantage to CD34⁺ cells. Genomic mutations of *RUNX1* were detected in 2 out of 39 (5.1%) MDS and 9 out of 25 (36%) MDS/MPN patients. Some patients had both a *RUNX1* gene mutation and *RUNX1a* overexpression (Figure 1A-B).

We next focused on the mechanism of *RUNX1a* overexpression. In MDS and MDS/MPN, splicing factor mutations are frequently found.¹⁶ We hypothesized that aberrant splicing of *RUNX1* might explain our

findings, and we therefore examined splicing factor mutations. *SRSF2*, *U2AF1*, and *SF3B1* were detected in 11 out of 47 MDS patients (23%) and 15 out of 27 MDS/MPN patients (56%) (Figure 2A-B; supplemental Table 2). In MDS, patients with splicing factor mutations did not show high *RUNX1a* expression; however, some MDS/MPN patients with these mutations, most of which were *SRSF2* mutations (supplemental Table 2), strongly overexpressed *RUNX1a*. Because *SRSF2* contributes to myelodysplasia by mutation-specific effects on exon recognition,¹⁷ it was hypothesized that the *SRSF2* mutation may affect the expression of *RUNX1a*. To investigate this, we analyzed *SRSF2*P95H-transduced TF-1 cells. Most of the clones exhibited higher expression of *RUNX1a* than mock-transduced cells, whereas *RUNX1b* expression was reduced in all of them (Figure 2C). Increased *RUNX1a* expression was confirmed by immunoblotting (Figure 2D). Furthermore, *SRSF2*P95H-transduced cells showed phenotypic changes, with higher CD11b and CD14 expression than mock cells (Figure 2E). The phenotypic changes were partially reverted by *RUNX1a* knockdown (Figure 2F), suggesting that *SRSF2*P95H may induce monocytic differentiation via *RUNX1a* overexpression, at least in part. *RUNX1* mutations in intron 6 and exon 7a were also analyzed. A 5' splice site change just after exon 6 (c.724+1_2dup) was detected in a CMML patient with *RUNX1a* overexpression. This may represent another mechanism responsible for *RUNX1a* overexpression. No mutations of exon 7a or changes in the 3' splice site just before exon 7a were detected. The possibility that other mechanisms are involved also needs to be considered.¹⁸

Pathogenic functions of *RUNX1a* have been reported in mouse models. However, this is not clear in patients with myeloid neoplasms. Here, we show for the first time that in addition to *RUNX1* mutations, overexpression of *RUNX1a* may have an important role in disease progression in MDS and MDS/MPN patients. Splicing factor mutations are suggested to contribute to the mechanism of *RUNX1* dysregulation.

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Authorship

Contribution: H.S. collected data and wrote the paper; Y.H. assembled and analyzed the data and wrote the paper; Y.O. and Y.K. collected data; N.S., N.D., K.O., and N.K. provided patient samples

Figure 1. *RUNX1a* overexpression in MDS, MDS/MPN, and AML patients with a history of MDS or MDS/MPN. (A-B) *RUNX1* expression levels by quantitative reverse-transcription polymerase chain reaction in CD34⁺ cells from patients with MDS (A) or MDS/MPN (CMML and MDS/MPN-unclassifiable) (B). Relative *RUNX1a* and *RUNX1b* expression was calculated as a ratio relative to *GAPDH* expression; the ratio of *RUNX1a* to *RUNX1b* expression was also calculated. RNA from normal bone marrow CD34⁺ cells served as a control, their RNA level being defined as unity. * $P < .05$; ** $P < .01$. Closed circles show *RUNX1*-mutated patients. BM, bone marrow. (C-D) Change in *RUNX1* expression levels over time in a single patient with MDS (C) or MDS/MPN (D). Patient (Pt) 20, 42, 14, and 32 showed disease progression, whereas patient 302, who was treated with azacitidine (AZA), showed hematological improvement. The diagnosis was based on WHO2016.¹⁹ (E) Enforced *RUNX1a* expression in CD34⁺ cells from patients. Growth patterns of the GFP-sorted cells cultured on MS5 stromal cells are shown. EB, excess blasts; N.S., not significant; PB, peripheral blood.

Figure 2. *RUNX1a* overexpression induced by splicing factor mutations. (A-B) *RUNX1a* expression levels in CD34⁺ cells from MDS (A) or MDS/MPN (B) patients with or without splicing factor (SF) mutations. ***P* < .01. (C) *RUNX1* expression levels of 13 *SRSF2* mutant (MT)–transduced clones were compared with those of 3 mock-transduced cells. Average transcript expression and standard deviation from triplicate experiments is shown. (D) *RUNX1* protein expression in TF-1 cells was verified by immunoblot, and the relative protein level was quantified. *RUNX1a*–transfected Plat-GP cells were used as a positive control for *RUNX1a* protein. (E) Representative flow cytometry analysis of mock-transduced cells and *SRSF2* mutant (MT)–transduced cells. (F) Phenotypic changes by *RUNX1a* knockdown in *SRSF2* mutant–transduced cells. *RUNX1a* knockdown was verified by relative *RUNX1a* expression to *GAPDH* expression. BM, bone marrow.

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Correspondence: Hironori Harada, Laboratory of Oncology, School of Life Sciences, Tokyo University of Pharmacy and Life Sciences, 1432-1 Horinouchi, Hachioji, Tokyo 192-0392, Japan; e-mail: hharada@toyaku.ac.jp.

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